EJGO European Journal of Gynaecological Oncology

Expression and clinical significance of Ki-67, E-cadherin, and mesothelin in serous borderline ovarian tumor

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Summary

Objective: This study aimed to investigate the expression of Ki-67, epithelial cadherin (E-cadherin), mesothelin, and their correlations with clinicopathological features of serous borderline ovarian tumors (SBOTs). *Materials and Methods:* Immunohistochemistry was conducted to investigate the expression of a cellular proliferation-related factor (Ki-67) and metastasis-related factors (E-cadherin and mesothelin) in 41 cases of SBOTs, 30 benign ovarian tumor tissues, and 30 malignant ovarian tumor tissues. *Results:* The results showed that the expression rate of Ki-67 (46.3%) in SBOTs was higher than that in benign tumors (6.7%), but lower than that in ovarian carcinomas (80%). SBOTs significantly differed from benign ovarian tumors ($p < 0.01$) and carcinoma ($p < 0.01$). The positive expression rate of Ki-67 was significantly correlated with FIGO stage and peritoneal implantation of SBOTs ($p < 0.01$). The expression rate of E-cadherin was significantly lower in ovarian carcinomas (56.7%) than in SBOTs (80.5%) and benign ovarian tumors (90%; $p < 0.05$). The mesothelin expression in ovarian carcinomas and SBOTs significantly differed from that in benign ovarian tumors ($p < 0.01$). The positive expression rate of mesothelin was related to the serum CA125 level ($p < 0.05$). The expression of Ki-67 was positively correlated with the expression of E-cadherin and mesothelin in SBOTs. *Conclusion:* SBOTs generally behave in a benign manner. Ki-67, E-cadherin, and mesothelin may play an important role in the oncogenesis and progression of SBOT. Patients with Ki-67 and mesothelin overexpression and low E-cadherin expression should be followed up.

Key words: serous ovarian borderline tumors, immunohistochemistry, Ki-67, E-cadherin, mesothelin.

Introduction

Borderline ovarian tumors (BOTs) were reported as a "semi-malignant disease" in 1929 by Taylor [1]. In 1971, BOTs were described as carcinomas with "low malignant potential" by the International Federation of Gynecology and Obstetrics (FIGO) [2]. In 1973, these tumors were considered as "borderline ovarian tumors" by the World Health Organization (WHO) [3]. Among these technical descriptions, the latter is the term used in current classification (2003) [4]. BOTs represent 10% to 20% of all epithelial ovarian malignancies [5]. The most common BOTs are serous BOTs (SBOTs), which account for two-thirds to three-fourths of these tumors [6]. BOTs differ from ovarian carcinoma in terms of the absence of stromal invasion. In contrast to ovarian cancer, BOTs are characterized clinically on the basis of the maximum overall survival, such as the five-year survival rates of approximately 100% in the early stage of the disease [7]. Nevertheless, approximately 25% of BOTs may develop a clinical aggressive behavior; of these BOTs, 3% to 15% recur as invasive carcinomas [8]. Therefore, the growth, development, and prognosis of SBOTs should be investigated.

Malignant proliferation is one of the distinct characteristics of malignant tumors. The Ki-67 protein is a cellular marker of proliferation. This marker is closely associated with cell proliferation. Ki-67 is also an excellent marker to determine the growth fraction of a specific cell population. For instance, the fraction of Ki-67-positive tumor cells is often correlated with the clinical course of cancers. Thus, the authors chose this biomarker to evaluate the cell proliferation of SBOTs in this study.

Tumor metastasis is a multi-step process; in this process, epithelial cadherin (E-cadherin) is a major homophilic cell-to-cell adhesion molecule that interconnects epithelial cells to inhibit the motility of individual cells in the matrix [9]. SBOTs with peritoneal implantation and stromal microinvasion may be associated with reduced cell-to-cell adhesion.

Mesothelin is a 40 kDa cell surface glycoprotein that is highly expressed in pancreatic cancers, ovarian cancers, mesotheliomas, and other cancers [10]. Le Page *et al.* showed that mesothelin is a marker independent of the grade and stage of ovarian serous tumors [11]; thus, mesothelin can be considered as a new marker of ovarian serous tumor.

This study aimed to evaluate the expression levels of Ki-67, E-cadherin, and mesothelin in ovarian serous cystadenomas, SBOTs, and ovarian serous cystadenocarcinomas. This study also aimed to analyze the correlation between these factors in SBOTs. The clinicopathological variables in SBOTs were also investigated.

Revised manuscript accepted for publication December 2, 2015

Table 1. — *Ki-67,E-cadherin, and mesothelin expression and correlation with different clinical pathological indexes in SBOTs.*

Group		Number	Mesothelin		E-cadherin		Ki-67	
			Positive (%)	*p*	Positive (%)	*p*	Positive (%)	*p*
Age (years)	≤ 35	17	14 (82.4)	0.296	14 (82.4)	1.000	9 (52.9)	0.537
	> 35	24	15 (62.5)		19 (90.5)		10 (41.7)	
Histological type	Typical	39	27 (69.2)	1.000	31 (79.5)	1.000	17 (43.6)	0.209
	Micropapillary	2	2 (100)		2 (100)		2 (100)	
FIGO Stage	I-II	34	22 (64.7)	0.085	28 (85.3)	0.606	12 (35.3)	0.002
	III-IV	7	7 (100)		5 (71.4)		7 (100)	
Peritoneal implant	Negative	35	23 (65.7)	0.156	29 (82.9)	0.518	13 (37.1)	0.004
	Positive	6	6 (100)		4 (66.7)		6 (100)	
Ascites	No	22	14 (63.6)	0.283	16 (72.7)	0.249	9 (40.9)	0.538
	Yes	19	15 (78.9)		17 (89.5)		10 (52.6)	
CA125 (KU/L)	≤ 35	10	2 (20.0)	0.000	10 (100)	0.147	4 (40.0)	0.704
	> 35	21	19 (90.5)		16 (76.2)		11 (52.4)	

Materials and Methods

Patients and tissue sampling

The investigation protocol was approved by the Institutional Ethics Committee prior to initiation. Tissue samples of 41 SBOTs, 30 benign ovarian serous cystadenocarcinomas, and 30 malignant ovarian serous cystadenomas were obtained from the Department of Pathology, Qi Lu Hospital affiliated with Shandong University and the Affiliated Hospital of BinZhou Medical University of Shan-Dong Province. Informed consent was obtained from all the patients.

All samples were routinely fixed with 10% formaldehyde and embedded with paraffin. The histological features of all cases were studied by hematoxylin-eosin staining (HE) and diagnosed by two clinical pathologists, strictly adhering to the current WHO criteria [4] for diagnosis of SBOT (typical pattern and micropapillary pattern), peritoneal implantation, and stromal microinvasion.

Immunohistochemical study

The streptavidin-peroxidase-biotin (SP) immunohistochemical method was performed on the paraffin sections to study the expression of Ki-67, E-cadherin, and mesothelin using a rabbit monoclonal antihuman Ki-67 antibody, mouse monoclonal antihuman E-cadherin antibody, and mouse monoclonal antihuman mesothelin antibody. In brief, the sections (five μm) were deparaffinized, rehydrated, and subjected to antigen retrieval using an autoclave (170 kPa) in EDTA buffer or sodium citrate. The endogenous peroxidase activity was blocked by incubating in 0.3% hydrogen peroxide. The slides were incubated in primary antibody overnight at 4°C, and then incubated with a secondary antibody conjugated with biotin. Finally, the sections were visualized with DAB (3, 3'-diaminobenzidine dihydrochloride) and counterstained with hematoxylin. The negative control for every experiment was made by replacing the primary antibodies with phosphate buffer solution (PBS).

The slides were examined independently by two pathologists. Immunohistochemical analysis revealed that Ki-67 expression was located in the nucleus of ovarian tumor cells; E-cadherin and mesothelin were expressed primarily in the cytoplasmic membrane of ovarian tumor cells.

The immunoreactivity of Ki-67 was graded in accordance with the following procedures. The intensity of cell staining was scored on a scale of 1 to 3, with 1 indicating weak staining, 2 indicating moderate staining, and 3 indicating the strongest staining. The extent of staining was calculated according to the percentage of positive cells as follows: 0, positive immunostaining of ≤ 5% of tumor cells; 1, positive immunostaining of 6% to 25% of tumor cells; 2, positive immunostaining of 26% to 50% of tumor cells; 3, positive immunostaining of 51% to 75% of tumor cells; and 4, positive immunostaining of > 75% of tumor cells. The percentage of cells (from 0 to 4) at each intensity was multiplied by the corresponding intensity (from 1 to 3) to obtain an immunostaining score ranging from 0 to 12: 0 as negative (-), 1 to 4 as weak positive (1+), 5 to 8 as moderate positive (2+), and 9 to 12 as strong positive (3+). The immunoreactivity of E-cadherin was graded according to the percentage of stained tumor cells: less than 10% as negative (-); 10% to 50% as weak positive (1+); 50% to 80% as moderate positive (2+); and ≥ 90% as strong positive (3+). Mesothelin positivity was scored as the percentage of tumor cells, and the results were categorized into four groups: negative (-): no positive immunostaining of tumor cells; weak positive (1+): < 20% of tumor cells stained positive; moderate positive (2+), 20% to 50% of tumor cells stained positive; and strong positive (3+): ≥ 50% of tumor cells stained positive.

Statistical analysis

Statistical analyses were performed using SPSS 20.0. Pearson's Chi-square test and Fisher's exact probabilities when an expected cell value was 5 or less were performed to analyze the statistical significance of Ki-67, E-cadherin, and mesothelin expression levels in the SBOT and the control groups, as well as the associations between the expression levels and the clinicopathological factors. Spearman's rank correlation coefficient was used to analyze the correlation between the expression levels of Ki-67, E-cadherin, and mesothelin in SBOT. A $p < 0.05$ was considered statistically significant.

Results

The pathological specimens of 101 women with serous ovarian tumor (30 with benign serous cystadenoma, 41 with SBOT, and 30 with malignant serous cystadenocarcinoma) were analyzed. The median age of women in the benign, borderline, and malignant groups was 44 (range, 17–85), 42 (range, 18–82), and 51 years (range, 23–68), respectively. In SBOT, the vast majority exhibited typical pattern; only two cases were diagnosed to have micropapillary pattern. Furthermore, 32 patients had Stage I disease; FIGO II and III were observed in two and seven patients, respectively; no

Table 2. — *Ki-67, E-cadherin, and mesothelin expression in serous cystadenocarcinoma, SBOT, and serous cystadenoma.*

Group	Number	Mesothelin					E-cadherin				Ki-67				
		-	+	2+	3+	*p*	-	+	2+	*p*	-	+	2+	3+	*p*
Cystadenoma	30	24	4	1	1	0.000*	3	20	7	0.335*	28	2	0	0	0.000*
SBOT	41	12	14	13	2	0.154#	8	27	6	0.030#	22	14	5	0	0.000#
Cystadenocarcinoma	30	4	12	8	6	0.000•	13	14	3	0.004•	6	15	8	1	0.000•

*Compared with the SBOT; #compared with cystadenocarcinoma; •compared with cystadenoma.

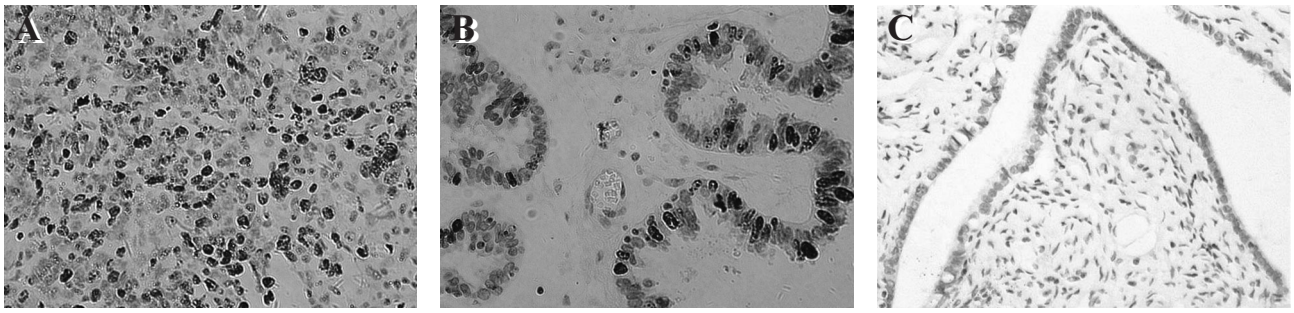

Figure 1. — Immunohistochemical staining of Ki-67 in benign, borderline, and malignant serous ovarian tumors. (A) Serous cystadenocarcinoma showeing strong positive signals (diffuse dark brown) in the nucleus of tumor cells. (B) Focal positive staining of the tumor cells is observed in SBOT. (C) Absence of Ki-67 expression in serous cystadenomas. Original magnifications: ×400.

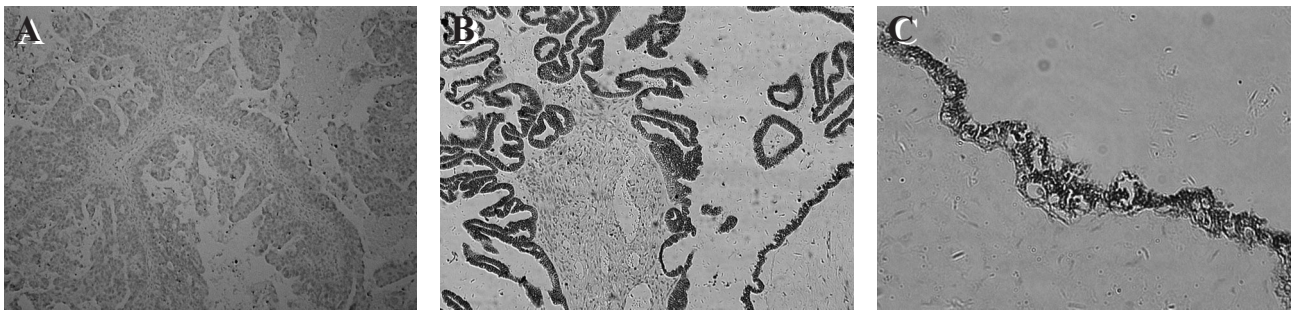

Figure 2. — Immunohistochemical staining of E-cadherin in benign, borderline, and malignant serous ovarian tumors. Negative E-cadherin expression in serous cystadenocarcinoma (A: original magnifications ×400), while strong positive expression of E-cadherin in tumor cell membrane are detected in SBOT (B: original magnifications ×100) and serous cystadenomas (C: original magnifications ×400).

Stage IV was reported. In addition, 14 (34.1%) patients had bilateral disease, consistent with literature reports [12]. Peritoneal implant and stromal microinvasion were detected in six (14.6%) and two (4.9%) patients, respectively. Serum CA-125 elevation was observed in 21 cases out of the 31 cases in preoperative testing. The ovarian tumors were classified and staged in accordance with the World Health Organization (WHO) and International Federation of Gynecology and Obstetrics (FIGO) criteria [13].

Ki-67, E-cadherin, and mesothelin expression and correlation with different clinicopathologic parameters in SBOTs

Table 1 shows the correlation between the Ki-67, E-cadherin, and mesothelin expression levels and the clinicopathological factors. The expression of Ki-67 was associated with FIGO stage ($p < 0.01$) and peritoneal implant ($p < 0.01$) but not with age, histological type, ascites, and serum CA125. No significant associations were observed between mesothelin expression and age, histological type, FIGO stage, peritoneal implant, and ascites. However, a significant difference was noted between the positive mesothelin rate and the serum CA125 level ($p < 0.05$). The E-cadherin expression was not associated with age, histological type, FIGO stage, peritoneal implant, ascite, and serum CA125 level.

Expression of Ki-67, E-cadherin, and mesothelin in serous ovarian tumors (Table 2)

Ki-67 mainly presented in the nucleus of ovarian tumor cells (Figure 1). The positive Ki-67 expression was detected in 6.7% (2/30) of benign, 46.3% (19/41) of borderline, and 80% (24/30) of malignant tumors. The expression of Ki-67 was significantly higher in malignant than that in borderline ($p < 0.01$) and benign tumors ($p < 0.01$) Moreover, significant differences were observed between bor-

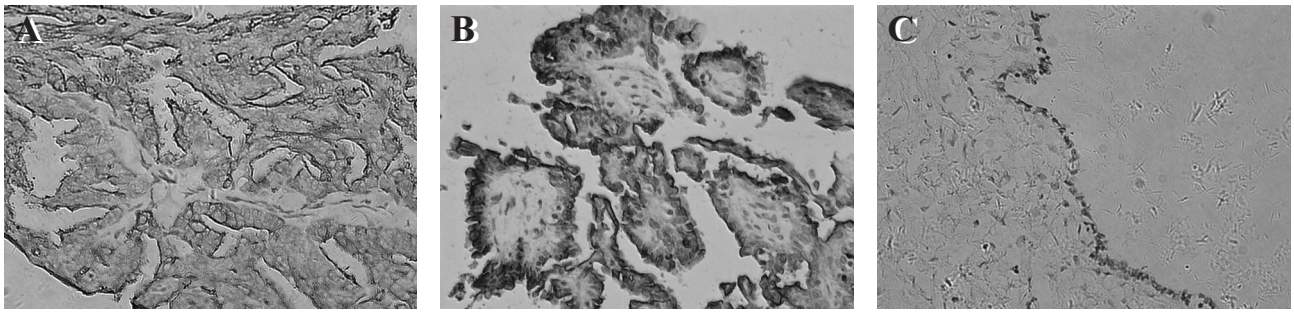

Figure 3. — Immunohistochemical staining of mesothelin in benign, borderline, and malignant serous ovarian tumors. Serous cystadenocarcinoma (A) and SBOT (B) show positive expression in the tumor cell membrane, while negative expression is observed in serous cystadenomas (C). Original magnifications: ×400.

Table 3. — *Associativity of Ki-67, E-cadherin and mesothelin expression in SBOTs.*

Ki-67	Number	Mesothelin		
		Positive	Negative	
Positive	19	17	2	$r = 0.358$
Negative	22	12	10	$p < 0.05$

Ki-67	Number	E-cadherin		
		Positive	Negative	
Positive	19	18	1	$r = 0.317$
Negative	22	15	7	$p \leq 0.05$

Mesothelin	E-cadherin		
	Positive	Negative	
Positive	22	7	$r = 0.179$
Negative	11	1	$p > 0.05$

derline and benign tumors ($p < 0.01$).

E-cadherin was mainly localized to the membrane of the ovarian tumors (Figure 2). Positive E-cadherin expression was detected in 90% (27/30) of benign, 80.5% (33/41) of borderline, and 56.7% (17/30) of malignant tumors. The expression of E-cadherin was significantly lower in malignant than that in SBOT ($p < 0.05$) and benign tumors ($p < 0.01$); no significant difference was observed between SBOT and benign tumors ($p > 0.05$).

Mesothelin was mainly localized in the membrane of the ovarian tumors (Figure 3). The positive mesothelin expression was also detected in 20% (6/30) of benign, 70.7% (29/41) of borderline, and 86.7% (26/30) of malignant tumors. The expression level of mesothelin was significantly higher in malignant tumors than in benign tumors ($p < 0.01$); no significant difference was observed between borderline and malignant tumors ($p > 0.05$).

Association of Ki-67, E-cadherin, and mesothelin expression in SBOTs (Table 3)

A significant positive correlation was found between Ki-67 and E-cadherin, Ki-67 and mesothelin, in SBOTs. Of the 19 with positive expression of Ki-67 that were examined, 18 exhibited E-cadherin ($p < 0.05$), 17 exhibited mesothelin expression. No significant positive correlation was observed between E-cadherin and mesothelin expression.

Discussion

SBOTs have low malignancy potential. Their histologic characteristics and biological behavior are intermediate between clearly benign and overtly malignant ovary tumors. Application of flow cytometry to borderline tumors has shown that aneuploid tumors are more likely to behave in a malignant manner [14]. The diagnosis of SBOTs relies on morphological features, such as complex papillary architecture, multilayered epithelium with tufting, no more than mild nuclear atypia, and modestly increased mitotic activity, but do not exert destructive stromal invasion [4]. In addition, SBOTs are apt to peritoneal implants and microinvasive, which are associated with an adverse prognosis. Thus, studying the biomarkers of growth, development, and prognosis of SBOTs is necessary for diagnosis and prediction of prognosis to guide the management of these patients.

Cell proliferation is an important aspect for tumorigenesis and progression. Ki-67 is covered in the proliferation cycle outside G0, which is a good indicator of the cell proliferation activity [15]. The present results show that Ki-67 in SBOTs is higher than in benign tumors, but lower than in malignant ovarian carcinomas. Significant difference was observed between borderline and benign tumors ($p < 0.05$) and carcinomas ($p < 0.01$). In serous cystadenocarcinoma, diffused distribution of Ki-67 positive expression was observed, indicating high cell proliferation activity, resulting in a series of malignant biological behavior. In SBOTs, the distribution of Ki-67 positive cells was focal,

located in the epithelial cells of papillary, indicating that high cell proliferation activity is not a characteristic of SBOTs. Compared with serous ovarian cystadenocarcinoma, SBOTs showed obvious characteristic of low proliferation index; hence, the clinical development process of SBOTs is inactive.

Given the different conclusions on the relationship of the expression and clinical prognosis of Ki-67, previous studies [16] showed that Ki-67 expression is not associated with recurrence and the clinical pathological characteristics, such as age and FIGO stage SBOTs. However, the present authors found a significant correlation between the positive expression rate of Ki-67 and FIGO stage in SBOTs ($p < 0.05$). They also found that SBOTs with high proliferation are apt to peritoneal implantation. In higher clinical stage of six cases with peritoneal implantation, all cases showed positive Ki-67 overexpression. This finding suggests that Ki-67 may facilitate SBOTs progression by promoting tumor growth. Hence, close follow up is essential for SBOTs patients with high Ki-67 expression.

E-cadherin, an important member of the calcium adhesion family and involved in cell adhesion of epithelial cells, is one of the important molecules for cellular connection. Ovarian tumor has unique growth and metastasis, wherein tumor cells fall off from the body of the tumor, and then into the ascites, which in turn is widely implanted in the peritoneum and omentum. Some studies have demonstrated that decreased expression or dysfunction of E-cadherin leads to tumor invasion and metastasis [17, 18].

The present experimental results suggest that the expression of E-cadherin in SBOTs showed significant difference with malignant cystadenocarcinoma, but not with benign cystadenoma, demonstrating that the lack of E-cadherin expression is associated with malignant progression of ovarian tumor. In six cases of SBOTs with peritoneal implantation, four cases showed positive expression. In one case with stromal microinvasion, E-cadherin presented negative expression, suggesting that the biological behavior of SBOTs tend to benign tumor even with extra-ovarian lesions. The present results showed that the expression of Ki-67 was positively correlated with that of E-cadherin. In addition, the expression levels of E-cadherin and Ki-67 in the four cases of SBOTs with peritoneal implantation were both positive, indicating that the intercellular adhesion was not significantly reduced, although the cell proliferation activity of SBOTs was high. On one hand, the SBOTs at the stage of transition from benign to malignant transformation are more inclined to benign tumor. On the other hand, the loss of E-cadherin may be a relatively late event in tumorigenesis, invasion, and metastasis of ovarian cancer [19].

The biological function of mesothelin has been extensively investigated. Other studies have noted a high mesothelin expression at both mRNA and protein levels in serous ovarian carcinoma samples [20]. Chang *et al.* first found that mesothelin is associated with cell adhesion; the cells transfected with mesothelin have stronger adhesion force than the control group [10]. Rump *et al.* think that mesothelin participates in regulating adhesion in ovarian epithelial carcinoma and mesothelial cell may play a key role in peritoneal metastasis of SBOTs [21]. Gilks *et al.* studied the gene expression pattern of SBOTs and serous carcinomas (S-Ca), and found that several genes that have been previously identified to be unregulated in ovarian carcinoma are expressed at higher levels in SBOTs. These genes include mucin-1, mesothelin, HE4, PAX-8, and apolipoprotein J [22]. In the present study, the expression of mesothelin was significantly higher in malignant tumors than in benign tumors ($p < 0.01$); no significant difference was observed between malignant and borderline tumors. The present finding is inconsistent with the expression at mRNA level reported in literatures, which may be attributed to the less number of specimens. However, Gilks *et al.* also mentioned that the mRNA expression level by hybridization may significantly differ from the protein expression level displayed by immunohistochemical analysis. The present results show that the positive expression rate of mesothelin increases in serous cystadenoma, SBOT, and serous cystadenocarcinoma; therefore, mesothelin plays an important role in the malignant transformation of serous ovarian tumors. The mesothelin expression was positively correlated with the Ki-67 overexpression; this result indicated that the high expression of mesothelin and Ki-67 may be related to malignant potential of SBOTs. In addition, CA125 is the main marker closely correlated with ovarian cancer [23]. Despite modest sensitivity and specificity, CA125 is implicated in the follow up of BOTs [24]. Abdominopelvic ultrasound and serum CA-125 assessment are also considered as the most appropriate follow-up methods in BOTs [25]. Mesothelin is also described as a novel CA125-binding protein, and CA125 may contribute to the metastasis of ovarian cancer to the peritoneum by initiating cell attachment to the mesothelial epithelium by binding to mesothelin [21]. The present authors found that the serum CA125 level of patients with SBOT was correlated with the positive mesothelin expression ($p < 0.01$); the combination of the two factors can help detect the malignant potential of SBOTs to some extent. However, they did not include the expression of CA125 protein in this study; hence, the role of mesothelin in the development of SBOTs must be continuously studied.

In summary, this study evaluated the expression of Ki-67, E-cadherin, and mesothelin in SBOTs. The results indicated that the behavior and prognosis of SBOTs were excellent. This research suggested that patients with overexpressed Ki-67 and mesothelin, and with low expression of E-cadherin should be subjected to follow up. The serum CA125 level and the mesothelin expression can also be an efficient tool to diagnose the invasive lesions of SBOTs. However, this study was limited by the relatively low number of cases. As such, further studies should be conducted to provide objective targets that can be used as a basis to di-

vide BOTs into benign and malignant groups, predict the corresponding prognosis, and implement appropriate clinical managements.

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