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High level of WAVE1 expression is associated with tumor aggressiveness and unfavorable prognosis of epithelial ovarian cancer

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ABSTRACT

Objectives. Wiskott–Aldrich syndrome protein family verprolin-homologous protein 1 (WAVE1) has been shown to promote cancer invasion and metastasis. However, no evidence has been found to identify the role of WAVE1 in epithelial ovarian cancer (EOC). This study aims to determine the effect of WAVE1 expression and investigate a possible relationship between WAVE1 and prognosis in EOC.

Methods. WAVE1 protein level was measured in 223 EOC specimens by immunohistochemical staining and 46 EOC specimens by Western blot analysis. Expression of WAVE1 in ovarian cancer cell lines was evaluated by Western blot analysis and immunofluorescence. Survival analysis was performed to assess the correlation between WAVE1 expression and survival.

Results. Immunohistochemical staining and Western blot analysis showed that WAVE1 was overexpressed in EOC compared with samples from a non-invasive ovarian tumor and normal ovaries ($P<0.05$). Furthermore, expression of WAVE1 was significantly associated with advanced FIGO stage, poor grade, serum Ca-125 and residual tumor size ($P<0.05$). By Western blot analysis, WAVE1 expression was detected in four ovarian cancer cell lines. Immunofluorescence was performed to demonstrate WAVE1 expression in SKOV3 and 3AO cell lines. Survival analysis showed that patients with low WAVE1 staining had a significantly better survival compared to patients with high WAVE1 staining ($P<0.05$). In multivariate analysis, WAVE1 overexpression, advanced stage and suboptimal surgical debulking were independent prognostic factors of poor survival.

Conclusions. Our present study finds that WAVE1 overexpression is associated with an unfavorable prognosis. WAVE1 is an independent prognostic factor for EOC, which suggests that it is a novel and crucial predictor for EOC metastasis.

Introduction

Epithelial ovarian cancer (EOC) is a common gynecological malignancy that is associated with an unfavorable outcome, and it is the fifth-leading cause of death in females [1]. Worldwide, nearly 204,000 women are diagnosed, and 125,000 die due to ovarian cancer each year [2]. Seventy-five percent of EOC patients are diagnosed at an advanced and metastatic stage because of the lack of sensitive screening tests or early symptoms [3]. To date, the cellular/molecular mechanisms of EOC metastasis remain poorly understood, even though extensive clinical and basic research efforts have been undertaken [4–7]. Identification of novel molecular markers and the cellular/molecular mechanisms of EOC could improve the prediction of metastasis, novel therapeutic methods and further understanding of EOC progression.

The initial and essential step of cancer cell metastasis is cell migration. Invasive cancer cells must use their remodeled actin cytoskeleton to migrate through the extracellular matrix (ECM) and enter the blood or lymphatic system [8,9]. Reorganization of actin filaments is tightly regulated by a variety of proteins including the Rho family small GTPases (Rac, Cdc42 and Rho A), actin-related protein 2/3 (Arp2/3) complex, myosin and the Wiskott–Aldrich syndrome protein (WASP) family protein. Although Rac, Cdc42 and Rho A are essential for cell movement, the WASP family proteins, which respond to upstream signals to activate the Arp2/3 complex, are directly involved in actin polymerization [10,11].

Wiskott–Aldrich syndrome protein family verprolin-homologous protein 1 (WAVE1), which belongs to WASP family protein, has been proposed as acting as an enhancer [12]. Various research studies have found that WAVE1 is critical for cancer cell migration, invasion and metastasis. Down-regulation of WAVE1 has been found to be related to decreased invasion of prostate cancer cells, and results suggest that it might be used as a molecular target for preventing cell metastasis [13]. WAVE1 protein was overexpressed in malignant melanoma cells, which showed higher Rac activity than non-invasive and non-metastatic cells [14]. A

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recent study has shown that WAVE1 is a novel regulator of apoptosis that is involved in the multidrug resistance of leukemia cells [15].

Our previous study using the innovative reverse capture antibody microarray to identify autoantibody biomarkers in the plasma samples of EOC patients suggested a significantly elevated expression of WAVE1 in EOC plasma compared with healthy controls [3]. However, no study has assessed the expression of WAVE1 in patients with EOC or investigated the role of WAVE1 in ovarian cancer cell invasion and metastasis. In the present study, we aimed to examine the expression pattern of WAVE1 in human EOC tissues and human ovarian cancer cell lines. In addition, we investigated a possible relationship between WAVE1 and prognosis in EOC.

Material and methods

Patients and specimens

In the present study, a total of 320 paraffin-embedded tissue samples consisting of 223 EOC tissues, 29 borderline tumor tissues, 44 benign tumor tissues and 24 normal ovaries were obtained from the archives of the Department of Pathology, Chongqing Medical University, between February 2001 and October 2011 (Table 1). Forty-six EOC specimens were collected between September 2009 and October 2011 and snap-frozen in liquid nitrogen immediately after resection, which were stored at −80 °C for Western blot analysis. In addition, 16 borderline tumor tissues, 32 benign tumor tissues and 22 normal ovaries (resected for non-ovarian diseases) from gynecological surgery were added as controls. None of the patients had received chemotherapy or radiotherapy before surgery.

Table 1
Association of WAVE1 expression with clinicopathological characteristics in 223 patients of EOC.

	No. of patients	WAVE1 expression		*P* value
	(n = 223)	Low no. (%)	High no. (%)	
Characteristics				
Age (years)				
<51.0	105	31 (29.5%)	74 (70.5%)	
≥51.0	118	29 (24.6%)	89 (75.4%)	0.407
Serum Ca-125 level (U/ml)				
<35	28	13 (46.4%)	15 (53.5%)	
≥35	195	47 (24.1%)	148 (75.9%)	**0.013**
FIGO stage				
I/II	72	39 (54.2%)	33 (45.8%)	
III/IV	151	21 (13.9%)	130 (86.1%)	<**0.001**
Grade				
1	46	34 (73.9%)	12 (26.1%)	
2	106	20 (18.9%)	86 (81.1%)	
3	71	6 (8.5%)	65 (91.5%)	
	Grades 2–3 versus 1			<**0.001**
Ascites (ml)				
<100	81	25 (30.9%)	56 (69.1%)	
≥100	142	35 (24.6%)	107 (75.4%)	0.315
Tumor type				
Serous	118	31 (26.3%)	87 (73.7%)	
Mucinous	40	14 (35.0%)	26 (65.0%)	
Clear cell	13	4 (30.8%)	9 (69.2%)	
Endometrioid	16	7 (43.8%)	9 (56.3%)	
Adenocarcinoma	36	4 (11.1%)	32 (88.9%)	
	Serous versus non-serous			0.821
Tumor size (cm)				
<5	39	14 (35.9%)	25 (64.1%)	
≥5	184	46 (25.0%)	138 (75.0%)	0.164
Tumor lesion no.				
Unilateral lesion	116	37 (31.9%)	79 (68.1%)	
Bilateral lesions	107	23 (21.5%)	84 (78.5%)	0.081
Residual tumor size(cm)				
<1	185	55 (29.7%)	130 (70.3%)	
≥1	38	5 (13.2%)	33 (86.8%)	**0.036**

Bold items highlight $P<0.05$.

The 223 EOC patients who provided samples for immunohistochemical staining had a mean age of 50.36 ± 11.12 years (range: 15–81 years). The 46 EOC patients who provided specimens for Western blot analysis ranged from 27 to 75 years with a mean age of 52.57 ± 10.59 years. The clinical and pathological characteristics of these patients are described in Tables 1 and 2. The patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO). All tissue blocks were reevaluated for histological type, graded by two senior pathologists and stained with hematoxylin-eosin. The study protocols have been approved by the local Ethics Committee. Prior informed consent was obtained from the patients according to the Declaration of Helsinki.

Immunohistochemical staining

The paraffin-embedded blocks were cut at 4 μm and mounted on slides. Sections were deparaffinized with xylene and rehydrated through graded alcohol. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min at room temperature. Antigen retrieval was done in the microwave for 15 min. After rinsing with PBS, normal goat serum was incubated for 20 min at 37 °C to eliminate nonspecific binding. Primary antibody and goat anti-human WAVE1 (1:100) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were applied to the sections overnight at 4 °C. Negative control slides were incubated with normal goat serum. After washing, sections were incubated with a biotinylated secondary antibody for 20 min at 37 °C. A PBS wash was followed by the SABC reagent (Boster, Wuhan, China) according to the instruction. Sections were stained by applying DAB and then counterstained with hematoxylin.

WAVE1 staining was mainly localized in both the cytoplasm and the cytomembrane. Staining intensity was graded on a 0–3 scale as

Table 2
Association of WAVE1 protein expression with clinicopathological characteristics in 46 cases of EOC.

Characteristics	No. patients	WAVE1 expression	
	(n = 46)	Protein	*P* value
Age (years)			
<51.0	18	0.904 ± 0.206	
≥51.0	28	0.947 ± 0.176	0.459
Serum Ca-125 level (U/ml)			
<35	10	0.800 ± 0.184	
≥35	36	0.970 ± 0.174	**0.011**
FIGO stage			
I/II	24	0.766 ± 0.061	
III/IV	22	1.109 ± 0.084	<**0.001**
Grade			
1	9	0.780 ± 0.175	
2	16	0.863 ± 0.121	
3	21	1.045 ± 0.169	
	Grades 2–3 versus 1		**0.006**
Ascites (ml)			
<100	13	0.893 ± 0.190	
≥100	33	0.945 ± 0.187	0.408
Tumor type			
Serous	27	0.970 ± 0.189	
Mucinous	7	0.822 ± 0.110	
Clear cell	3	0.726 ± 0.071	
Endometrioid	5	0.832 ± 0.171	
Adenocarcinoma	4	1.125 ± 0.029	
	Serous versus non-serous		0.086
Tumor size (cm)			
<5	7	1.007 ± 0.212	
≥5	39	0.916 ± 0.182	0.243
Tumor lesion no.			
Unilateral lesion	25	0.901 ± 0.179	
Bilateral lesions	21	0.964 ± 0.196	0.266
Residual tumor size(cm)			
<1	37	0.868 ± 0.174	
≥1	9	1.104 ± 0.139	**0.019**

Bold items highlight $P<0.05$.