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MiR-1231 correlates tumor prognosis and inhibits cell growth in ovarian cancer

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the expression characteristics of miR-1231 in ovarian cancer (OC), and to further explore its effects on cell proliferation capacity of OC cells.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (QRT-PCR) was performed to detect miR-1231 expression in 116 paired of OC and matched adjacent normal tissues. The association of miR-1231 expression and clinicopathological features and prognosis was analyzed. Furthermore, the effects of miR-1231 on cell proliferation and cell cycle of OC cells were evaluated by functional assays.

RESULTS: In the study, the results exhibited that miR-1231 expression was lower in ovarian cancer tissues compared with adjacent normal tissues. Lower miR-1231 expression was associated with tumor clinical stage and lymph node invasion in patients. Survival plots by K-M survival analysis showed that lower miR-1231 expression predicted a poor outcome in ovarian cancer patients. Moreover, multivariate analysis implied that miR-1231 expression was an independent maker of overall survival (OS). Functional assays showed that upregulation of miR-1231 expression inhibited cell proliferation and cell cycle progression.

CONCLUSIONS: We revealed that miR-1231 expression was lower in ovarian cancer tissues cell lines. Lower miR-1231 expression predicted a poor outcome in ovarian cancer patients and upregulation of miR-1231 expression inhibited cell growth.

Key Words:
MicroRNAs, MiR-1231, Ovarian cancer, Prognosis, Cell proliferation.

Introduction

Ovarian cancer (OS) is the third most common malignancy and the second highly lethal malignancy in the female reproductive system worldwide[1]. Due to the limitations of diagnostic methods, it is difficult to be diagnosed at the early stage, the five-year survival rate was low for all patients[2,3]. Thus, it is urgent to figure out the pathological mechanism of OC and find effective molecular targets for diagnosis and treatment.

MicroRNA (miRNA) is endogenous small non-coding RNA with a length of 19 to 25 nucleotides, which can regulate target gene expression by binding to 3'UTR[4]. MiRNAs have been involved in many cancer-related biological processes, including cell proliferation, apoptosis, invasion and metastasis, and prognosis in ovarian cancer[5]. MiR-135b-5p affected malignant behaviors of ovarian cancer cells by targeting KDM5B[6]. MiR-186 bidirectionally regulates cisplatin sensitivity of ovarian cancer cells *via* suppressing targets PIK3R3 and PTEN and upregulating APAF1 expression[7]. MiR-202-5p suppressed cell proliferation, migration and invasion in ovarian cancer *via* regulating HOXB2[8]. However, the miR-1231 expression and relative functional implications in OC remain unknown.

In previous report, Zhang et al[9] showed that microRNA-1231 exerts a tumor suppressor role by regulating the EGFR/PI3K/AKT axis in glioma. MiR-1231 expression is downregulated in prostate cancer with prognostic and functional implications[10]. In the study, we aim to investigate the clinical roles of miR-1231 expression in ovarian cancer. Our results exhibited that miR-1231 expression was lower in ovarian cancer tissues and cells. Lower miR-1231 expression was associated with tumor clinical stage and lymph node invasion in patients. K-M survival analysis showed that lower miR-1231 expression predicted a poor outcome in ovarian cancer patients. Functional assays showed that upregulation of miR-1231 expression inhibited cell proliferation and cell cycle progression. Thus, these results indicated that miR-1231 was a prognostic of cervical cancer and regulates cell growth.

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Patients and Methods

Patients and Tissues

A 116 cases of OC tissues and adjacent normal tissues were obtained from Department of Gynecology and obstetrics, Taizhou Central Hospital between 2012 and 2017. The patients were 47.6 ± 12.6 years old. Clinical, demographic, and pathologic data were obtained from the medical the patients. All enrolled patients were initially diagnosed and treated for gynecological diseases except pregnancy, did not received medication or radiation therapy before surgery, and did not have other organ or system severe disorders or malignant neoplasm diseases. Patients who did not meet the above criteria were excluded. Two pathologists confirm the histological characteristics of all ovarian tissue specimens. All of subjects provided informed consent, and the study was approved and supervised by the Research Ethics Committee of Taizhou Central Hospital.

Cell Lines Culture

The human OC cell lines SKOV3, OVCA433, OV2008, and A2780 and human ovarian surface epithelial cell line (HOSEpiC) were cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Waltham, MA, USA) in a humidified tissue culture incubator containing 5% CO_2 at 37°C.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from OC cell lines using the miRNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The cDNA was synthesized with the QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA). Quantitative Real Time-Polymerase Chain Reaction (QRT-PCR) was performed on a 7500 Fast Real-Time PCR System (Life Technologies, Shanghai, China) using a QuantiFast SYBR Green PCR kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. 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 related primers used in this study were prepared by Shanghai Sangon Biotechnology company. The primer sequences used in this study were as follow: miR-1231 (Forward) 5'-GCCAGTGTCTGGGCGGAC-3', miR-1231(Reverse)5'-GTGCAGGGTCCGAGGT-3',GAPDH (Forward) 5'-GTCAACGGATTTGGTCTGTATT-3', GAPDH (Reverse) 5'-AGTCTTCTGGGTGGCAGTGAT-3'.

Cell Counting Kit-8 Assay (CCK-8 Assay)

Cells were seeded on 96-well plates at a density of 3000 cells per well with 100 μL medium containing 10% serum. Cell Counting Kit-8 solution (CCK-8, Dojindo, Kumamoto, Japan) solution (10 μL) was added to each well after 0, 24, 48, 72 and 96 hours. Cell proliferation was detected using a microtiter plate reader (Tecan Infinite 200 PRO; Salzburg, Austria) and the absorbance was at 450 nm.

Flow Cytometry Detection of Cell Cycle

The transfected cells were digested by enzyme and centrifuged at 1000 g for 5 min. After washed with PBS, the cells were fixed with precooled 70% ethanol at 4°C for 24 h. Next, the cells were treated with propidium iodide (PI) and RNase A (Multi Sciences, Hangzhou, China) at 37°C for 30 min. Cell cycle was analyzed using a flow cytometer (FACScan; BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

Data analyses were performed using SPSS version 20.0 (version 20.0: SPSS Corp., Armonk, NY, USA) software. Clinicopathological characteristics were analyzed by the chi-square test. The Kaplan Meier curves and log rank test for the study of prognosis. The differences between the groups were compared using a two-tailed Student's *t*-test. All *p*-values were determined from 2-tailed tests and differences with a p-value < 0.05 were considered to be statistically significant.

Results

The Expression of MiR-1231 Is Downregulated in OC Tissues

The expression of miR-1231 was examined in 116 cases of human OC tumor tissues and adjacent normal ovarian tissues from OC patients by qRT-PCR analysis. The results showed that the expression of miR-1231 was significantly downregulated in human OC tumor tissues, compared to adjacent normal ovarian tissues (Figure 1A, $p<0.05$). Next, we assessed the association between miR-1231 expression and clinicopathological factors in OC patients. We observed that lower miR-1231 expression was significantly associated with lymph node metastasis and poor FIGO stage ($p<0.05$, Table I). However, there was no association between miR-1231 expression and age, sex, tumor size and so on (all $p>0.05$, Table I).

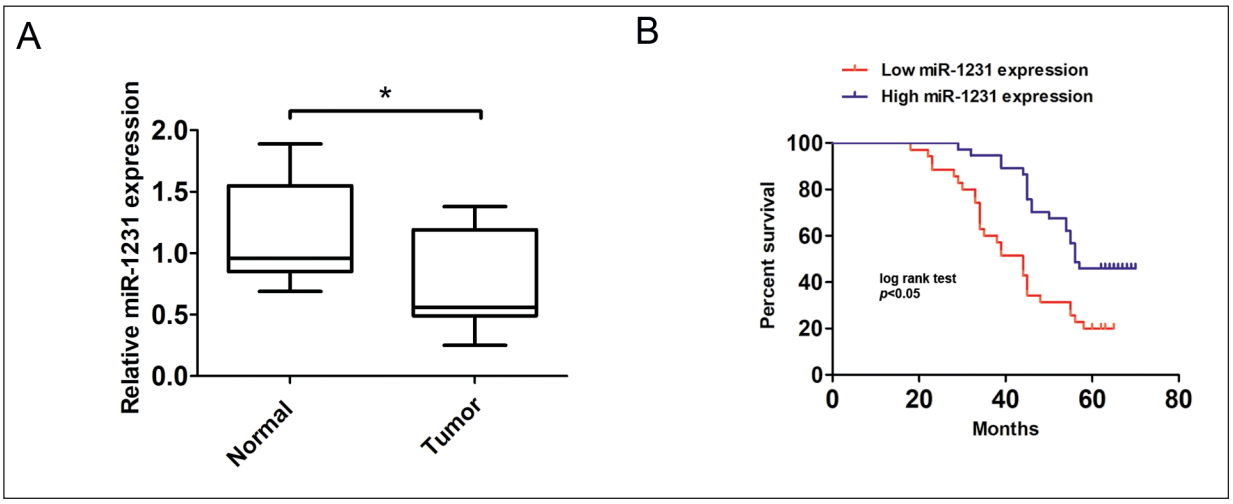

Figure 1. Low expression of miR-1231 in tumor tissues of OC patients. **A**, QRT-PCR was used to detect the expression of miR-1231 in OC and adjacent tissues. **B**, Survival plots by K-M survival analysis showed that lower miR-1231 expression predicted a poor outcome in ovarian cancer patients. *Represents $p<0.05$ compared with adjacent tissues.

Low Expression of MiR-1231 Predicts Poor Prognosis in OC Patients

Furthermore, we evaluated the prognostic value of miR-1231 expression in patients. Kaplan-Meier method and log rank test demonstrated that lower miR-1231 expression showed shorter overall survival rate than patients with higher miR-1231 expression group (Figure 1B, log rank test, $p < 0.05$). Moreover, Multivariate COX analysis results showed that FIGO stage ($p < 0.001$, Table II), lymph node metastasis ($p<0.001$, Table II) and lower miR-1231 expression ($p < 0.001$, Table II) were independent poor prognostic factor for OC patients. Thus, these results indicated that miR-1231 could act as a prognostic predictor in OC patients.

Table I. The association between miR-1231 expression and clinicopathological parameters.

Factors	miR-1231 expression			
	Total (n=116)	Higher (n=57)	Lower (n=59)	*p*-value
Age				0.697
≤50	53	25	28	
>50	63	32	31	
Serum CA125				0.064
≤319	57	33	24	
>319	59	24	35	
Tumor size				0.264
≤4 cm	59	32	27	
>4 cm	57	25	32	
Tumor grade				0.164
Well	57	23	34	
Moderately	36	20	16	
Poor	23	14	9	
FIGO stage				0.001*
I-II	68	42	25	
III-IV	48	15	33	
Lymph node metastasis				0.001*
No	67	44	23	
Yes	49	13	36	

*$p<0.05$.

Table II. Multivariate Cox regression analysis of parameters determining OS.

Factors	Overall survival (OS)	
	HR (95% CI)	p
Age (years)	0.598(0.186-1.223)	0.876
Serum CA125	1.012 (0.678-1.873)	0.545
Tumor size (cm)	0.783 (0.466-1.822)	0.763
Tumor grade	1.051 (0.764-1.956)	0.387
FIGO stage	2.622 (1.455-4.288)	0.001*
Lymph node metastasis	2.433 (1.677-4.112)	0.001*
Lower miR-1231 expression	2.467 (1.466-4.688)	0.001*

*$p<0.05$.

MiR-1231 Overexpression Inhibits Cell Proliferation, Cell Cycle Progression in OC

Next, we detected the expression of miR-1231 in four human ovarian cancer cell lines, including SKOV3, OVCA433, OV2008, and A2780 cells and a human ovarian surface epithelial cell line (HOSEpiC). The results showed that miR-1231 expression was lower in human ovarian cancer cell lines, including SW626, A2780, SKOV3 andOVCAR3 cells compared to HOSEpiC cells (Figure 2A, $p < 0.05$). Furthermore, we performed function assays by transfecting miR-1231 mimic into A2780 and SKOV3 cells (Figure 2B, $p < 0.05$). The cell proliferation ability was detected by CCK-8 cell proliferation assay. The results showed that overexpression of miR-1231 significantly inhibited cell proliferation ability compared to miR-NC group in A2780 and SKOV3 cells (Figure 2C-2D, $p < 0.05$). The cell cycle analysis showed that overexpression of miR-1231 significantly reduced the cell number of S phase compared to miR-NC group in A2780 and SKOV3 cells (Figure 3A-3B $p < 0.05$). Thus, our results indicated that miR-1231 overexpression inhibited cell growth in OC.

Discussion

Dysregulation of miRNAs expression plays critical roles in tumor initiation and progression[11,12]. However, the expression and function of miRNAs in ovarian carcinogenesis and progression remains largely elusive. Some researchers have reported some miRNAs to be involved in OC. Such as, upregulation of miR-874-3p and miR-874-5p inhibits epithelial ovarian cancer malignancy *via* SIK2[13]. MiR-532-5p is a prognostic marker and suppresses cells proliferation and invasion by targeting TWIST1 in epithelial ovarian cancer[14]. MicroRNA-183 correlates cancer prognosis, regulates cancer proliferation and bufalin sensitivity in epithelial ovarian cancer[15]. However, the miR-1231 expression and relative functions implications in OC remain unknown.

Low expression of miR-1231 was reported in patients with glioma and its prognostic significance. MiR-1231 is downregulated in prostate cancer with prognostic and functional implications[10]. MicroRNA-1231 exerts a tumor suppressor role by regulating the EGFR/PI3K/AKT axis in glioma[9]. A miR-1231 binding site polymorphism in the 3'UTR of IFNAR1 is associated with hepatocellular carcinoma susceptibility[16].

In the study, we aim to investigate the clinical roles of miR-1231 expression in ovarian cancer. Our results exhibited that miR-1231 expression was lower in ovarian cancer tissues and cells. Lower miR-1231 expression was associated with tumor clinical stage and lymph node invasion in patients. Survival plots by K-M survival analysis showed that lower miR-1231 expression predicted a poor outcome in ovarian cancer patients. Moreover, we implied that miR-1231 expression was an independent maker and functional assays showed that upregulation of miR-1231 expression inhibited cell proliferation and cell cycle progression. Thus, these results indicated that miR-1231 was a prognostic of cervical cancer and regulates cell growth.

Conclusions

Our results exhibited that miR-1231 expression was lower in ovarian cancer tissues and implied that miR-1231 expression was an independent maker of overall survival. Functional assays

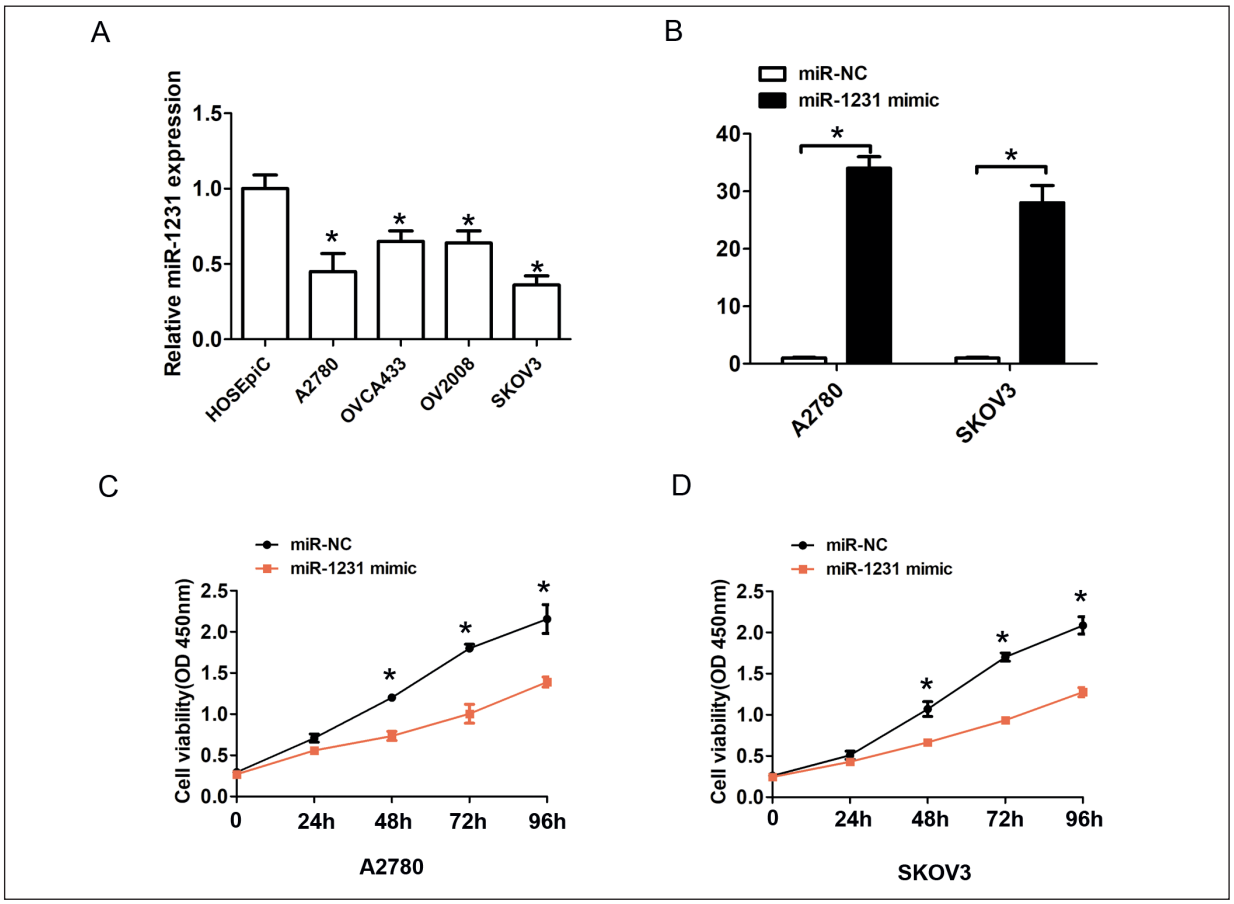

Figure 2. MiR-1231 overexpression inhibits cell proliferation in A2780 and SKOV3 cells. **A**, The expression of miR-1231 was detected in the human epithelial OC cell lines SKOV3, OVCA433, OV2008, and A2780 and the HOSEpic cell in A2780 and SKOV3 cells. **B**, The expression of miR-1231 was detected in A2780 and SKOV3 cells when cells were transfected with miR-1231 mimic. **C-D**, The cell proliferation ability was detected when cells were transfected with miR-1231 mimic in A2780 and SKOV3 cells. *Represents $p<0.05$ compared with adjacent tissues.

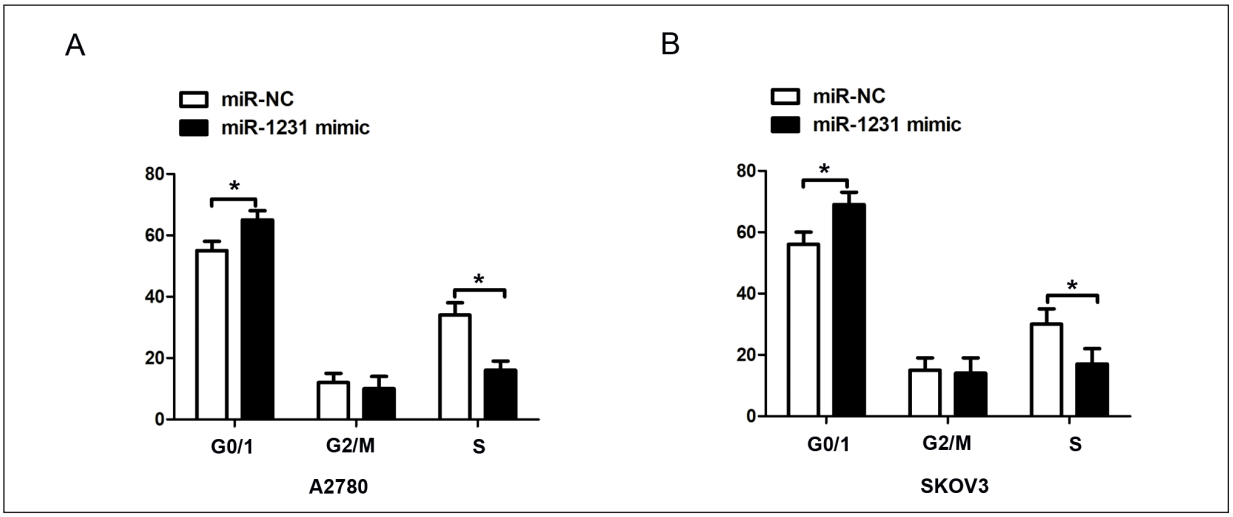

Figure 3. MiR-1231 overexpression inhibits cell cycle in A2780 and SKOV3 cells. **A**, Flow detection of cell cycle was shown when cells were transfected with miR-1231 mimic in A2780 cells. **B**, Flow detection of cell cycle was shown when cells were transfected with miR-1231 mimic in SKOV3 cells. *Represents $p<0.05$ compared with adjacent tissues.

showed that upregulation of miR-1231 expression inhibited cell proliferation and cell cycle progression. Thus, these results indicated that miR-1231 was a prognostic of cervical cancer and regulates cell growth.

Conflict of Interests

The authors declare that they have no conflict of interests.

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