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
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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 participants 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, dis-tant 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 ddH₂O. 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’-AACGCTTCACGAATTGC GT-3’. The relative expression quantity of miR-433 was calculated using the formula 2^{-ΔΔCt}. 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.
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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 (HR=2.973; 95% CI: 1.260-7.012) as well as FIGO stage (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-

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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).

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

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<td>FIGO stage</td>
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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

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