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Original Article

microRNA-217 was downregulated in ovarian cancer and was associated with poor prognosis

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Abstract: Background: MicroRNA-217 (miR-217) has been found to be down-regulated in ovarian cancer tissues. In the present study, we aimed to investigate the clinical significance of miR-217 in ovarian cancer. Methods: We performed quantitative RT-PCR analysis to detect the expression level of miR-217 in ovarian cancer tissues and normal ovarian tissues. The relationship between the expression level of miR-217 and clinic-pathological factors was analyzed using the Chi-square test or Fisher's exact test, as appropriate. Survival curves were constructed with the Kaplan-Meier method and compared by log-rank tests. A Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. Results: miR-217 was significantly decreased in ovarian cancer tissues when compared with that in the paired normal ovarian tissues (P<0.001). Low miR-217 expression was significantly associated with tumor differentiation (P=0.01), lymph nodes metastasis (P=0.002), and FIGO stage (P<0.001). Kaplan-Meier analysis showed that the ovarian cancer patients who had a lower expression level of miR-217 suffered poorer 5 year overall survival rates (P=0.006). Multivariate regression analysis indicated that differentiation (P=0.019), lymph nodes metastasis (P=0.009), FIGO stage (P=0.011) and miR-217 expression level (P=0.038) were independent prognostic factors for patients with ovarian cancer. Conclusions: Down-regulation of miR-217 was significantly associated with poor prognosis in patients with ovarian cancer.

Keywords: Ovarian cancer, miR-217, expression, prognosis

Introduction

Ovarian cancer is the fifth-leading cause of death among all cancers worldwide, and it is the leading cause of death in gynaecological cancer [1]. The poor ratio of survival is primarily attributed to lacking of specific symptoms and effective methods for the early detection in ovarian cancer patients [2]. There is an urgent need to clarify the mechanisms underlying the pathogenesis of ovarian cancer and to develop novel and effective methods for its diagnosis, treatment, and prognosis.

MicroRNAs (miRNAs) are small, endogenous RNA molecules, which play key roles in diverse pathways by targeting messenger RNAs (mRNAs), including those involved in developmental processes and cell growth, differentiation, and apoptosis [3-5]. Accumulating evidence has implicated miRNAs in the development of many cancer types, as either oncogenes or tumor suppressors [6-8]. MicroRNA-217 (miR-217) has been found to be down-regulated in osteosarcoma [9], gastric cancer [10], colorectal cancer [11], renal cell carcinoma [12], hepatocellular carcinoma [13], pancreatic cancer [14], and lung cancer [15], which plays as a tumor suppressor. The expression of miR-217 has also been investigated in ovarian cancer. Li et al reported that miR-217 expression was downregulated in ovarian cancer tissue and inversely correlated with advanced FIGO stage, high histological grading and lymph node metastasis. Function analysis revealed that the ectopic expression of miR-217 in ovarian cancer cells inhibited cell proliferation, migration and invasion in vitro, as well as suppressed tumor growth in vivo [16]. In the present study, we aimed to investigate the clinical significance of miR-217 in ovarian cancer.

Table 1. Association between miR-217 expression and patients' clinicopathologic features

Clinicopathologic variables	N	miR-217 level		*P* value
		Low (n=65)	High (n=64)	
Age (years)				
≤65	55	25	30	0.376
>65	74	40	34	
Serum CA125 (U/ml)				
≤675	61	28	33	0.38
>675	68	37	31	
Histologic type				
Serous	81	44	37	0.277
Non-serous	48	21	27	
Differentiation				
G1-2	93	40	53	0.01
G3	36	25	11	
Lymph nodes metastasis				
Yes	27	21	6	0.002
No	102	44	58	
FIGO stage				
I-II	42	11	31	<0.001
III-IV	87	54	33	

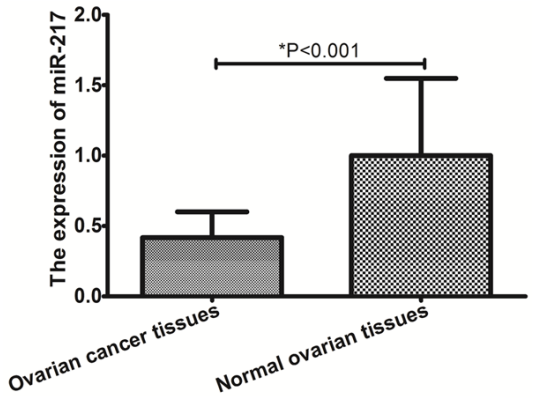

Figure 1. miR-217 expression level by qRT-PCR.

Materials and methods

Tissue samples

Samples of human ovarian cancer tissues and paired-adjacent normal ovarian tissues were obtained from 129 patients who underwent surgery resection at the Department of Obstetrics and Gynecology, the Third Xiangya Hospital of Central South University between April 2008 and January 2015. All the samples were biopsy materials before any therapy and were frozen and stored at -80°C until RNA extraction. Follow-up information was available for all patients. Tumor staging was established according to the International Federation of Gynecology and Obstetrics (FIGO) system. Overall survival was defined as the time from the day of diagnosis to death or, for living patients, the date of last follow up. The clinicopathological information of these patients was shown in **Table 1**.

Quantitative real-time PCR

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA was generated using the GoScript Reverse Transcription system (Promega, Madison, WI, USA) according to the manufacturer's protocol. 1 mL of RNA was used to measure the expression of miR-217 by quantitative RT-PCR (qRT-PCR) with the TaqMan miRNA reverse transcription kit and the TaqMan miRNA assay-specific RT primers for miR-217 according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA). U6 snRNA was used for miRNA control. The relative expression of miR-217 was computed by $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The relationship between the expression level of miR-217 and clinic pathological factors was analyzed using the Chi square test or Fisher's exact test, as appropriate. Survival curves were constructed with the Kaplan-Meier method and compared by log-rank tests. A Cox proportional hazards regression analysis was used for univariate and multivariate analyses of prognostic values. $P<0.05$ was considered to indicate a statistically significant difference. Statistical analysis was carried out using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

Decreased miR-217 expression in ovarian cancer tissues

We performed quantitative RT-PCR analysis to detect the expression level of miR-217 in ovari-

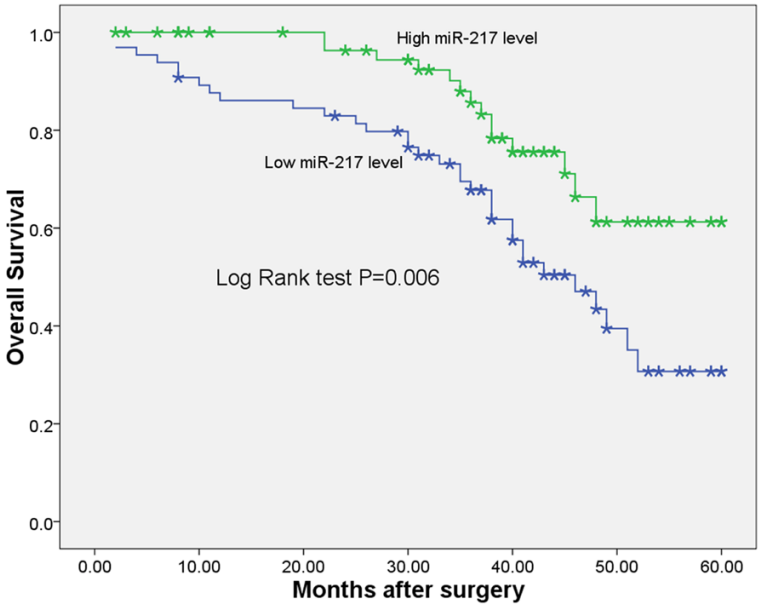

Figure 2. Kaplan-Meier Log-rank survival analysis for overall survival of ovarian cancer patients according to miR-217 expression.

Table 2. Multivariate analyses of parameters associated with overall survival of 129 patients with ovarian cancer

Variable	Hazard ratio	95% CI	*P*-value
Age (years)	1.281	0.572-1.823	0.572
Serum CA125 (U/ml)	2.116	0.827-2.774	0.169
Histologic type	0.819	0.271-1.298	0.797
Differentiation	3.991	1.283-9.017	0.019
Lymph nodes metastasis	4.192	2.827-11.993	0.009
FIGO stage	3.785	2.085-10.295	0.011
miR-217 expression level	2.553	1.247-7.829	0.038

an cancer tissues and normal ovarian tissues. Our results showed that miR-217 was significantly decreased in ovarian cancer tissues when compared with that in the paired normal ovarian tissues (P<0.001, shown in **Figure 1**). To evaluate the correlation between miR-217 expression and clinicopathological characteristics, the 129 ovarian cancer patients were classified into two groups according to the median expression of miR-217.

Correlation of miR-217 expression with clinicopathological features of ovarian cancer

We compared the clinicopathological factors of the low miR-217 expression group (n=65) and high miR-217 expression group (n=64). As shown in **Table 1**, low miR-217 expression was significantly associated with tumor differentiation (P=0.01), lymph nodes metastasis (P=0.002), and FIGO stage (P<0.001). However, no statistically significant correlation was observed between miR-217 expression and patient's age, serum CA-125 level, and histologic type (all P>0.05).

Relationship between miR-217 expression level and the prognosis of ovarian cancer

We used Kaplan-Meier method to evaluate whether there was any association between miR-217 expression and survival rate. Our results showed that the ovarian cancer patients who had a lower expression level of miR-217 suffered poorer 5 year overall survival rates (P=0.006, shown in **Figure 2**). The univariate and multivariate analyses were conducted to identify factors related to patient prognosis. Multivariate regression analysis indicated that differentiation (P=0.019), lymph nodes metastasis (P=0.009), FIGO stage (P=0.011) and miR-217 expression level (P=0.038) were independent prognostic factors for patients with ovarian cancer (shown in **Table 2**).

Discussion

Ovarian cancer is the malignancy with the highest mortality rate in women. Despite the low prevalence rate, most patients with advanced disease experience tumor recurrence and die eventually [17]. Therefore, there is an urgent need to clarify the mechanisms underlying the pathogenesis of ovarian cancer and to develop novel and effective methods for its diagnosis and treatment. And the identification of tissue-specific biomarkers with prognostic and therapeutic significance is an important strategy.

A large body of evidences suggested that miRNAs were involved in the manipulation of proliferation, differentiation, cell cycle, apoptosis, authophagy and metabolism. Recently, more and more reports have demonstrated that miR-

NAs are aberrantly expressed in many human cancers, functions as oncogenes and tumor suppressors [6-8]. miR-217 has been found to be downregulated in osteosarcoma [9], gastric cancer [10], colorectal cancer [11], renal cell carcinoma [12], hepatocellular carcinoma [13], pancreatic cancer [14], and lung cancer [15], which plays as a tumor suppressor. The expression of miR-217 has also been investigated in ovarian cancer. Li et al reported that miR-217 expression was downregulated in ovarian cancer tissue and inversely correlated with advanced FIGO stage, high histological grading and lymph node metastasis. Function analysis revealed that the ectopic expression of miR-217 in ovarian cancer cells inhibited cell proliferation, migration and invasion in vitro, as well as suppressed tumor growth in vivo [16].

The clinical significance and prognostic value of miR-217 have been investigated in several cancers. For example, Sun et al found that miR-217 was significantly downregulated in osteosarcoma cell lines and clinical specimens. Decreased miR-217 expression was significantly associated with large tumor size, positive distant metastasis, and advanced clinical stage. Low miR-217 expression in osteosarcoma was an independent predictor of poor survival [18]. Chen et al found that miR-217 levels were lower in gastric cancer tissue compared with the adjacent normal tissue. Low levels of miR-217 were associated with aggressive tumor phenotypes and poor overall survival in gastric cancer patients [19]. However, the clinical significance and prognostic value of miR-217 in ovarian cancer are still not known.

In the present study, our results showed that miR-217 was significantly decreased in ovarian cancer tissues when compared with that in the paired normal ovarian tissues. Low miR-217 expression was significantly associated with tumor differentiation, lymph nodes metastasis, and FIGO stage. We then used Kaplan-Meier method to evaluate whether there was any association between miR-217 expression and survival rate. Our results showed that the ovarian cancer patients who had a lower expression level of miR-217 suffered poorer 5 year overall survival rates. The univariate and multivariate analyses were conducted to identify factors related to patient prognosis. Multivariate regression analysis indicated that miR-217 expression level was independent prognostic factor for patients with ovarian cancer.

In conclusion, our results suggested that miR-217 expression might be downregulated in ovarian cancer tissues and was associated with aggressive features of this malignancy. Detection of tissue miR-217 levels might have clinical potentials as a non-invasive prognostic biomarker for patients with ovarian cancer.

Disclosure of conflict of interest

None.

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