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Original Article

Decreased expression of *microRNA-433* is associated with the prognosis of epithelial ovarian cancer

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Abstract: Background: *MicroRNA-433* (*miR-433*), possessing tumor suppressive activity, has been found to be down-regulated in different types of cancer. However, its clinical significance in epithelial ovarian cancer (EOC) is still unclear. Therefore, the aim of this study was to detect the *miR-433* expression and its prognostic value in patients suffering from EOC. Methods: The *miR-433* expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR) analysis in 115 EOC tissues and 45 normal tissues. Then, the associations of *miR-433* expression with clinicopathologic characteristics as well as overall survival of EOC patients were determined by Chi-square test and Kaplan-Meier method respectively. Besides, the prognostic value of *miR-433* was estimated via Cox regression analysis. Results: The expression of *miR-433* in EOC tissues were significantly lower than that in normal tissues ($P<0.05$). In addition, low *miR-433* expression was found to be closely correlated with tumor size ($P=0.050$), advanced FIGO stage ($P=0.009$), and recurrence ($P=0.002$). Moreover, the Kaplan-Meier analysis demonstrated that EOC patients with low *miR-433* expression had a poorer overall survival than those with high *miR-433* expression ($P=0.000$). Furthermore, the multivariate analysis identified *miR-433* ($P=0.013$; HR=2.973; 95% CI=1.260-7.012) was an independent prognostic factor for EOC patients. Conclusion: For the first time, the current study offered convincing evidence that the expression of miR-433 was decreased in EOC and it might be associated with tumor progression of EOC. Therefore, *miR-433* may be an independent prognostic marker for EOC patients.

Keywords: Epithelial ovarian cancer, *MiR-433*, prognosis

Introduction

Epithelial ovarian cancer (EOC), as the most common subtype of ovarian cancer, is the most lethal gynecological malignancy cancer and the fifth leading cause of cancer-related deaths among women worldwide [1, 2]. More than 70% patients with EOC are diagnosed at the advanced stages because of its mild and diffuse symptoms or ineffective tumor biomarkers in the early days [3]. Just for that the mortality of EOC is very high. Even though there has been great improvement on traditional treatments, such as surgery supplemented with radiotherapy and chemotherapy. The prognosis of EOC is still very poor with a five-year survival rate below 40% [4]. Therefore, it is urgently needed to discover new potential molecule markers to improve the prognosis of patients suffering EOC.

MicroRNAs (miRNAs), a class of highly conserved, single-stranded, small non-coding RNA molecules, are known to regulate endogenous gene expression through translation repression and messenger RNA cleavage after targeting the 3'-UTR [5]. It has been widely accepted that miRNAs play key roles in various biological processes, including cell cycle, apoptosis, hematopoietic cell differentiation, metabolism, neural development and metastasis [6-8]. Numerous researches have also found the aberrant expression of miRNAs in various cancer types and have described the association of miRNA deregulation with the initiation and progression of human cancers [9]. Growing evidence has also indicated the possible use of miRNA expression profiles to distinguish the normal and neoplastic tissues, leading to the identification of prognostic markers. In human ovarian cancer, multiple miRNAs with aberrant expression have

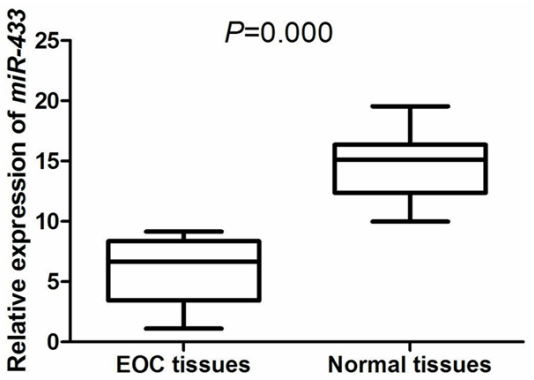

Figure 1. *MiR-433* expression was decreased in EOC tissues compared with normal tissues (*P*=0.000).

been identified such as *miR-145*, *miR-100*, *miR-132*, *miR-200c*, *miR-141*, *miR-203*, and *miR-221* [10-15]. However, to our knowledge, the expression pattern and clinical significance of *miR-433* in EOC have not yet been reported.

In the present study, we aimed to investigate the expression level of *miR-433* in clinical EOC specimens and normal tissues, and analyze the association of *miR-433* with the clinical features of the patients. In addition, we also decided to estimate the prognostic value of *miR-433* in EOC patients.

Materials and methods

Patients and tissues samples

A total of 115 female patients (aged 24~59 years old with a median age of 37.3) with epithelial ovarian carcinoma were selected from Gynecology and Obstetrics Hospital of Weifang University China from 2010-2014. None of these patients had received preoperative chemotherapy. 45 normal healthy people who underwent hysterectomy for benign disease during the same time period were used as controls. The study was approved by the Ethics Committee of the institution. And written informed consents were signed by all participators in advance.

The tumor tissues and normal healthy tissues were obtained and frozen in liquid nitrogen, immediately. Then the frozen tissues were stored at -80°C for RNA extraction. The clinicopathologic characteristics included age, tumor size, FIGO stage, lymph node metastasis, distant metastasis, and recurrence were recorded in a database. All patients were staged based on the International Federation of Gynecology and Obstetrics (FIGO) staging system [16]. A follow-up was conducted via a telephone or questionnaires and lasted for 5 years. The overall survival time was defined from the day of surgery to the day of death. Patients who died from unexpected events or other diseases were excluded from our study.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from tumor tissues and healthy tissues using TRIzol reagent (Life Technologies), respectively. The first-strand cDNA synthesis was performed with the Superscript III kit (Life Technologies). Real-time PCR reaction was conducted by the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The final reaction volume of 20 μl included: 0.5 μl cDNA template, 10 μl TaqMan Master Mix (Applied Biosystems, Paisley, UK), 1 μl mix containing primers and probes, 8.5 μl ddH_2O. The *RNU6B* small nuclear RNA was amplified as an internal control. Primer sequences used in this study were as follow: for *miR-433,* F-5'-GGATCATGATGGGCTCCT-3', R-5'-CAGTGCGTGTCGTGGAGT-3'; for *RNU6B,* F-5'-CTCGCTTCGGCAGCACA-3', R-5'-AACGCTTCACGAATTTGC GT-3'. The relative expression quantity of *miR-433* was calculated using the formula $2^{-\Delta\Delta Ct}$. Each experiment was conducted in triplicate.

Statistical analysis

Statistical analysis was conducted using the SPSS statistics software package (IBM SPSS Statistics Data Editor 18). The data were stated as mean ± standard deviation (SD). The difference of *miR-433* expression between tumor tissues and healthy tissues was estimated by students' test. The association between *miR-433* expression and clinicopathologic characteristics was evaluated by Chi-square test. Kaplan-Meier and Cox regression analysis were used to analyze the relationship between the *miR-433* expression and overall survival as well as the prognosis of EOC, respectively. When $P<0.05$, the difference was considered to be statistically significant.

Table 1. The relationship between *miR-433* expression and clinicopathological parameters

Parameters	Cases (n)	*MiR-433* expression		x^2	*P* values
		Low	High		
Age (years)					
≤40	62	40	22	0.029	0.864
>40	53	35	18		
Tumor size (cm)					
≤4	49	27	22	3.851	0.050
>4	66	48	18		
FIGO stage					
I/II	50	26	24	6.813	0.009
III/IV	65	49	16		
Lymph node metastasis					
Absent	65	40	25	0.892	0.345
Present	50	35	15		
Distant metastasis					
No	101	66	35	0.006	0.938
Yes	14	9	5		
Recurrence					
No	80	45	35	9.318	0.002
Yes	35	30	5		

Results

Downregulation of miR-433 in human EOC tissues

We conducted qRT-PCR to detect the *miR-433* expression in EOC tissues and healthy tissues. As shown in **Figure 1**, the expression level of *miR-433* in EOC tissues (6.094 ± 2.577) was found to be obviously decreased compared to that in normal tissues (14.590 ± 2.480) (*P*<0.05). Therefore, we inferred that *miR-433* might be a tumor suppressor.

Association of *miR-433* expression with clinicopathologic characteristics of EOC patients

The median expression of *miR-433* was used as a cutoff point to divide all 115 patients into two groups: the low *miR-433* expression group (n=75) and the high *miR-433* expression group (n=40). The association between *miR-433* expression and clinicopathologic characteristics was analyzed by Chi-square test. It proved that the expression of *miR-433* was significantly influenced by tumor size (*P*=0.050), FIGO stage (*P*=0.009), and recurrence (*P*=0.002) (**Table 1**). However, there was no relationship between *miR-433* and other parameters including age, lymph node metastasis, and distant metastasis (*P*>0.05, **Table 1**). In addition, the expression level of *miR-433* was significantly lower in EOC patients with advanced FIGO stage (III/IV) (4.727 ± 2.514) than those with low FIGO stage (I/II) (7.872 ± 1.220; *P*=0.000; **Figure 2**). These findings might reveal that *miR-433* participated in the development of EOC and it contributed to the tumor progression.

Correlation of miR-433 expression with overall survival of EOC patients

The association between *miR-433* expression and overall survival of EOC patients was investigated by Kaplan-Meier analysis and log-rank test. As shown in **Figure 3**, EOC patients with low *miR-433* expression tend to have shorter overall survival time than those with high *miR-433* expression (Log-rank test, *P*<0.001). Cox regression analysis indicated that low *miR-433* expression and FIGO stage affected the overall survival of EOC patients. Besides, *miR-433* expression (*P*=0.013; HR=2.973; 95% CI: 1.260-7.012) as well as FIGO stage (*P*=0.022; HR=2.448; 95% CI: 1.135-5.278) were important clinical factors and could be valuable prognostic indicators for patients with EOC (**Table 2**).

Discussion

EOC is the main type of ovarian cancer and 5-year survival rate ovarian cancer patients is less than 40% in the past 30 years [17, 18]. Besides, the morbidity and mortality are often strengthened by transcoelomic which is the most common route of metastasis in EOC [19]. Moreover, the biological and phenotypic heterogeneity of EOC patients are caused by the complex genomic rearrangements and structural variations which are observed in the ovarian cancer genome. Therefore it is difficult to exploit whole-genome information to determine patients more accurately for prognosis of EOC until now.

MiRNAs have been confirmed to be related too much progress of various cancers. The differential expression of miRNAs between tumor tis-

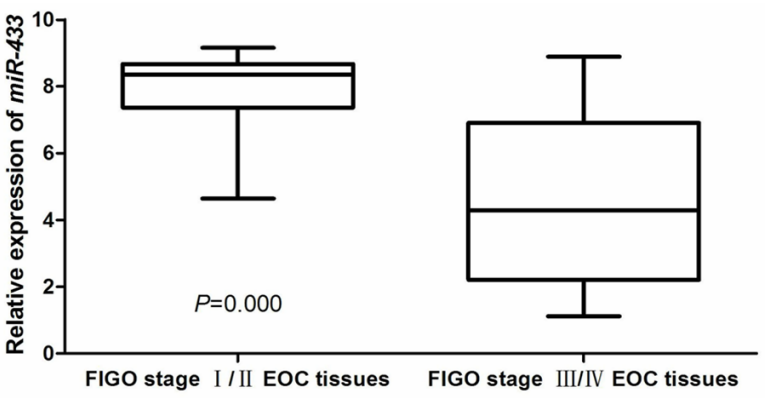

Figure 2. The expression level of *miR-433* was significantly lower in EOC patients with advanced FIGO stage (III/IV) than those with low FIGO stage (I/II) (*P*=0.000).

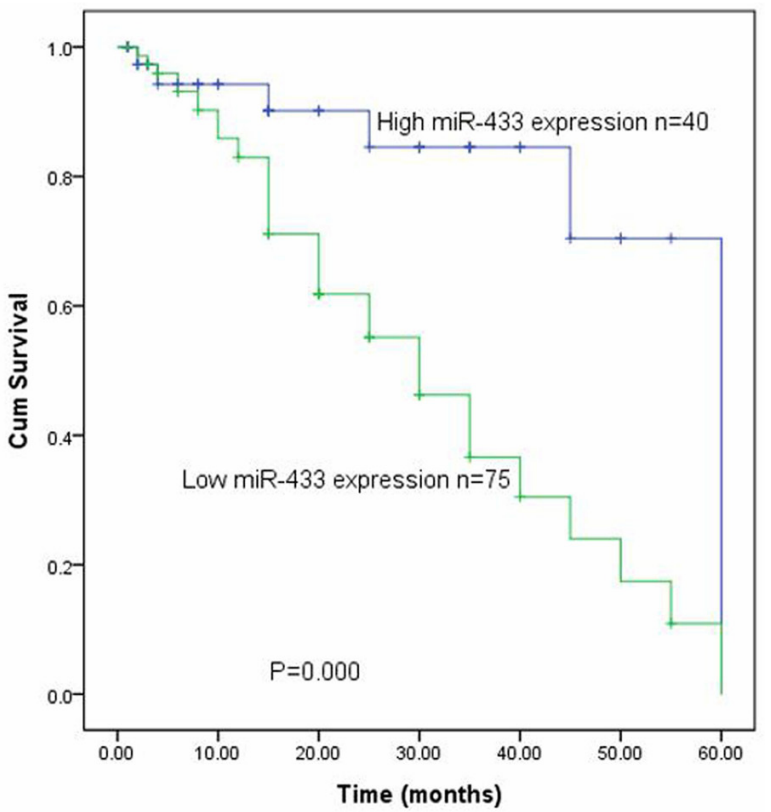

Figure 3. Kaplan-Meier analysis for the correlation between *miR-433* expression and overall survival of patients with EOC. The overall survival of EOC patients with low *miR-433* expression lived shorter than those with high *miR-433* expression. Log-rank test showed the result had statistical significance (*P*<0.001).

sues and healthy tissues make them either act as an oncogene or tumor suppressor in different cancers. Now, more than 1900 human miRNAs regulating about 60% of the genes in mammals have been identified [20]. *MiR-433* located at 14q32.2 of chromosome and had been confirmed to play roles in various cancers. For instance, *miR-433* was found to be decreased significantly in human gastric carcinoma and it could suppress hepatocellular carcinoma cells migration via regulating CREB1 [21, 22]. According to Gotanda et al. the overexpression of *miR-433* could induce the sensitivity to 5-FU in Hela cells of cervical cancer by suppressing the expression of TYMS [23]. Lin et al. and Valerio et al. have revealed that the level of *miR-433* were up-regulated in myeloproliferative neoplasms and lung dysplasia, respectively [24, 25]. Guo et al. has reported that *miR-433* has been attributed with tumor suppressor functions in gastric cancer cells [26]. These findings demonstrate that the dysregulation of *miR-433* may participate in human malignancy and carcinogenesis. Besides, in the study of Karolina et al., the aberrant expression of *miR-433* was considered to adversely affect intracellular signaling to mediate chemoresistance in ovarian cancer cells by driving cellular senescence [27].

In the present study, we investigated the *miR-433* expression with qRT-PCR analysis in EOC tissues. In addition, based on the calculation of relative expression, we analyzed the relationship of *miR-433* with the clinicopathologic characteristics of EOC patients. The results indicated that the *miR-433* expression was decreased in EOC tissues compared with normal tissues, which was consistent with previous investigations focused on

Table 2. The univariate and multivariate analysis for the prognostic factors with cox regression analysis

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* values	HR (95% CI)	*P* values
Low *miR-433* expression	2.739 (1.114-6.734)	0.028	2.973 (1.260-7.012)	0.013
FIGO stage	2.394 (1.079-5.310)	0.032	2.448 (1.135-5.278)	0.022

HR, Hazard ratio, 95% CI, 95% confidence interval.

other human malignancies. In addition, the present study also proved that *miR-433* expression was tightly related to tumor size, FIGO stage and recurrence. Meanwhile, we found that a low level of *miR-433* expression was more frequently detected in tumors with advanced FIGO stage. Therefore, we inferred that *miR-433* might play a crucial role in EOC carcinogenesis and progression.

To investigate the prognostic role of *miR-433* in EOC, we performed Kaplan–Meier and Cox regression analyses. The results revealed that EOC patients with a low level of *miR-433* expression had poorer overall survival compared to those with high *miR-433* expression levels. To further evaluate the prognostic value of *miR-433* in EOC, we performed Cox regression analysis adjusting for age, lymph node metastasis, distant metastasis, FIGO stage and recurrence of the patients. The results proved that decreased *miR-433* expression was a vital factor in the prognosis of EOC. These results indicated that *miR-433* could constitute a molecular prognostic marker for patients with EOC, and be used for identifying high risk individuals who were good candidates to receive aggressive treatment.

In summary, *miR-433* expression is decreased in EOC and associated with tumor progression. The present study also demonstrated for the first time that *miR-433* was an independent prognostic factor for patients with EOC.

Disclosure of conflict of interest

None.

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References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Bast RC Jr, Hennessy B and Mills GB. The biology of ovarian cancer: new opportunities for translation. Nat Rev Cancer 2009; 9: 415-428.

[3] Seidman JD, Horkayne-Szakaly I, Haiba M, Boice CR, Kurman RJ and Ronnett BM. The histologic type and stage distribution of ovarian carcinomas of surface epithelial origin. Int J Gynecol Pathol 2004; 23: 41-44.

[4] Mirandola L, M JC, Cobos E, Bernardini G, Jenkins MR, Kast WM and Chiriva-Internati M. Cancer testis antigens: novel biomarkers and targetable proteins for ovarian cancer. Int Rev Immunol 2011; 30: 127-137.

[5] Shukla GC, Singh J and Barik S. MicroRNAs: Processing, Maturation, Target Recognition and Regulatory Functions. Mol Cell Pharmacol 2011; 3: 83-92.

[6] Filipowicz W, Bhattacharyya SN and Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008; 9: 102-114.

[7] Zhou G, Shi X, Zhang J, Wu S and Zhao J. MicroRNAs in osteosarcoma: from biological players to clinical contributors, a review. J Int Med Res 2013; 41: 1-12.

[8] Liang W, Gao B, Fu P, Xu S, Qian Y and Fu Q. The miRNAs in the pathgenesis of osteosarcoma. Front Biosci (Landmark Ed) 2013; 18: 788-794.

[9] Esquela-Kerscher A and Slack FJ. Oncomirs-microRNAs with a role in cancer. Nat Rev Cancer 2006; 6: 259-269.

[10] Liang H, Jiang Z, Xie G and Lu Y. Serum microRNA-145 as a novel biomarker in human ovarian cancer. Tumour Biol 2015; 36: 5305-5313.

[11] Peng DX, Luo M, Qiu LW, He YL and Wang XF. Prognostic implications of microRNA-100 and its functional roles in human epithelial ovarian cancer. Oncol Rep 2012; 27: 1238-1244.

[12] Chung YW, Bae HS, Song JY, Lee JK, Lee NW, Kim T and Lee KW. Detection of microRNA as novel biomarkers of epithelial ovarian cancer from the serum of ovarian cancer patients. Int J Gynecol Cancer 2013; 23: 673-679.

[13] Gao YC and Wu J. MicroRNA-200c and microRNA-141 as potential diagnostic and prognostic biomarkers for ovarian cancer. Tumour Biol 2015; 36: 4843-50.

[14] Wang S, Zhao X, Wang J, Wen Y, Zhang L, Wang D, Chen H, Chen Q and Xiang W. Upregulation of microRNA-203 is associated with advanced

tumor progression and poor prognosis in epithelial ovarian cancer. Med Oncol 2013; 30: 681.
[15] Hong F, Li Y, Xu Y and Zhu L. Prognostic significance of serum microRNA-221 expression in human epithelial ovarian cancer. J Int Med Res 2013; 41: 64-71.
[16] Zeppernick F and Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynecol Obstet 2014; 290: 839-842.
[17] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
[18] Wang B, Liu SZ, Zheng RS, Zhang F, Chen WQ and Sun XB. Time trends of ovarian cancer incidence in China. Asian Pac J Cancer Prev 2014; 15: 191-193.
[19] Tan DS, Agarwal R and Kaye SB. Mechanisms of transcoelomic metastasis in ovarian cancer. Lancet Oncol 2006; 7: 925-934.
[20] Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011; 12: 861-874.
[21] Luo H, Zhang H, Zhang Z, Zhang X, Ning B, Guo J, Nie N, Liu B and Wu X. Down-regulated miR-9 and miR-433 in human gastric carcinoma. J Exp Clin Cancer Res 2009; 28: 82.
[22] Yang Z, Tsuchiya H, Zhang Y, Hartnett ME and Wang L. MicroRNA-433 inhibits liver cancer cell migration by repressing the protein expression and function of cAMP response element-binding protein. J Biol Chem 2013; 288: 28893-28899.
[23] Gotanda K, Hirota T, Matsumoto N and Ieiri I. MicroRNA-433 negatively regulates the expression of thymidylate synthase (TYMS) responsible for 5-fluorouracil sensitivity in HeLa cells. BMC Cancer 2013; 13: 369.
[24] Lin X, Rice KL, Buzzai M, Hexner E, Costa FF, Kilpivaara O, Mullally A, Soares MB, Ebert BL, Levine R and Licht JD. miR-433 is aberrantly expressed in myeloproliferative neoplasms and suppresses hematopoietic cell growth and differentiation. Leukemia 2013; 27: 344-352.
[25] Del Vescovo V, Meier T, Inga A, Denti MA and Borlak J. A cross-platform comparison of affymetrix and Agilent microarrays reveals discordant miRNA expression in lung tumors of c-Raf transgenic mice. PLoS One 2013; 8: e78870.
[26] Guo LH, Li H, Wang F, Yu J and He JS. The Tumor Suppressor Roles of miR-433 and miR-127 in Gastric Cancer. Int J Mol Sci 2013; 14: 14171-14184.
[27] Weiner-Gorzel K, Dempsey E, Milewska M, McGoldrick A, Toh V, Walsh A, Lindsay S, Gubbins L, Cannon A, Sharpe D, O'Sullivan J, Murphy M, Madden SF, Kell M, McCann A and Furlong F. Overexpression of the microRNA miR-433 promotes resistance to paclitaxel through the induction of cellular senescence in ovarian cancer cells. Cancer Med 2015; 4: 745-758.