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Original Article

Immunohistological analysis of stress-induced phosphoprotein 1 in ovarian cancer patients with low serum cancer antigen 125 levels

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Abstract

Objective: Stress-induced phosphoprotein 1 (STIP1) was recently identified as a potential tumor marker for human ovarian cancer. This study further evaluates the usefulness of STIP1 in ovarian tumor patients with normal CA125 serum levels.
Materials and Methods: STIP1 and CA125 were immunohistochemically analyzed in 84 primary ovarian cancer and 30 benign ovarian tumors in patients with serum CA125 levels < 35 U/mL before surgery. Histoscores (0–300) were calculated as staining intensities (0–3) multiplied by percentage of tumor tissue (0–100%).
Results: The cell types of the 84 cancers included 11 serous, 10 clear-cell, 51 mucinous, and 12 endometrioid carcinomas. There were 55 patients with invasive cancer and 29 with borderline ovarian tumors. The histoscores of STIP1, but not of CA125, in invasive cancer (mean ± SD, 186.3 ± 82.5) were significantly ($p < 0.0001$) higher than those seen in borderline ovarian tumors (86.2 ± 85.5). When the STIP1 histoscore was set at 183.8, invasive cancers ($n = 55$) were identified from benign tumors ($n = 30$) with a sensitivity of 56.4%, a specificity of 93.3%, a positive predictive value of 93.9%, and a negative predictive value of 53.8%. Results of receiver operating characteristics analysis showed that the area under curve of the STIP1 histoscore was 0.755, which was superior to that of CA125 (0.599).
Conclusion: STIP1 histoscores may be useful in detecting invasive human ovarian cancer in patients with low serum CA125 levels.

Keywords: borderline tumor; cancer antigen 125; carcinoma; ovary; stress-induced phosphoprotein 1

Introduction

Epithelial ovarian cancers are the most lethal malignancies in women [1]. Early detection of ovarian cancer and successful treatment remain challenging for gynecologists. To identify genetic risk factors of ovarian cancer, genome-wise association study approaches [2] have been used, identifying several risk variants [3–5]. Gene expression variation caused by such genetic variants has been recently confirmed in some ovarian cancer risk alleles [6]. To improve therapeutic efficacy of advanced ovarian cancer [7], microarray analysis of gene expression [8] of cancer have been extensively used as a step toward personalized medicine.

Epithelial ovarian cancers are histologically divided into serous, mucinous, endometrioid, and clear-cell carcinoma. Usually, serous tumors are the most common [9], but an increased prevalence of clear-cell carcinoma has been reported in Japan and Taiwan [10,11] compared to that of western countries [12]. Clear-cell cancers are generally considered as endometriosis-associated ovarian cancer, where mutations of *ARID1A* have been identified [13]. During the treatment of

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ovarian cancer, the association with endometriosis often imposes additional diagnostic difficulties [14] and surgical challenges [15] on gynecologists.

Measurement of serum cancer antigen (CA)125 is a standard practice for the complete assessment of a pelvic mass [16]. Serum CA125 levels $\geq$ 35 U/mL are clinically considered abnormal. However, the sensitivity of CA125 for detecting epithelial ovarian cancer is only 85% using this criterion [17]. Elevated CA125 was found less frequently in patients with stage I disease, mucinous or borderline tumors [17–19], and premenopausal women [20]. Therefore, more proteins that may be used as complementary markers for CA125 in these situations are necessary.

Through a systemic search of biomarkers of human ovarian cancer by comparing proteomes between tumor interstitial fluid and normal interstitial fluid, we have identified stress-induced phosphoprotein 1 (STIP1) as a candidate tumor marker [21]. Our previous results showed that serum levels of STIP1 are significantly higher in patients with ovarian cancer than in age-matched healthy controls. Combined use of CA125 and STIP1 may increase early detection of ovarian cancer [21]. The potential of serum STIP1 for detecting human ovarian cancer was supported by an independent group [22]. Subsequently, we have reported that secreted form of STIP1 promotes proliferation of ovarian cancer cells via binding the cell membrane receptor ALK2 and activating the SMAD-ID3 signaling pathway [23]. Although we have recently reported that tissue levels of STIP1 can be used as a prognostic biomarker for the survivals of ovarian cancer patients [24], evaluation of the clinical use of STIP1 as a marker in patients with ovarian cancer is still incomplete. For instance, immunohistochemistry analysis of STIP in the patients with low CA125 serum levels (<35 U/mL) has not yet been reported.

In this study, we collected patients in our hospital with diagnosed ovarian cancer from 2000 to 2005. We aimed to compare the tissue expression of STIP1 versus CA125 in patients with ovarian cancer, focusing on the patients with preoperative CA125 levels < 35 U/mL.

Materials and methods

Patients

Clinical specimens for this study were obtained from two studies (Fig. 1). Specimens of Group A were from a prospective study, and detailed results of serum CA125 and STIP1 levels were previously published [21]. Group B was a retrospective study on consecutive patients between 2000 and 2005 in the tumor databank of Chang Gung Memorial Hospital. Thirty samples of patients with benign tumors served as controls. Exclusion criteria were: (1) patients who underwent neoadjuvant therapy before definite surgeries or who were referred from outside hospitals after initial surgeries; and (2) undifferentiated carcinomas that were arisen from teratomas.

Immunohistochemistry

Using procedures that were reported previously [21,23–25], archival formalin-fixed paraffin-embedded ovarian cancer slides of Chang Gung Memorial Hospital were

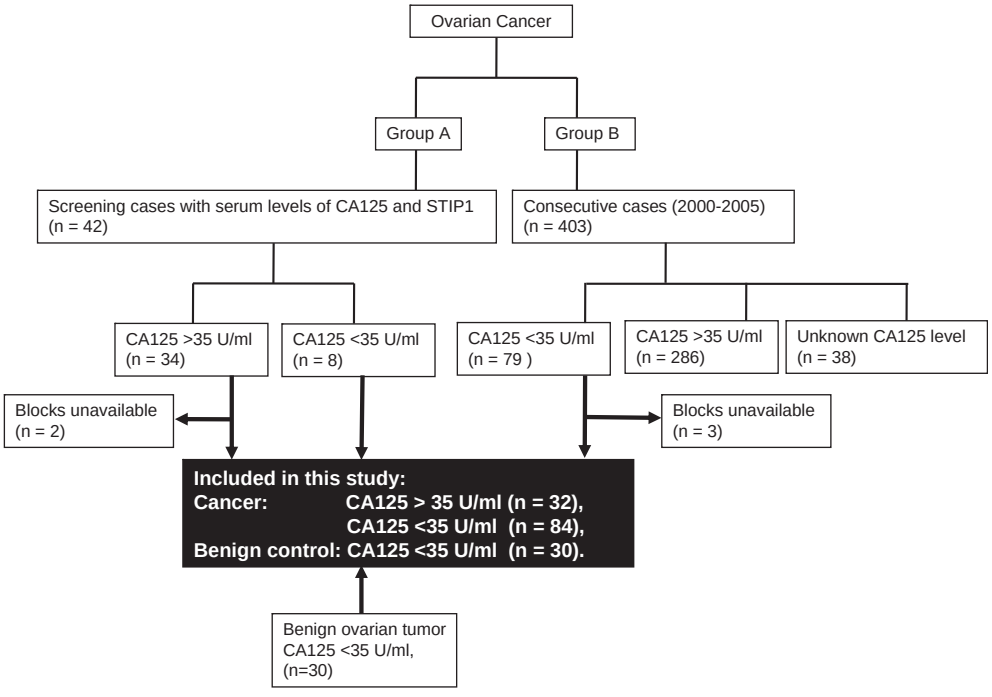

Fig. 1. Study design and analytical steps of this study. Note that two paraffin blocks in Group A and three paraffin blocks in Group B were unavailable for immunohistochemical studies.

analyzed. The slides of 4-μm thick sections were deparaffinized in xylene and rehydrated with a series of graded ethanol. Sections were then stained with a primary mouse anti-human STIP1 monoclonal antibody (1:1800; Abnova Corp., Taipei, Taiwan) or CA125 (SPM111, 1:400; Thermo Fisher, Rockford, IL, USA) using an automated IHC stainer with the Ventana Basic DAB (3,3-diaminobenzidine) Detection kit (Tucson, AZ, USA) according to the manufacturer's protocol. Counterstaining was performed with hematoxylin. The slides were examined independently by two pathologists (L.Y.L and C.H.) without knowing the clinicopathological information. The overall immunohistochemical score (histoscore) in this study was the percentage of positive cells multiplied by its staining intensity (0 = negative, 1 = weak, 2 = moderate, 3 = strong), and ranged from 0 to 300 (100% multiplied by 3) [23,24].

Statistical analysis

Differences in histoscore between two groups were compared using the Mann–Whitney *U* test, and the Kruskal–Wallis test was used if more than two groups were compared. Receiver operating characteristic curve analysis was used to provide sensitivities for the given specificity in the use of histoscores of STIP1 and CA125. Logistic regression was used to compare the risk factors of individual variables for invasive cancer. Cox's proportional hazards model was used in a multivariate analysis for the covariates selected from univariate analyses. Multivariate logistic regression was used to identify the significant risk factors for invasive ovarian cancer. The multivariate adjusted odds ratios and 95% confidence intervals were given. For all analyses, p values of <0.05 were considered statistically significant. The data were analyzed by the SPSS 17.0 statistical package.

Results

Characteristics of study population

A total of 116 patients with ovarian cancer were included in this study: 32 had serum CA125 levels > 35 U/mL, and 84 patients had serum CA125 < 35 U/mL (Fig. 1). In the group of ovarian cancer patients with CA125 levels < 35 U/mL, 92.9% (78/84) were stage I, 60.7% (51/84) were mucinous type, and 34.5% (29/84) were borderline ovarian tumors (BOT; Table 1). The noncancer control group ($n = 30$) consisted of mucinous cystadenoma ($n = 10$), serous cystadenoma ($n = 10$), dermoid cyst ($n = 5$), fibroma-thecoma ($n = 5$).

Immunohistochemical analysis of STIP1 and CA125 in ovarian cancer tissues

Overall, the histoscores of STIP1 in ovarian cancer patients were significantly higher than the histoscores of CA125. In the patients with serum CA-125 levels > 35 U/mL ($n = 32$), both the mean histoscores of STIP1 and CA125 were significantly higher than those of patients with serum CA-125 < 35 U/mL ($n = 84$; Table 2). However, stratifying the patients with normal serum CA-125 into invasive cancer and BOT pathological entities, the mean STIP1 histoscore of invasive cancer was significantly higher than BOT ($p < 0.0001$), but this was not seen with the CA125 histoscore ($p = 0.667$; Table 2). Differential distribution of histoscores between patients with invasive cancer and those with BOT is illustrated in Fig. 2. The mean STIP1 histoscore for the 30 benign samples was 103.6 ± 80.7.

Table 1
Characteristics of the ovarian cancer patients with normal serum CA125 ($n = 84$).

	N	*%*
Age Median (range)	44.4 (16–81)	NA
Mean ± SD	47.0 ± 15.92	NA
Stage		
I	78	92.9
II	2	2.4
III	4	4.8
IV	0	0
Histologic type		
Serous	11	13.1
Mucinous	51	60.7
Endometrioid	12	14.3
Clear	10	11.9
Grade[a]		
I	22	26.2
II	18	21.4
III	5	6.0
Borderline malignancy	29	34.5

NA = not applicable; SD = standard deviation
[a] Clear cell carcinoma and borderline malignancy are not graded.

STIP1 protein is a potential marker for detecting invasive ovarian cancer

To determine the usefulness of STIP1 as a histological marker for ovarian cancer, a receiver operating characteristic was used curve to analyze the histoscores of samples of ovarian cancer and benign ovarian tumors. The area under the curve (AUC) was used to compare the value of the two methods (STIP1 vs. CA125 histoscores) for discriminating cancer from benign tumors. The highest AUC (0.755) was achieved by using STIP1 histoscore to discriminate invasive cancer ($n = 55$) from benign tumors ($n = 30$). The AUC was decreased to 0.649 when the STIP1 histoscore was used to discriminate the total number of ovarian cancer (invasive plus

Table 2
Histoscores of STP1 and CA125 in epithelial ovarian cancer.

Serum CA125 (U/mL)	STIP1 histoscore[a]	*p*	CA125 histoscore[a]	*p*
≥35 ($n = 32$)	287.3 ± 20.6	<0.0001[b]	85.6 ± 67.1	<0.0001[b]
<35 ($n = 84$)	151.9 ± 95.9		27.4 ± 50.8	
Invasive ($n = 55$)	186.3 ± 82.5	<0.0001[b]	34.7 ± 59.2	0.667[b]
BOT[a] ($n = 29$)	86.2 ± 85.5		13.4 ± 24.3	

BOT = borderline ovarian tumor.
[a] Histoscore = percentage × intensity.
[b] Mann-Whitney *U* test.

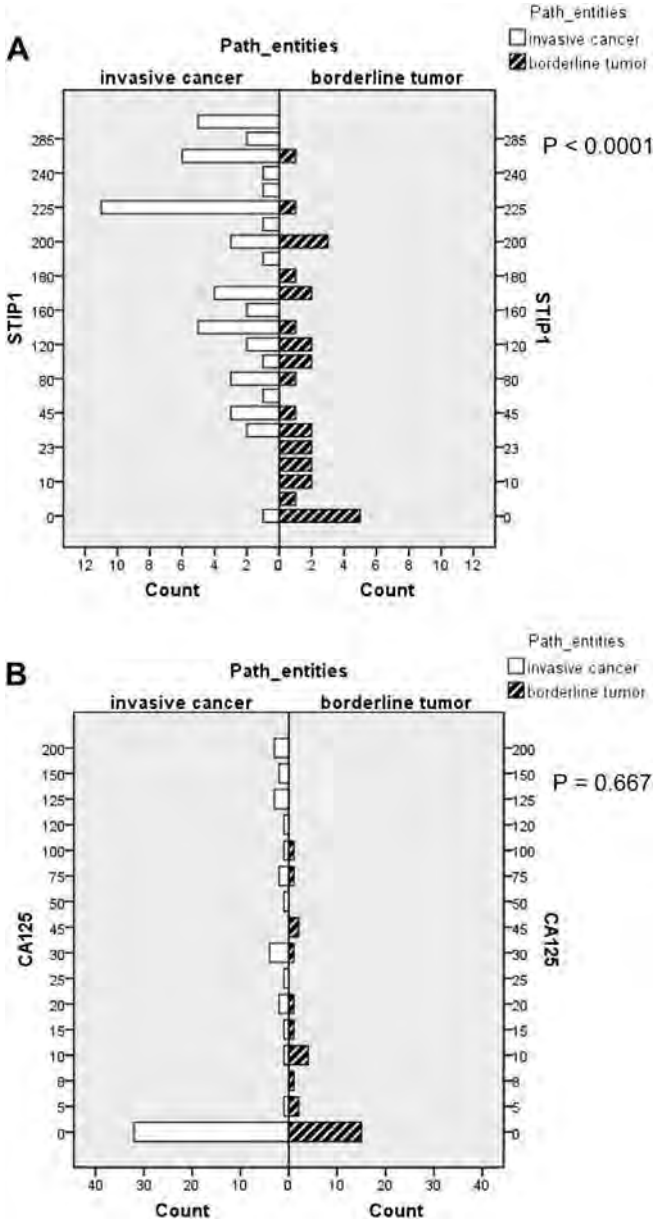

Fig. 2. Differential distribution of histoscores of (A) STIP1 and (B) CA125 between patients with invasive ovarian cancer ($n = 55$) and those with borderline tumor ($n = 29$). All of these patients ($n = 84$) had serum CA125 levels < 35 U/mL.

BOT, $n = 84$) from benign tumors ($n = 30$). The AUCs of CA125 histoscore were low, suggesting that it is of minimal use for these in distinguishing ovarian cancer from benign tumors (Table 3). In the 84 cases of ovarian cancer and 30 benign tumors, the histoscore that could result in the maximal sum of specificity and sensitivity was selected as the cutoff point. The cutoff points were 183.8 for STIP1 and 2.5 for CA125 (Table 3). Using these cutoff points, sensitivity, specificity, positive predictive value, and negative predictive value are summarized in Table 3.

Correlations between STIP1 histoscores and clinicopathological parameters

The correlations between STIP1 histoscores and clinicopathological parameters of 84 patients with normal CA125 are summarized in Table 4. Old age ($\geq$ 50 years), advanced stage, tumors with nonmucinous (serous carcinoma, clear-cell carcinoma, or endometrioid carcinoma), and invasive cancer entities were significantly associated with higher histoscores. High (II and III) grades of the tumors were marginally associated with higher histoscores (Table 4). Results of both univariate and multivariate analyses also indicated that high STIP1 histoscores were significantly associated with invasive cancers (Table 5).

Patient outcome

Of the 84 ovarian cancer patients with normal CA125, 14 patients had recurrences or persistent disease. Nine of them died of the disease (Table 6). All patients with stage IIIC disease succumbed to their disease. All except one had STIP1 that stained strongly (intensity $\geq$ 2). This particular patient (OV-316) had undergone laparoscopic surgery for a tumor that was suspected to be benign, but final pathology results revealed papillary serous ovarian cancer. She subsequently received staging laparotomy within one month but had a recurrence 1 year later.

Discussion

STIP1 (GeneID 10963; HPRD 05454) is a 62.6 kDa protein that has been found in melanoma [26], hepatocellular carcinoma [27], glioma [28], and pancreatic cancer [29]. STIP1

Table 3
Values of immunohistochemical analyses of STIP1 and CA125 in ovarian tumor patients with normal serum CA125.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC of ROC
STIP1 (cut-off value: 183.8)						
Invasive cancer and BOT ($n = 84$) vs. benign tumor ($n = 30$)	42.9	93.3	94.7	36.8	56.1	0.649
Invasive cancer only ($n = 55$) vs. benign tumor ($n = 30$)	56.4	93.3	93.9	53.8	69.4	0.755
CA125 (cut-off value: 2.5)						
Invasive cancer and BOT ($n = 84$) vs. benign tumor ($n = 30$)	44.0	73.3	82.2	31.9	51.8	0.596
Invasive cancer only ($n = 55$) vs. (benign tumor ($n = 30$)	41.8	73.3	74.2	40.7	54.9	0.599

AUC = area under curve; BOT = borderline ovarian tumor; NPV = negative predictive value; PPV = positive predictive value; ROC = receiver operating characteristics.

Table 4
Clinicopathological characteristics and STIP1 histoscore in ovarian cancer patients with normal serum CA125.

Characteristics	Patient ($n = 84$)	STIP1 score	p
Age			
<50	47 (56.0%)	124.6 ± 95.4	0.002[b]
≥50	37 (44.0%)	186.3 ± 85.9	
Stage			
I	78 (92.9%)	145.7 ± 95.6	0.017[b]
≥II	6 (7.1%)	230.0 ± 61.2	
Histologic type			
Mucinous	51 (60.7%)	114.5 ± 88.6	<0.0001[c]
Serous	11 (13.1%)	191.8 ± 97.2	
Clear cell + endometrioid	22 (26.2%)	218.2 ± 65.3	
Grade[a]			
I	22 (48.9%)	151.6 ± 86.4	0.049[b]
II and III	23 (51.1%)	199.5 ± 71.7	
BOT vs Invasive cancer			
BOT	29 (34.5%)	86.2 ± 85.5	<0.0001[b]
Invasive cancer	55 (65.5 %)	186.3 ± 82.5	

BOT = borderline ovarian tumor.
[a] Clear cell carcinoma and borderline malignancy are not graded.
[b] Mann-Whitney U test.
[c] Kruskal-Wallis test.

protein contains nine tetratricopeptide repeats motifs clustering into three domains, which form scaffolds that mediate formation of different protein complexes. Thus STIP1 participates in various biological processes, including RNA splicing, transcription, protein folding, signal transduction, and cell cycle regulation [30,31]. As a phosphoprotein, STIP1 is phosphorylated by Cdc2 kinase, which is accompanied by cytoplasmic translocation of STIP1 [31]. The presence of STIP1 in various types of cancer suggests that STIP1 has antiapoptotic or progrowth nature, or both. Knockdown of STIP1 was shown to suppress the invasiveness of pancreatic cancer cells [29]. A glioblastoma cell line has been shown to secrete STIP1 into culture medium, and recombinant STIP1 can induce the proliferation of glioma cells by activating the ERK and PI3K pathways [28]. We also previously showed that STIP1 secreted by ovarian cancer cells promotes cell proliferation [21] and that cancer STIP1 levels can be used as a prognostic biomarker for ovarian cancer patients [23]. Herein we demonstrate the usefulness of STIP1 histoscores in ovarian cancer patients with serum CA125 levels < 35 U/mL.

Table 6
Ovarian cancer patients with CA125 less than 35 U/ml who died of the disease ($n = 9$).

Patient	Cell type	Stage	Grade	Relapse	Age at diagnosis	Histoscore	
						STIP1	CA125
OV-0563	Endometrioid	IIIC	2	Persistent	63	225	0
OV-0181	Clear cell	IIIC		Persistent	52	270	0
OV-0137	Endometrioid	IC	2	Relapse	53	150	25
OV-0049	Clear cell	IIC		Persistent	56	225	0
OV-0439	Mucinous	IA	1	Relapse	34	270	0
OV-0316	Papillary serous	IC	1	Relapse	30	30	200
OV-0542	Papillary serous	IIIC	3	Persistent	66	240	200
OV-0614	Papillary serous	IIIC	3	Persistent	44	300	0
OV-0328	Mucinous	IC	2	Persistent	80	225	0

Of note, STIP1 histoscores were highest in the clear-cell and endometrioid cancer groups (Table 4). Clear-cell tumor is a distinct histological type of epithelial ovarian cancer, which is frequently diagnosed at early stages but often recur even after primary chemotherapy [32]. According to Cancer Registry of the Department of Health in Taiwan [10], the prevalence of histological types of ovarian cancer are different from that seen in western countries. In Taiwan, serous carcinomas remained the most common cell type, and the percentages of serous, mucinous, endometrioid, clear-cell, and undifferentiated was 34.5%, 16.5%, 14.8%, 14.5%, and 19.6%, respectively. Notably, the incidence of clear-cell carcinomas in Taiwan (14.5%) was higher than the 5–10% seen in Western countries, but similar to that in Japan, which was 20–25% [12]. In the consecutive 403 cases in our institution between 2000 and 2005 (Group B of Fig. 1), 21.6% (79/365) of cases had serum CA125 < 35 U/mL. Of the 79 cases with CA125 < 35 U/mL, one-third (23 cases) were found to be clear-cell and endometrioid carcinoma. A biomarker to complement CA125 is urgently needed for this group of patients.

Table 5
Univariate and multivariate analyses regarding detection of invasive ovarian cancer from borderline ovarian tumor.

Characteristics	Univariate analysis					Multivariate analysis		
	BOT	IC	OR	95% CI	p	OR	95% CI	p
Age								
<50	18	29	1		0.413			
≥50	11	26	1.47	0.59–3.67				
Stage								
I	29	49	—	—	—			
≥II	0	6						
Histologic type								
Mucinous	28	23	1		0.001	1	3.53–229.7	0.002
Others	1	32	38.96	4.94–307.30		28.47		
STIP1 histoscore								
<183.8	24	24	1		0.001	1	1.0–12.0	0.049
≥183.8	5	31	6.20	2.06–18.65		3.48		

BOT = borderline ovarian tumor; CI = confidence interval; IC = invasive cancer; OR = odds ratio.

Clear-cell and endometrioid carcinomas have been linked to endometriosis [9], but the molecular pathology of clear-cell carcinoma remains unclear. Endometrioid carcinomas are reported to be characterized by K-RAS activation and PTEN dysfunction [33]. On the contrary, tumorigenesis of clear-cell carcinoma is heterogeneous, involving loss of heterozygosity [34], dysfunctions of signaling pathways of early mitotic inhibitor-1 [35] and mutations in mammalian target of rapamycin [36] and K-RAS mutation [37]. Clinically, about 60% of patients with stage I clear-cell carcinomas show resistance to chemotherapy [38]. Based on our previous findings that treatment of ovarian cancer cells with STIP1 significantly induces ERK phosphorylation, promotes DNA synthesis, and increases Ki-67 immunoreactivity in ovarian cancer cells [21], we speculate that STIP1 may be involved in development of chemotherapy resistance by clear-cell carcinomas.

CA125 is a useful serum marker that is elevated in 85% of nonmucinous epithelial ovarian cancers [39]. We have had a similar detection rate (79.4%) of epithelial ovarian cancer in our cohort (Fig. 1). Although the lack of elevation in serum CA125 is more common in mucinous and borderline ovarian tumors [17–19], serum levels of CA125 usually correlate with the size of residual cancer after debulking surgery [40]. In the small portion of invasive cancers with normal CA125 serum levels, elevated STIP1 histoscores resulted in a 69.4% accuracy rate of cancer detection (Table 3). Because there were only 42 cases with known serum levels of both CA125 and STIP1 in this study (Fig. 1), correlation of cancer expression of STIP1 and serum STIP1 in patients with ovarian cancer is yet to be confirmed by further studies with larger sample sizes.

In conclusion, this is the first immunohistochemical study on tissue expression of STIP1 in ovarian cancer patients with normal serum CA125 levels. Since Taiwan and Asia have an unexplained higher incidence of clear-cell carcinoma, the potential use of STIP1 in nonmucinous tumors, including clear-cell carcinoma and endometrioid carcinoma, warrants further investigation.

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