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Original Article

WWOX acts as a novel diagnostic biomarker for ovarian cancer patients

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Abstract: Objective: The WW domain containing oxidoreductase (*WWOX*) was reported to be associated with progression of ovarian cancer. The purpose of this study was to detect the diagnostic value of *WWOX* in ovarian cancer. Methods: 104 patients confirmed with ovarian cancer and 84 age-matched healthy volunteers were enrolled in this study. The relative expression of *WWOX* in plasma specimens was detected by fluorescence quantitative real-time PCR (qRT-PCR) and the corresponding protein level was analyzed by western blot assay. Besides, the relationship between *WWOX* expression and clinical characteristics of ovarian cancer patients was evaluated by Chi-square test. Moreover, the diagnostic value of *WWOX* in ovarian cancer was analyzed by receiver operating characteristic (ROC) analysis. Results: Decreased expression of *WWOX* was detected in the plasma specimens of ovarian cancer patients, compared with the healthy control group (0.326±0.191 vs 0.695±0.135, *P*=0.001). In addition, the protein level in cancer patients was lower than that in healthy group. *WWOX* expression level was associated with histological stage (*P*=0.000), FIGO stage (*P*=0.000), lymph node metastasis (*P*=0.001) and recurrence (*P*=0.000). Moreover, the diagnosis analysis indicated that *WWOX* could be a diagnostic marker for ovarian cancer with the sensitivity of 76%, the specificity of 95.2% and the AUC value was 0.937. The cut-off value of *WWOX* for ovarian cancer diagnosis was 0.405. Conclusion: *WWOX* is a valuable biomarker for ovarian cancer diagnosis and it might be a potential therapeutic target for the cancer.

Keywords: *WWOX*, ovarian cancer, diagnosis

Introduction

Ovarian cancer is one of the most common gynecologic malignancies with highest mortality in women worldwide [1]. The patients with ovarian cancer are often diagnosed at late stage, due to few clinical symptoms at the early stage thus the survival for the patients is poor [2, 3]. Early detection of ovarian cancer is a promising strategy for reducing mortality, especially for serious subtypes [4]. Therefore, specific biomarkers for diagnosis are urgently needed to improve the clinical outcomes of ovarian cancer patients.

The WW domain containing oxidoreductase (*WWOX*) is localized in the common fragile site, FRA16D (locus 16q23.3-24.1) [5]. In the previous studies, *WWOX* was reported to be a tumor suppressor gene and its abnormal expression was associated with tumor progression [6]. Lin et al. had reported that *WWOX* could inhibit prostate cancer progression via regulating cyclin D1 in cell cycle lead to G1 arrest [7]. In the study of Yang et al., alteration of *WWOX* gene was proved to be associated with nasopharyngeal carcinoma progression [8]. In cervical cancer, the under expression of *WWOX* was detected to be correlated with tumor development [9]. In addition, the function roles of the gene in ovarian cancer had been reported in the previous investigation. *WWOX* was reported to regulate cell cycle and apoptosis in ovarian cancer cell lines [10], besides methylation status of the gene correlated with the formation and progression of epithelial ovarian cancer [11]. These studies indicated that the *WWOX* gene could inhibit the occurrence of ovarian cancer. However, the diagnostic value of the gene in ovarian cancer had been rarely reported. The purpose of the present study was to evaluate the diagnostic significance of *WWOX* in ovarian cancer, and further understand the clinical effect of the gene to provide valuable treatment for the disease.

Table 1. Sequences of primers used in this study

Primers		Sequences
WWOX	Forward	5'-GATAATCCGACCAAGCCAAC-3'
	Reverse	5'-ACTGCTTCACTCGCCCTTG-3'
GAPDH	Forward	5'-TATTGGGCGCCTGGTCACCA-3'
	Reverse	5'-CCACCTTCTTGATGTCATCA-3'

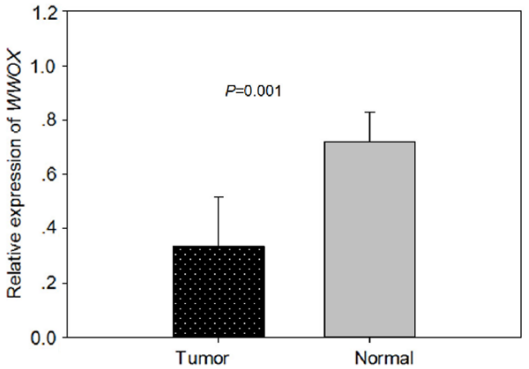

Figure 1. Relative expression of *WWOX* in plasma specimens collected from ovarian cancer patients and healthy volunteers (*GAPDH* as internal control).

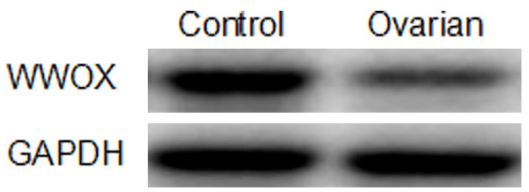

Figure 2. Western blot assay for the analysis of *WWOX* protein expression. The expression level of WWOX in ovarian cancer patients was lower than in control group.

In this study, we aimed to detect the relative expression of serum *WWOX* in patients with ovarian cancer and analyze its association between clinical characteristics, in order to evaluate the diagnostic value of *WWOX* in ovarian cancer.

Materials and methods

Plasma specimens

A total of 104 patients confirmed with ovarian cancer in Xi'an Fourth Hospital and 84 healthy volunteers were enrolled in the present study from November 2013 to January 2015. 10 ml blood specimens were collected from every participator before they had taken any food in the morning. The blood was kept in a tube containing EDTA at 4°C, and then the tubes were centrifuged at 2,500 rpm for 5 min, aiming to get plasma. All of the plasma specimens were stored in -80°C until used.

Total RNA extraction and qRT-PCR

Trizol reagent (Invitrogen, Carlsbad, CA) was used for extracting total RNA from plasma specimens according to the manufacturer's instructions. The residual DNA in RNA samples were treated with DNase. The concentration and quantity of the extracted RNA were detected by UV absorbance (A260/A280) and 1% agarose gel electrophoresis, respectively.

The relative expression of *WWOX* in collected plasma specimens were detected by fluorescence quantitative real-time PCR (qRT-PCR). The cDNA was synthesized by Prime Scrip RT reagent kit (Takara, China) and the qRT-PCR was performed with SYBR Green assay (Takara, China). *GAPDH* acted as internal control in the present study and the data was analyzed by $2^{-\Delta\Delta Ct}$ method. The sequence of primers designed by Primer 5.0 was listed in **Table 1**.

Western blot analysis

Proteins were extracted from plasma specimens. Then the proteins were separated via 12% SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane, blocked and incubated with rabbit monoclonal anti-*WWOX* and anti-GAPDH primary antibody for 3 h at room temperature. Following washed three times with western washing buffer, the membrane was incubated with secondary antibody for 40 min at room temperature. An enhanced chemiluminescence kit (Pierce Chemical) was used for chemiluminescent assay.

Statistical analysis

The differences of *WWOX* expression levels in collected plasma specimens were analyzed by student's T test. The relationship between *WWOX* expression and clinical characteristics of patients with ovarian cancer was evaluated by Chi-square test. Besides, receiver operating characteristic (ROC) analysis was used to assess the diagnostic significance of *WWOX* in ovarian cancer. Statistical analysis was performed with SPSS 18.0 software and Sigma Plot software was used for drawing. $P<0.05$ was considered as statistical significance.

Table 2. Relationship between *WWOX* expression and clinical characteristics

Characteristics	Total number (n)	*WWOX* expression		X^2	*P*
		High (n)	Low (n)		
Age				0.116	0.733
≥45	75	39	36		
<45	29	14	15		
Tumor location				0.346	0.556
Unilateral	52	25	27		
Bilateral	52	28	24		
Histological grade				14.086	0.000
G1+G2	48	34	14		
G3	56	19	37		
FIGO stage				24.047	0.000
I+II	52	39	13		
III+IV	52	14	38		
Lymph node metastasis				11.119	0.001
Yes	52	18	34		
No	52	35	17		
Recurrence				12.717	0.000
Yes	57	20	37		
No	47	33	14		

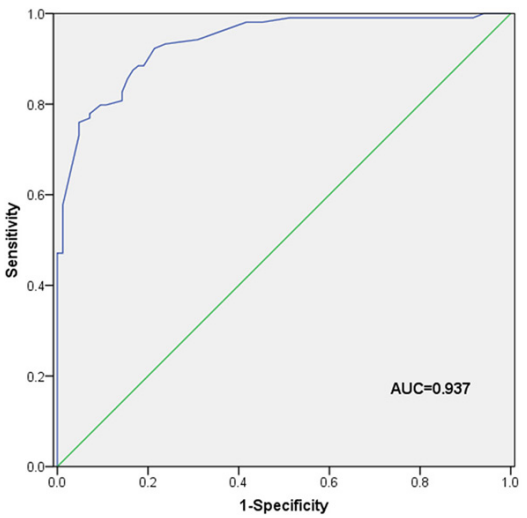

Figure 3. ROC curve analysis for diagnostic significance of *WWOX* in ovarian cancer. The AUC was 0.937 with sensitivity of 76% and specificity of 95.2%.

Results

Relative expression of WWOX and corresponding protein level

Relative expression of *WWOX* in plasma of ovarian cancer patients was detected by qRT-PCR and the results were shown in **Figure 1**. The expression level of *WWOX* in plasma specimens collected from ovarian cancer patients was 0.326±0.191, while that in the control groups was 0.695±0.135. There was a significant difference between the two groups (*P*= 0.001).

The corresponding protein level was detected by western blot assay and the results suggested that the protein level in plasma of ovarian cancer patients was lower than that in the control groups (**Figure 2**).

Relationship between WWOX expression and clinical characteristics

The average age of 104 patients with ovarian cancer was 49.4 years old, while the mean age of the healthy control group was 51.2. The clinical characteristics of the patients were listed in **Table 2**. In order to analyze the association between *WWOX* expression and clinical features, the enrolled patients were divided into two groups according to their average expression level of *WWOX*. From the results of Chi-square test, we found that expression level of *WWOX* was significantly associated with histological stage (*P*=0.000), FIGO stage (*P*=0.000), lymph node metastasis (*P*=0.001) and recurrence (*P*=0.000). However, there was no significant correlation between *WWOX* expression and age or tumor location (*P*>0.05 for all).

Diagnostic value of WWOX in ovarian cancer patients

The diagnostic value of *WWOX* was evaluated by ROC analysis. The results shown in **Figure 3** suggested that it could be distinguished from the ovarian cancer patients and healthy controls according to *WWOX* expression and the cut-off for diagnosis was 0.405. The AUC value of *WWOX* in ovarian cancer was 0.937 with the sensitivity of 76% and the specificity of 95.2%.

Discussion

WWOX acting as a tumor suppressor was reported to take part in the progression of sev-

eral types of cancer. Li et al. had reported that *WWOX* was deleted or attenuated in about 29-63% breast cancer tissues and the aberrant expression was associated with prognosis of breast cancer patients [12]. In the study of Chen et al. decreased expression of *WWOX* was detected in nasopharyngeal carcinoma and the expression level of *WWOX* was associated with the tumor progression [13]. Besides, *WWOX* was also detected in hepatocellular carcinoma by Li et al. and its expression level was correlated with Wnt/β-catenin signaling pathway [14]. Previous studies have reported that *WWOX* abnormally expressed in ovarian cancer [15] and the aberrant expression might be a promising marker for diagnosis of the disease.

In this study, the relative expression of *WWOX* in serum of ovarian cancer patients and healthy volunteers was detected. The expression level of the gene in patient's serum was lower than that in the healthy control groups. A further study indicated that the expression level was associated with histological stage, lymph node metastasis, FIGO stage and recurrence. These results suggested that *WWOX* expression was associated with ovarian cancer progression. The functional roles of *WWOX* in ovarian cancer were also proved in the previous studies. Paige et al. had reported that *WWOX* could suppress gene polymorphisms in ovarian cancer and be associated with tumor pathology and prognosis [16]. In the study of Xiong et al. over expression of *WWOX* in ovarian cancer cell line A2780 was proved to inhibit cell growth and induce apoptosis [17].

Patients with ovarian cancer were often diagnosed at late stage due to few diagnostic symptoms. The clinical outcomes of patients with ovarian cancer would be improved if patients diagnosed at early stage before metastasis [18]. Early diagnosis of ovarian cancer based on the techniques such as transvaginal ultrasound, physical examination, and blood-based tests [19]. The most widely used method was blood-based test and there were many serum factors involved in the test. For example, Teresa et al. had indicated that human epididymis protein 4 (*HE4*) was significantly associated with differential and early diagnosis of ovarian cancer [20]. Carbohydrate antigen 125 (*Ca-125*), the only one accepted tumor marker for ovarian cancer, could take part in diagnosis and monitoring of patients with ovarian cancer [21]. Besides, there were many other useful markers for ovarian cancer diagnosis reported in the previous studies, such as macrophage-colony stimulating factor (*M-CSF*), *VEGF,* soluble mesothelin-related peptide (*SMRP*), kallikrein family, matrix metalloproteinase-7 *(MMP-7*), CC chemokine ligand 18 (*CCL18*) and CC chemokine ligand 11 (*CCL11*) [22-26]. However, the sensitivity and specificity of the identified serum factors were unsatisfactory for early detection of ovarian cancer. Even if Ca-125 was the most widely used marker, it only detected less than 50% ovarian cancer patients at early stage [27]. Therefore, novel biomarkers with high sensitivity and specificity were greatly needed. In this study, we evaluated the diagnostic significance of *WWOX* in ovarian cancer and the results of ROC curve showed that *WWOX* could act as a diagnostic marker for the cancer. The tumor suppressor gene *WWOX* might enhance the sensitivity and specificity of early detection for ovarian cancer.

In this study, although we have proved that *WWOX* could act as a tumor suppressor, a further study is needed to analyze the molecular mechanisms of the gene involved in ovarian cancer. Besides, even we have proved *WWOX* was a potentially diagnostic marker for ovarian cancer; the functional roles of the gene in the clinical practice are needed to be verified. *WWOX* combined with other serum factors might be more useful for early detection of ovarian cancer in clinical practice.

In conclusion, abnormal expression of *WWOX* is detected in plasma of ovarian cancer patients and the expression level is associated with tumor progression. Besides, *WWOX* may be a potential biomarker for ovarian cancer diagnosis, which might help improve clinical outcomes of ovarian cancer patients.

Disclosure of conflict of interest

None.

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