Epithelial membrane protein 1 expression in ovarian serous tumors

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Abstract. The present study aimed to analyze the clinical significance of epithelial membrane protein 1 (EMP1) expression in ovarian serous tumors. A total of 84 cases of ovarian serous tumor (50 patients with malignant ovarian serous tumors and 34 patients with borderline and benign serous tumors) were retrospectively analyzed. Differences in the expression levels of EMP1 between the malignant and non-malignant tumor groups were evaluated by immunohistochemical staining. In addition, the association between EMP1 expression and prognostic factors in malignant ovarian serous tumors was investigated. The expression levels of EMP1 were significantly reduced in all the 50 malignant ovarian serous tumors, compared with the 34 non-malignant ovarian serous tumors (P<0.000). Reduced expression of EMP1 was correlated with high grade (P=0.009) and stage (P<0.000) of malignant tumors. EMP1 expression was not observed to be correlated with any other investigated parameters, including surgery, type of operation and chemotherapy response (P>0.005). These results indicated that EMP1 may have a significant role as a negative regulator in ovarian serous tumors, and reduced EMP1 expression in serous tumors may be associated with increased disease severity.

Introduction

Ovarian cancer is the seventh most common cancer in women worldwide, with ~239,000 cases and 151,000 mortalities recorded in 2012 (1). Previous studies have revealed that nulliparous women possess an increased risk of developing epithelial ovarian cancer (EOC), while women who have previously given birth, breastfed, undergone tubal ligation or received oral contraceptives possess a reduced risk of developing EOC (2). Patients exhibiting EOC have been diagnosed with certain molecular abnormalities. However, the role of these molecular abnormalities in early malignant transformation remains to be elucidated (2). A previous study detected cytogenetic abnormalities, mutations in the proto-oncogene p53 and overexpression of pro-apoptotic genes (2). Lai *et al* (3) reported an association between epithelial membrane protein 1 (EMP1) expression and tumor development and progression. In particular, EMP1 was proposed to participate in the development and progression of non-small cell lung cancer via activation of the phosphoinositide 3-kinase/AKT signaling pathway (3). To the best of our knowledge, no previous studies describing the association between epithelial ovarian tumors and EMP1 expression have been reported to date. Serous tumors are the most frequently observed epithelial tumors of the ovaries (2). In the present study, the expression levels of EMP1 in patients with ovarian serous tumors were investigated using immunohistochemistry, and the association between EMP1 expression and certain clinical features of these patients was analyzed.

Materials and methods

Patient samples. Following approval from the ethics committee of Ondokuz Mayıs University (Samsun, Turkey; approval no. 568.2014), informed consent was obtained from all patients enrolled in the study. The present retrospective study included 84 cases of ovarian serous tumor who had been diagnosed between 2005 and 2013 at the Department of Pathology of the Faculty of Medicine of Ondokuz Mayıs University. Each sample had undergone routine hematoxylin (cat. no. 105175) and eosin staining (cat. no. 115935; Merck KGaA, Darmstadt, Germany), and all hematoxylin and eosin-stained slides were re-examined to confirm the original diagnosis. A total of 82 serous tumors were classified into three groups: i) Serous adenocarcinoma (n=50); ii) borderline serous tumor (n=16); and iii) benign serous tumor (n=18). All borderline serous tumors were categorized as classical type, and micropapillary serous borderline tumors were excluded from the study. All specimens were routinely fixed in formalin (cat. no. 104003; Merck KGaA) and processed in paraffin wax (cat. no. 107337; Merck KGaA). Representative samples were selected for immunohistochemistry, and the association between EMP1 expression and tumor type, grade,

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Table I. Clinicopathological features of patients with ovarian serous tumors.

Variable	Total patients, n (%)
Age, years	
Mean ± standard deviation	57.6±11.9
CA125 levels at diagnosis, U/ml	
Median (range)	719 (18-5,000)
Surgery	
TAH-BSO	40 (83.3)
Frozen pelvis	8 (16.7)
Surgery success	
Optimal	16 (32.0)
Suboptimal	34 (68.0)
Stage	
I	11 (22.0)
II	12 (24.0)
III	15 (30.0)
IV	12 (24.0)
Chemotherapy response	
Complete	30 (62.5)
Stable disease	3 (6.2)
Progression	2 (4.2)
Partial	13 (27.1)
Grade	
Low	13 (26.0)
High	37 (74.0)

CA125, cancer antigen 125; TAH, total abdominal hysterectomy; BSO, bilateral salpingo-oophorectomy.

Table II. Expression of epithelial membrane protein 1 in patients with malignant vs. non-malignant ovarian serous tumors.

Score	Malignant, n (%)	Non-malignant, n (%)	Total, n (%)
0	22 (88.0)	3 (12.0)	25 (100.0)
1	5 (83.3)	1 (16.7)	6 (100.0)
2	14 (53.8)	12 (46.2)	26 (100.0)
3	9 (33.3)	18 (66.7)	27 (100.0)
Total[a]	50 (59.5)	34 (40.5)	84 (100.0)

[a]χ^2=17.861 (Pearson's χ^2 test); P<0.0001 (degrees of freedom=3).

stage and other prognostic parameters in EMP1$^+$ patients was investigated.

Immunohistochemical staining. Sections (4-μm) were prepared from routinely processed paraffin blocks. Slides were placed into an oven at 56˚C for 1 h. All immunohistochemical stains were performed with BOND-MAX

Table III. Association between epithelial membrane protein 1 expression and tumor grade in malignant ovarian tissues.

Score	Low grade, n (%)	High grade, n (%)	Total, n (%)
0	1 (4.5)	21 (95.5)	22 (100.0)
1	1 (20.0)	4 (80.0)	5 (100.0)
2	6 (42.9)	8 (57.1)	14 (100.0)
3	5 (55.6)	4 (44.4)	9 (100.0)
Total[a]	13 (26.0)	37 (74.0)	50 (100.0)

[a]χ^2=21.965 (Pearson's χ^2 test); P=0.009 (degrees of freedom=6).

Table IV. Association between epithelial membrane protein 1 expression and tumor stage in malignant ovarian tissues.

Score	Stage I, n (%)	Stage II, n (%)	Stage III, n (%)	Stage IV, n (%)	Total, n (%)
0	0 (0.0)	1 (4.5)	10 (45.5)	11 (50.0)	22 (100.0)
1	1 (20.0)	0 (0.0)	3 (60.0)	1 (20.0)	5 (100.0)
2	4 (28.6)	8 (57.1)	2 (14.3)	0 (0.0)	14 (100.0)
3	6 (66.7)	3 (33.3)	0 (0.0)	0 (0.0)	9 (100.0)
Total[a]	11 (22.0)	12 (24.0)	15 (30.0)	12 (24.0)	50 (100.0)

[a]χ^2=43.543 (Pearson's χ^2 test); P<0.0001 (degrees of freedom=9).

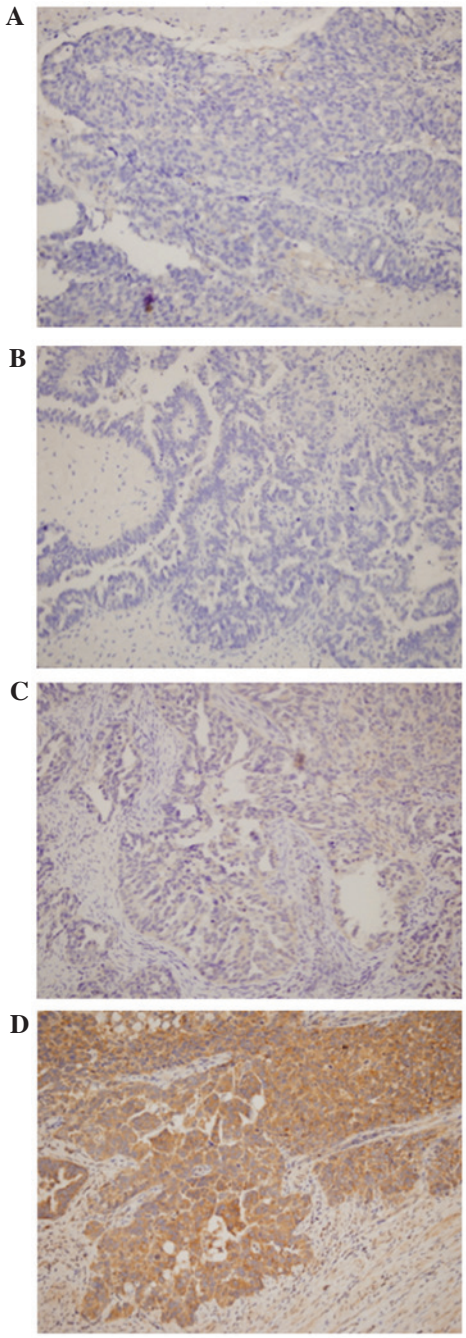

Figure 1. Light microscopy images of epithelial membrane protein 1 (EMP1) expression in high-grade ovarian serous adenocarcinoma samples. (A and B) Representative samples of ovarian serous adenocarcinoma demonstrating no expression of EMP1. Representative images of (C) weak and (D) strong staining for EMP1 (3,3'-diaminobenzidine staining; magnification, x200).

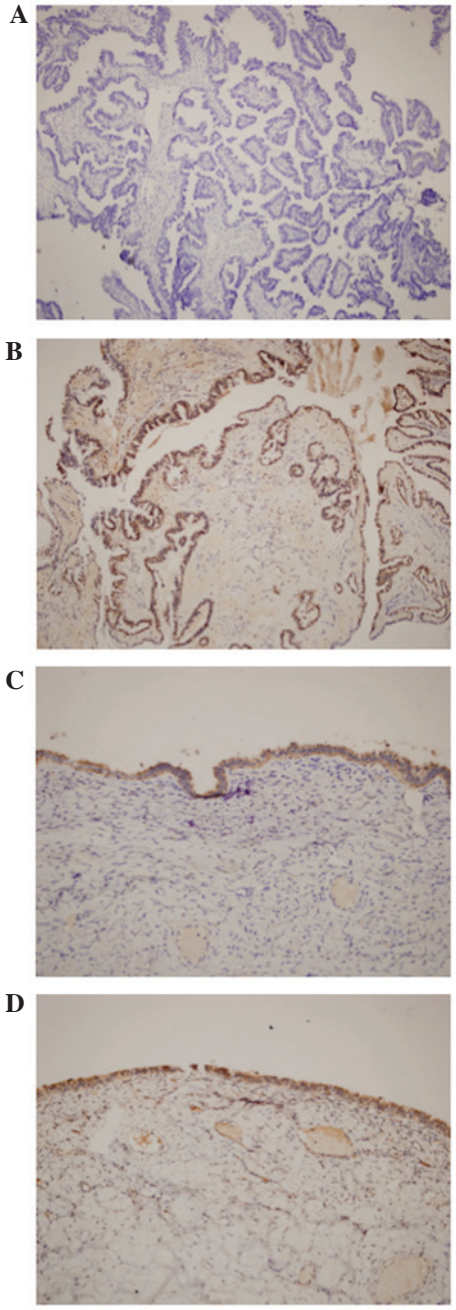

Figure 2. Light microscopy images of epithelial membrane protein 1 (EMP1) expression in borderline and benign ovarian serous tumor samples. Representative images of (A) weak and (B) strong staining for EMP1 in borderline serous tumor samples. (C and D) Strong staining for EMP1 in two representative benign serous tumor samples (3,3'-diaminobenzidine staining; magnification, x200).

autostainer (Leica Microsystems, Inc., Buffalo Grove, IL, USA). Antigen retrieval was performed by incubating the slides in citrate buffer (Thermo Fisher Scientific, Waltham, MA, USA) until the temperature reached 95˚C. Slides were then incubated for 30 min at room temperature with primary rabbit anti-human EMP1 antibody (cat. no. orb10588; polyclonal; 1:100 dilution; Biorbyt Ltd., Cambridge, UK), and revealed with the chromogen 3,3'-diaminobenzidine (cat. no. 901-DB801-082914; Biocare Medical, Inc., Concord, CA, USA).

Assessment of immunohistochemical staining. Pathologists assessed the stained slides blindly, with no knowledge of the pathological diagnosis, using light microscopy (Olympus BX51; Olympus Corporation, Tokyo, Japan). Cytoplasmic staining was considered to indicate positivity for EMP1. Each slide was evaluated according to the extent and intensity of staining. Samples were categorized as negative if <5% of tumor cells were positive for EMP1. Staining extent was assessed as the percentage of $EMP1^+$ cells present in the sample, and scored semi-quantitatively using the following 0-3 scale: i) 0, <5%; ii) 1, 5-25%; iii) 2, 26-50%; and iv) 3, 51-100%. Staining intensity was categorized into three groups based on the intensity of chromogen staining exhibited by the tumor cells: i) 0, negative; ii) 1, weak staining (light yellow); iii) 2, moderate staining (yellowish-brown); and iv) 3, strong staining (brown). By adding the staining extent and intensity scores, a final score for EMP1 expression was calculated. Samples were divided into four groups, according to their final scores, as follows: i) 0, negative; ii) 2, weak staining; iii) 3-4, moderate staining; and iv) 5-6, strong staining. A final score of 1 was not possible based on the scoring system used.

Statistical analyses. All statistical analyses were performed with SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). χ^2 test was utilized to determine the P-values for the clinicopathological features analyzed. $P<0.05$ indicated a statistically significant difference.

Results

Demographic characteristics and response rates. The characteristics of 50 malignant ovarian serous adenocarcinoma patients, who were evaluated in the present study, are summarized in Table I. The mean age of the patients was 57.6 years, with a median CA125 level at diagnosis of 719 U/ml (range, 18-5,000 U/ml). A total of 13 patients exhibited low-grade disease, while 37 patients exhibited high-grade disease. Of the 50 patients with malignant ovarian serous adenocarcinoma evaluated, the characteristics of 48 patients with available demographic characteristics and chemotherapy response rates were also summarized in Table I. A complete response was observed in 30 patients (62.5%), stable disease in 3 patients (6.2%), progression in 2 patients (4.2%) and partial response in 13 patients (27.1%).

EMP1 expression. EMP1 was expressed in all the 50 cases of malignant ovarian serous adenocarcinoma. However, the expression levels of EMP1 in these patients were significantly reduced, compared with those observed in the 34 borderline and benign serous tumor cases ($P<0.0001$; Table II). Reduced expression of EMP1 was correlated with high grade ($P=0.009$; Table III) and stage ($P<0.0001$; Table IV; Figs. 1 and 2) of cancer. EMP1 expression was not correlated with any of the other investigated parameters, including surgery, operation type or chemotherapy response ($P>0.005$).

Discussion

The EMP1 gene encodes four ~18 kDa transmembrane domains (4). EMP1 differs from EMP2 and EMP3 in terms of hydrophobic groups (4). Zoidl *et al* (5) revealed that EMP1 was significantly expressed in undifferentiated embryonic stem cells, while poorly expressed in differentiated adult cells, and appeared to be involved in prolonging the transition of Schwann cells from G to $S/G_2/M$ phase. The EMP membrane glycoprotein family is considered to be associated with cell proliferation and differentiation (6). Previous studies have demonstrated that the EMP1 gene is expressed in several normal tissues, including the colon, lung, testis and ovary (7-9). Sun *et al* (6-8), identified that the protein levels of EMP1 were significantly reduced in nasopharyngeal carcinoma, breast and prostate cancer, compared with normal tissue, and were correlated with T stage, lymph node metastasis and clinical stage in these tumors. In addition, Sun *et al* (9) identified that the expression levels of EMP1 were significantly reduced in gastric cancer tissues, compared with normal tissues, and observed that reduced EMP1 expression in gastric cancer was associated with increased disease severity. Similarly, the present study revealed that the expression levels of EMP1 were significantly reduced in patients with malignant serous tumors, compared with patients exhibiting benign and borderline serous tumors. In addition, this decrease in EMP1 expression in high-grade and advanced-stage tumors indicated that EMP1 expression may be associated with tumor grade and stage. Gnirke and Weidle (10) demonstrated that EMP1 expression was correlated with cell invasion and metastatic characteristics in several human mammary carcinoma cell lines. Wang *et al* (11) reported that the EMP1 gene may act as a regulatory factor in cell signaling, communication and adhesion. Zhang *et al* (12) observed that the downregulation of the EMP1 gene was correlated with lymph node metastasis. Sun *et al* (6) reported that the protein levels of caspase-9 increased significantly with the levels of EMP1, which indicated that the mitochondrial-dependent apoptosis pathway may be involved in EMP1-induced apoptosis. Vascular endothelial growth factor C (VEGFC) promotes the proliferation of endothelial cells, increases vascular permeability, and acts as an essential factor for tumor angiogenesis, invasion and metastasis (6). Sun *et al* (6) demonstrated that VEGFC expression was significantly decreased following transfection with EMP1, which suggested that EMP1 was able to inhibit tumor angiogenesis by suppressing VEGFC expression and tumor metastasis.

In conclusion, EMP1 may possess a significant role in ovarian cancer cell proliferation, apoptosis, invasion and metastasis, and may be involved in a number of biological functions. Further extensive studies on EMP1 are required to aid the development

of novel therapy options for the treatment of ovarian cancer, and the potential identification of novel prognostic factors.

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