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MiR-206 inhibits epithelial ovarian cancer cells growth and invasion via blocking c-Met/AKT/mTOR signaling pathway

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ABSTRACT

Background: MicroRNAs play important roles in the pathogenesis of various kinds of tumors. However, there are few studies on the expression profile and function of miRNAs in epithelial ovarian cancer. In this study, we performed microRNA array to compare the expression profile of microRNA in ovarian cancer tissues with noncancerous tissues.

Methods: qRT-PCR was used to further confirm the microRNA expression levels in epithelial ovarian cancer tissues and cell lines. The function of microRNA was analyzed by overexpressing microRNA mimics followed by the analysis of cell cycle, proliferation, and metastasis. The downstream target of miR-206 was found and western blot analysis was performed to measure the activation of the downstream signaling pathway.

Results: In this study, we found the expression of miR-206 was significantly down-regulated in epithelial ovarian cancer tissues and epithelial ovarian cancer cell lines. In epithelial ovarian cancer patients, downregulation of miR-206 was associated with metastasis and poor prognosis. In epithelial ovarian cancer cell lines, miR-206 contributed to the cell cycle regulation, cell apoptosis, and cancer cell metastasis. MiR-206 mimics inhibited cancer cell proliferation and metastasis, and induced cell apoptosis. Moreover, our results demonstrated that miR-206 directly targeted c-Met and repressed the activation of downstream AKT/mTOR signaling pathway.

Conclusion: Our results demonstrated that miR-206 was down-regulated in epithelial ovarian cancer tissues and cell lines. MiR-206 inhibits the development of epithelial ovarian cancer cell by directly targeting c-Met and inhibiting the c-Met/AKT/mTOR signaling pathway.

1. Introduction

Ovarian cancer is the most lethal tumor type of female gynecologic malignancies with lower incidence rate but higher mortality rate than cervical cancer and endometrial cancer. The 5-year survival rate of ovarian cancer worldwide is 90% in early stage patients while less than 30% in advanced stage patients with 70% of the patients were found at late stage [1]. The composition of ovarian tissue is very complex. The most common ovarian cancer is epithelial ovarian cancer (EOC), accounting for 50%–60% of primary ovarian tumors. Because the ovary is deep in the pelvic cavity and lacks specific symptoms and signs, it is difficult to find and diagnose at the early stage of disease. Approximately 70% of patients are diagnosed with advanced stage (III, IV). Although the survival rate of patients with early ovarian cancer is relatively high, the survival rate of advanced patients is significantly reduced to 20%–30% [2].

MicroRNAs (miRNAs), a kind of non-coding RNAs, are important posttranscriptional regulators involved in various biological processes [3,4]. MiRNAs regulate gene expression by inhibition of translation or destabilization of mRNA transcript [5]. With such vital function, miRNAs are important protein expression regulators regulating approximately 30% of human gene transcriptions which control many important physiological processes such as cell differentiation, proliferation, and programmed cell death [6]. More and more evidences show that miRNAs are closely related to the occurrence and development of tumors by targeting important genes, indicating the importance of miRNAs in the development of tumors [7–9]. The miRNA expression profiles between tumor cells and normal tissue-derived cells are significantly different. MiRNAs were found to be mis-regulated in various cancers including breast cancer, lung cancer, lymphoma, leukemia, liver cancer, and ovarian cancer etc. [10].

It was found that in EOC tissues several miRNAs were up-regulated including miR-200a, miR-141, miR-200c, and miR-200b [11]. The identified miRNAs are associated with specific bio-pathological features

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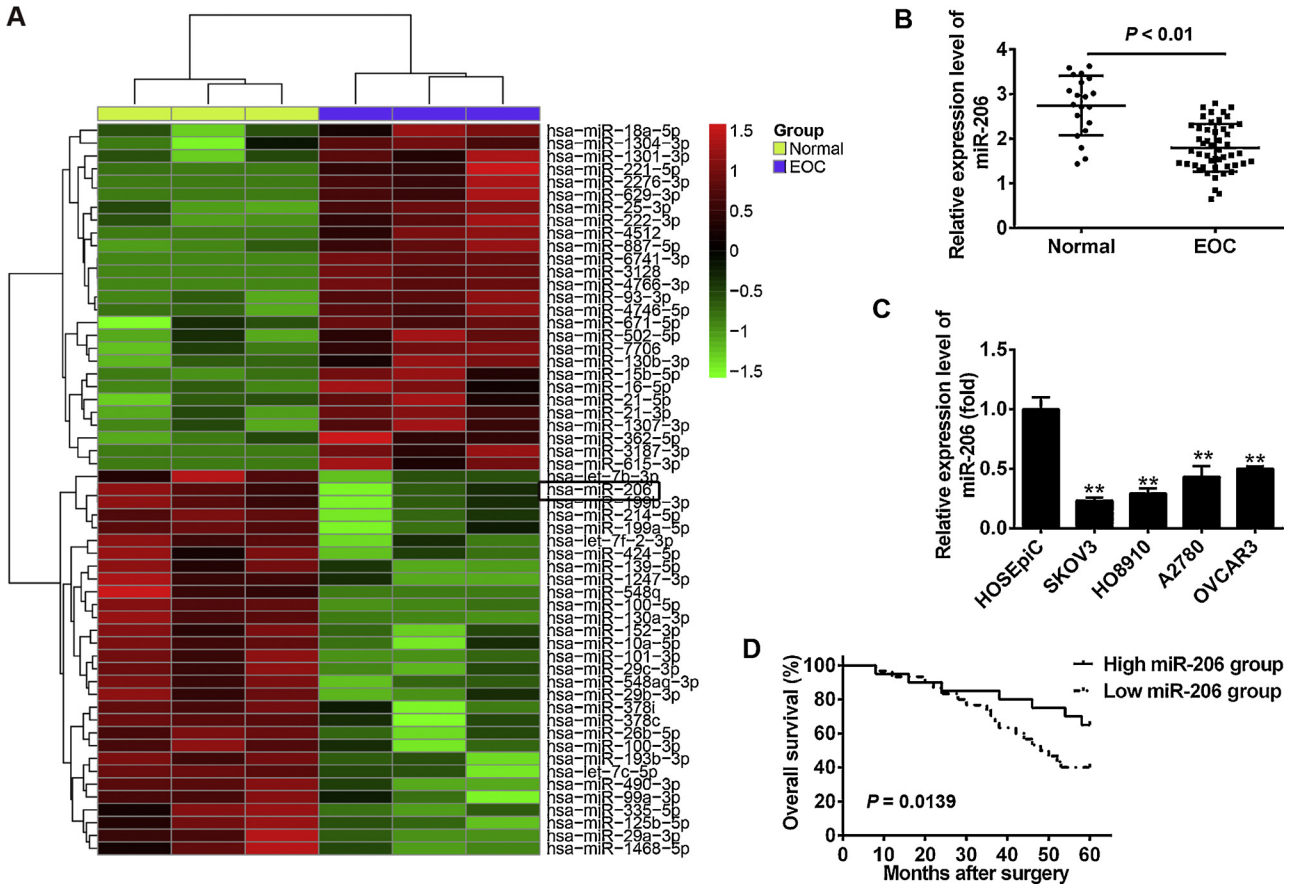

Fig. 1. MiR-206 downregulation correlates with human EOC progression. (A) The heatmap of microRNA profiling in human EOC tissues. (B) MiR-206 expression was measured in human EOC tissues (n = 50) and normal tissues (n = 20). (C) MiR-206 expression was measured in EOC cell lines. (D) The survival analysis was done by Kaplan-Meier curves and log-rank test. Error bars indicate SD. $^{**}P < 0.01$, Student's *t*-test.

Table 1
Correlation between miR-206 and clinicopathological features of epithelial ovarian cancer tissues.

Feature	Total n = 50	miR-206		*P* value
		High No. cases	Low No. cases	
Age at presentation (years)				0.6431
≤45	23	10	13	
> 45	27	10	17	
Lymph node metastasis				0.0184*
Absent	30	16	14	
Present	20	4	16	
Clinical stage				0.0353*
I–II	16	3	13	
III–IV	34	17	17	
Distant metastasis				0.0115*
Absent	32	17	15	
Present	18	3	15	
Histological type				0.4705
Serous	40	17	23	
Non-serous	10	3	7	
Residual tumor after surgery				0.1800
≤1 cm	33	11	22	
> 1 cm	17	9	8	

* $P < 0.05$.

such as tissue type, lymph vessel and organ infiltration, ovarian surface involvement. It was found that miR-21, miR-125a, miR-125b, miR-100, miR-145, miR-16 and miR-99a were differentially expressed in serous ovarian cancer [12]. The up-regulation of miR-200, miR-141, miR-18a, miR-93, and miR-429 and the down-regulation of let7b and miR-199a were associated with poor prognosis [12]. Dahiya et al. also proved that several miRNAs were differentially expressed in both cancer tissues and cell lines. It can be seen that the difference in the expression of miRNA between ovarian cancer tissues/cells and normal tissues/cells has certain universality [13]. The clues for screening the function and mechanism of miRNA research also open up a lot of space for clinical research and application. While the molecular mechanisms of how miRNAs work in EOC are still lacking.

In this study, we performed microRNA profiling and found differently expressed miRNAs in ovarian cancer tissues compare to noncancerous tissues. Among which, miR-206 was the most significantly down-regulated in EOC tissues. We also found miR-206 downregulation was correlated with metastasis and poor prognosis. Moreover, miR-206 was demonstrated to be downregulated in EOC cell lines and contributed to the cell cycle regulation, cell apoptosis, and cancer cell metastasis. Overexpression of miR-206 inhibited cancer cell proliferation and metastasis, and induced cell apoptosis. Furthermore, our results proved that miR-206 directly targeted c-Met and repressed the activation of downstream AKT/mTOR signaling pathway.

2. Materials and methods

2.1. Human EOC tissues collections

All the EOC tissues and noncancerous tissues glioma were surgically resected from the First Affiliated Hospital of Wenzhou Medical University with informed consent. All specimens were handled and made anonymous according to the ethical and legal standards.