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Downregulation of miR-1294 associates with prognosis and tumor progression in epithelial ovarian cancer

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Abstract. – OBJECTIVE: Increasing evidence has revealed that microRNAs (miRNAs) act as key players in the regulation of tumor growth and metastasis in epithelial ovarian cancer (EOC). However, the clinical role and functional effects of miR-1294 in EOC remain unknown.

PATIENTS AND METHODS: We examined the expression of miR-1294 in 69 cases of EOC tissues and cell lines by quantitative Real-time polymerase chain reaction (qRT-PCR). The associations of miR-1294 expression with clinicopathologic features and overall survival of EOC patients were analyzed. Biological functional effects of miR-1294 expression on cell growth were analyzed using Cell Counting Kit-8 (CCK8) assays and flow cytometry assays *in vitro*.

RESULTS: In the present study, we identified that miR-1294 expression was lower in 76 specimens of EOC compared to adjacent normal tissues. Lower miR-1294 expression was related to FIGO stage, lymph node metastasis and shorter overall survival rate in EOC patients. Multivariate Cox analysis demonstrated that miR-1294 expression was an independent prognostic indicator of EOC patients. Gain function assays showed that miR-1294 overexpression inhibited cell proliferation and cell cycle progression in EOC.

CONCLUSIONS: Our results indicated that miR-1294 acted as the prognostic biomarker and potential target of EOC treatment.

Key Words:
Epithelial ovarian cancer, microRNAs, miR-1294, Prognosis, Cell growth.

Introduction

Epithelial ovarian cancer (EOC) remains the most common type of malignant tumor of the female reproductive system worldwide[1]. Due to tumor relapse and distant metastases of this disease at an advanced stage, the 5-year survival rate for patients is about 40%[2,3]. Cancer screening and early detection show the potential to greatly reduce the mortality and morbidity from EOC[4]. Thus, to investigate potential biomarkers for early diagnosis and therapeutic targets of EOC is urgent. MicroRNAs (miRNAs), a family of short non-coding RNAs, affect target gene expression at the post-transcriptional level[5]. MicroRNAs affect cancer development including cell proliferation, invasion and metastases[6]. MicroRNAs have emerged to be identified as potential biomarkers for EOC prognosis and therapeutic targets. For instance, microRNA-183 expression correlates cancer prognosis, regulates cancer proliferation and bufalin sensitivity in epithelial ovarian cancer[7]. MiR-595 expression is downregulated in EOC tissues and low miR-595 expression level is significantly associated with shorter overall survival[8]. Decreased expression and increased expression of miR-100 and miR-203 could be correlated with progression and poor prognosis of EOC[9]. To the best of our knowledge, researches[10] have identified miR-1294 as a tumor suppressor in some tumors. For instance, miR-1294 inhibits the proliferation and enhances the chemosensitivity of glioma to temozolomide via the direct targeting of TPX2. Down-regulation of miR-1294 is related to dismal prognosis of patients with esophageal squamous cell carcinoma through elevating c-MYC expression[11]. In the present study, we found that miR-1294 expression was an independent prognostic indicator of EOC patients. MiR-1294 overexpression inhibited cell

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proliferation and cell cycle progression. Thus, these results suggested that miR-1294 may act as prognostic biomarker and novel target of EOC treatment.

Patients and Methods

Patients and Tissue Samples

A total of 69 paired of human EOC tumor tissues and adjacent normal ovarian tissues were obtained from patients that underwent surgical resection at the Department of Gynecology, Jiujiang Maternal and Child Health Care Hospital in Jiangxi Province between March 2011 and April 2014. The patients were 49.1 ± 15.5 years old. The stage of the tumors was determined according to the International Federation of Gynecology and Obstetrics (FIGO) staging system. No patients received chemotherapy or radiotherapy before surgery. All tissue samples were stored at -80°C for further RNA analysis. The present study was approved by Ethics Committee of Jiujiang Maternal and Child Health Care Hospital. Written informed consent was obtained from all participants in the study.

Cell Lines Culture and Transfection

Four human ovarian cancer cell lines including SW626, A2780, SKOV3 and OVCAR3 cells and a human ovarian surface epithelial cell line (HOSEpiC) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All of the cells were cultured in Dulbecco's modified eagle's medium (DMEM, Gibco, Gaithersburg, MD, USA) and supplemented with 10% fetal bovine serum (FBS, Gibco, Gaithersburg, MD, USA) at 37°C under a humidified atmosphere containing 5% CO_2.

RNA Extraction and Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted by using TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. The complementary DNA was synthesized from 1 μg of total RNA using the Prime Script RT Reagent kit (TaKaRa, Dalian, China). Expression level analysis of miR-1294 was performed using SYBR Premix ExTaq™ II (TaKaRa, Dalian, China). The miR-1294 expression was detected on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The reaction for qRT-PCR was 95°C for 10 min, followed by 40 cycles of 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s. The U6 expression was used as internal controls. The primers for miR-1294 were as follow: miR-1294-forward: 5'-TATGATCTCACCGAGTCCT-3', miR-1294-reverse: 5'-TATGATCTCACCGAGTCCT-3'. Fold changes were calculated using the $2^{-\Delta\Delta Ct}$ methods.

Cell Counting Kit-8 Assay

Transfected SKOV3 and OVCAR3 (2×10^4/well) cells were seeded into 96-well plates and incubated in Dulbecco's Modified Eagle Medium (DMEM) at 37°C and 5% CO_2 atmosphere. Cells were cultured for 24, 48, 72, and 96 hours. Subsequently, CCK8 solutions were added to each well and were incubated for 2 additional hours. Then, cell proliferation was detected using a microtiter plate reader (Tecan Infinite 200 PRO; Salzburg, Austria) and the absorbance was at 450 nm.

Cell Cycle Analysis

For cell cycle analysis, the miR-1294 mimic or miR-NC-transfected cells were harvested 48 h post-transfection. Cells were fixed with ice-cold 70% ethanol overnight at 4°C. After that, cells were stained with 50 mg/ml PI, treated with 50 mg/ml RNase. Cell cycle was analyzed using a flow cytometer (FACScan; BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

All statistical analyses were performed using SPSS (version 18.0: SPSS, Inc., Chicago, IL, USA). The results are presented as the mean ± standard deviation (SD). Differences between two groups were analyzed via the Student's *t*-test. Differences between more than two groups were analyzed using one-way analysis of variance followed by post-hoc Tukey analysis. A $p < 0.05$ was considered as statistical significance.

Results

MiR-1294 Expression is Downregulated in Epithelial Ovarian Cancer Tissues and Cells

The expression of miR-1294 was assessed in 69 cases of human EOC tumor tissues and adjacent normal ovarian tissues from EOC patients.

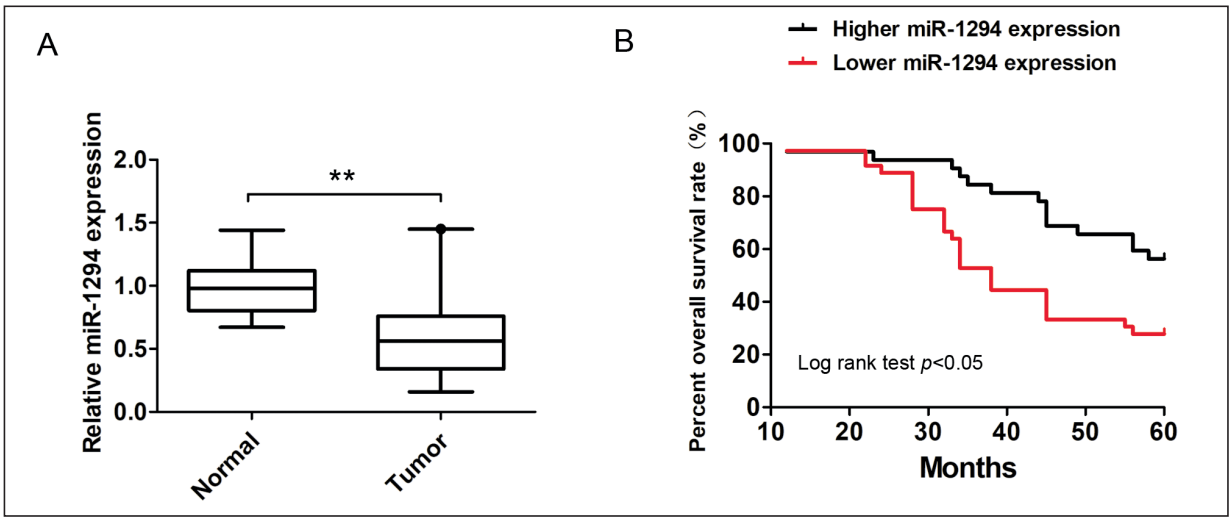

Figure 1. MiR-1294 expression is downregulated in EOC tissues and associated with prognosis. ***(A)*** MiR-1294 expression was detected using qRT-PCR in 69 cases EOC tissues and adjacent normal tissues. U6 was used as an internal control. ***(B)*** Lower miR-1294 expression showed a poor prognosis compared to higher miR-1294 expression in EOC patients. $^{**}p < 0.01$.

It was observed that the expression of miR-1294 was significantly downregulated in human EOC tumor tissues and adjacent normal ovarian tissues (Figure 1A, $p < 0.05$). In addition, we assessed the association between miR-1294 expression and clinicopathological characteristics of EOC patients. As shown in Table I, it was observed that lower miR-1294 expression was significantly associated with advanced FIGO stage ($p = 0.006$) and lymph node metastasis ($p = 0.001$). However, there was no correlation between miR-1294 expression and age, serum CA125 level, grade, tumor size and so on of EOC patients (all $p > 0.05$, Table I).

Table I. Clinicopathological variables and miR-1294 expression in epithelial ovarian cancer.

Parameters	Total (n = 69)	miR-1294 expression		p-value
		Higher (n = 33)	Lower (n = 36)	
Age (years)				0.543
≤ 55	35	18	17	
> 55	34	15	19	
Serum CA125				0.422
≤ 319	30	16	14	
> 319	39	17	22	
Tumor size				0.737
≤ 4 cm	32	16	16	
< 4 cm	37	17	20	
Grade				0.848
G1	28	13	15	
G2+G3	41	20	21	
FIGO stage				0.006*
I-II	30	20	10	
III-IV	39	13	26	
Lymph node metastasis				0.001*
Negative	31	21	9	
Positive	38	11	27	

*$p < 0.05$.

The Prognostic Value of miR-1294 Expression in EOC

The prognostic value of miR-1294 for the overall survival of patients with EOC was calculated during a follow-up period of 60 months. Kaplan-Meier method and log rank test showed that lower miR-1294 expression displayed shorter overall survival rate than patients with higher miR-1294 expression group (Figure 1B, log rank test, $p < 0.05$). Moreover, we performed multivariate Cox survival analyses. Multivariate analysis results showed that FIGO stage ($p = 0.002$, Table II), lymph node metastasis ($p = 0.001$, Table II) and lower miR-1294 expression ($p = 0.001$, Table II) were independent poor prognostic factor for EOC patients. Thus, these results indicated that miR-1294 may act as a prognostic biomarker of EOC.

Increased miR-1294 Expression Inhibits Cell Growth in EOC

We detected the expression of miR-1294 in four human ovarian cancer cell lines including SW626, A2780, SKOV3 and OVCAR3 cells and a human ovarian surface epithelial cell line (HOSEpiC). The results showed that miR-1294 expression was lower in human ovarian cancer cell lines including SW626, A2780, SKOV3 and OVCAR3 cells compared to HOSEpiC cells (Figure 2A, $p < 0.05$). Furthermore, we performed gain function assays by transfecting miR-1294 mimic into SKOV3 and OVCAR3 cells (Figure 2B-2C, $p < 0.05$). The CCK8 cell proliferation assay showed that overexpression of miR-1294 significantly inhibited cell proliferation ability compared to miR-NC group in SKOV3 or OVCAR3 cells (Figure 3A-3B, $p < 0.05$). The cell cycle analysis showed that overexpression of miR-1294 significantly reduced the cell number of S phase compared to miR-NC group in SKOV3 or OVCAR3 cells (Figure 3C-3D, $p < 0.05$). Thus, these results suggested that increased miR-1294 expression inhibited cell growth in EOC.

Discussion

Recently, marked improvement had been made for investing the underlying molecular mechanisms of EOC [12]. Due to the lack of early, safe and noninvasive detecting methods, patients with diagnosed at the late stage showed high malignancy. MicroRNAs have been found to acts as diagnosed and prognostic biomarkers. In EOC progression, miR-9 functions as a tumor inhibitor of cell proliferation in epithelial ovarian cancer through targeting the SDF-1/CXCR4 pathway[13]. Downregulation of miR-429 contributes to the development of drug resistance in epithelial ovarian cancer by targeting ZEB1[14]. MicroRNA-298 inhibits malignant phenotypes of epithelial ovarian cancer by regulating the expression of EZH2[15]. MiR-26b/KPNA2 axis inhibits epithelial ovarian carcinoma proliferation and metastasis through downregulating OCT4[16]. Thus, this evidence showed that microRNAs play crucial roles in EOC progression. However, the role of miR-1294 in EOC remains unknown. In the present study, we identified that miR-1294 was lower in EOC specimens compared to adjacent normal tissues. Furthermore, we found that lower miR-1294 expression was related to FIGO stage, lymph node metastasis and shorter survival rate in EOC patients. Multivariate Cox analysis also demonstrated that miR-1294 expression was an independent prognostic indicator of EOC patients. These results indicated that miR-1294 may act as a biomarker for predicting EOC prognosis.

Table II. Multivariate Cox regression analysis of parameters determining OS in EOC patients.

Parameters	Overall survival (OS)	
	HR (95% CI)	p
Age (years)	0.588 (0.146-1.205)	0.955
Serum CA125	0.821 (0.636-1.446)	0.523
Tumor size	0.766 (0.414-1.855)	0.711
Grade	0.967 (0.622-1.665)	0.498
FIGO stage	2.105 (1.022-3.699)	0.002*
Lymph node metastasis	2.355 (1.366-3.878)	0.001*
Lower miR-1294 expression	2.664 (1.659-4.205)	0.001*

*$p < 0.05$.

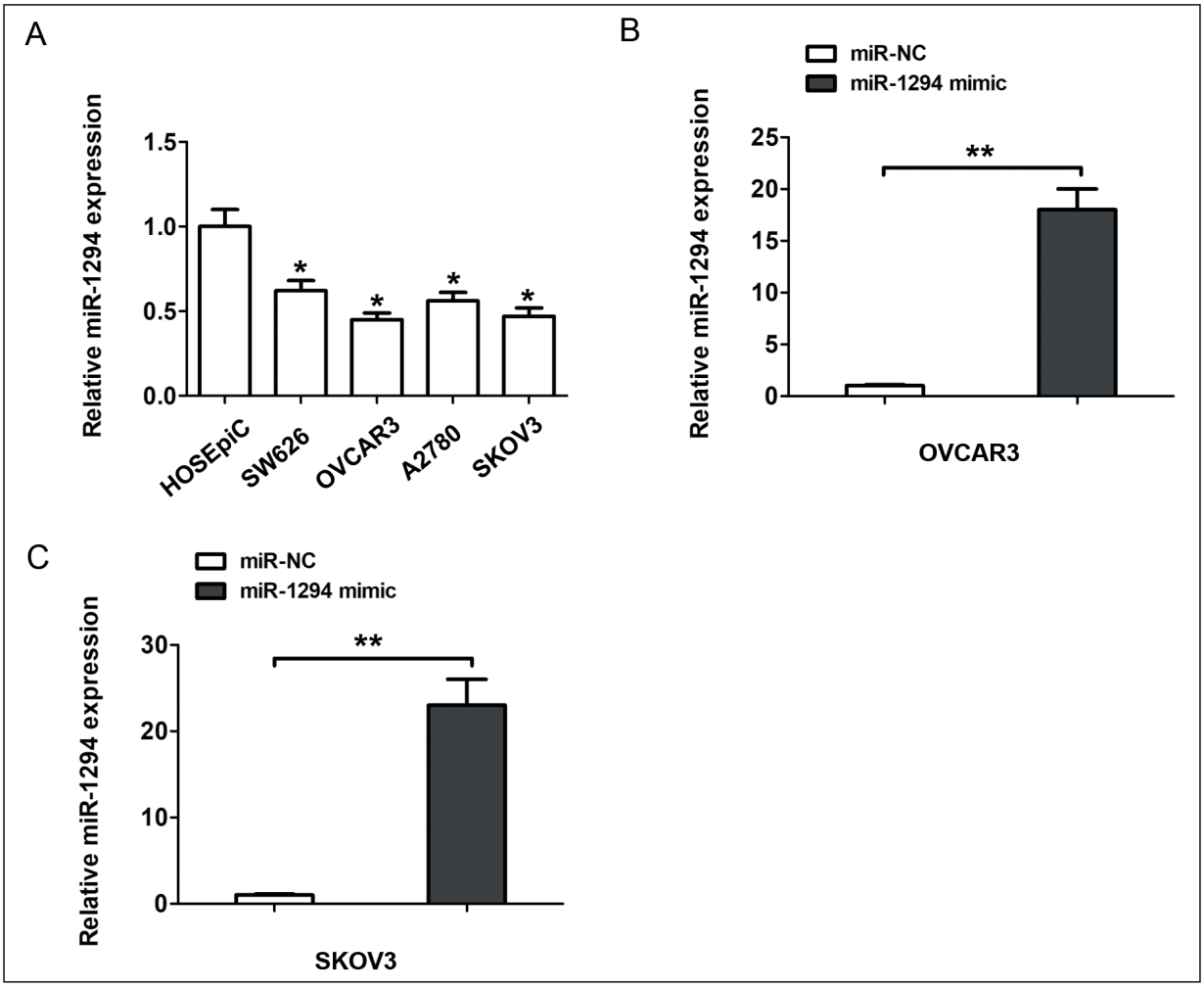

Figure 2. MiR-1294 expression was low in EOC cells. ***(A)*** MiR-1294 expression was detected using qRT-PCR in SW626, A2780, SKOV3 and OVCAR3 cells compared to HOSEpiC cells. U6 was used as an internal control. ***(B-C)*** MiR-1294 expression was detected using qRT-PCR in SKOV3 and OVCAR3 cells after cells were transfected with miR-1294 mimic or miR-NC. U6 was used as an internal control. *$p < 0.05$, **$p < 0.01$.

Moreover, we performed gain function assay to detect the effects of miR-1294 expression on cell proliferation and cell cycle progression. The CCK8 cell proliferation assay and cell cycle analysis showed that overexpression of miR-1294 significantly inhibited cell proliferation ability compared to miR-NC group in SKOV3 and OVCAR3 cells. Thus, these above results suggested that increased miR-1294 expression inhibited cell growth in EOC.

Conclusions

We provided evidence that lower expression of miR-1294 was correlated with poor prognosis in patients with EOC. Besides, miR-1294 overexpression inhibited cell proliferation. Therefore, these results indicated that miR-1294 acts as a potential new molecular marker for detecting the EOC prognosis and target of EOC treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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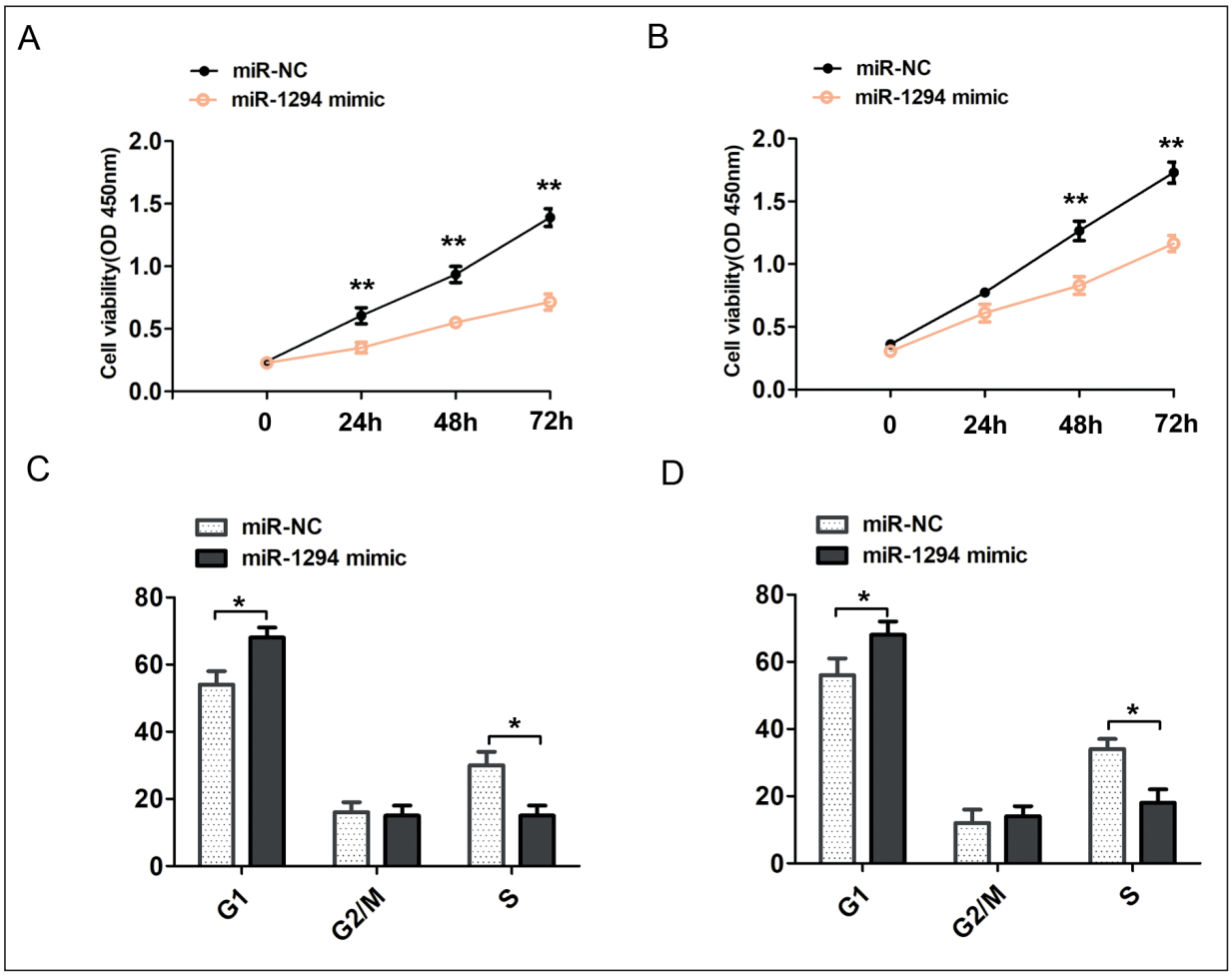

Figure 3. MiR-1294 overexpression suppressed cell proliferation in EOC cells. ***(A-B)*** CCK8 cell proliferation assay was used to evaluate the cell proliferation ability after cells were transfected with miR-1294 mimic or miR-NC. ***(C-D)*** Cell cycle analysis assay was used to evaluate the cell cycle after cells were transfected with miR-1294 mimic or miR-NC. *$p < 0.05$, **$p < 0.01$.

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