The role of actin bundling protein fascin in the progression of ovarian neoplasms

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Summary

Purpose of investigation: The aim of the study was to investigate the role of fascin in tumor progression and to investigate the role of fascin on endothelial cell migration and angiogenesis in ovarian neoplasms.

Methods: In the study, 94 malign epithelial ovarian neoplasms, 13 borderline epithelial ovarian neoplasms, 25 serous and mucinous cystadenomas and four normal ovarian tissues were examined by means of immunohistochemistry, using monoclonal antihuman fascin antibody, clone IM20.

Results: Total stromal fascin score in cases of borderline and malign epithelial ovarian tumors was significantly higher compared to normal ovaries and benign epithelial ovarian tumors (.000, $p < 0.001$). There was no statistically significant difference in terms of total epithelial fascin scores of samples between groups (.080, $p > 0.05$). Presence of vascular invasion (.000, $p < 0.001$), psammomatous calcifications (.001, $p = 0.001$), and lymphocytic infiltration (.000, $p < 0.001$) were significantly higher in malign neoplasms. There was no significant difference in terms of mean microvessel count and homogeneous or heterogeneous fascin expression of microvessels between the benign and malign groups (respectively $p = .228$ and $p = .143$).

Conclusions: This study suggests that up-regulation of fascin in tumoral tissue may promote invasion of ovarian carcinoma by cell-matrix adhesion.

Key words: Fascin; Immunohistochemistry; Cystadenoma; Epithelial ovarian neoplasms; Angiogenesis.

Introduction

Epithelial ovarian carcinoma accounts for 80% to 90% of all ovarian malignancies. It is the most lethal of all gynecologic cancers. Many patients with ovarian cancer come to medical attention with advanced stage of disease [1] . The development of malignant neoplasms is a complex process involving the ability of tumor cells to overcome cell-cell and cell-matrix adhesion and to invade surrounding tissue. Tumor invasion and metastasis are controlled by degradation of the extracellular matrix components. Recently, the role of matrix proteinases and tissue metalloprotease inhibitors in ovarian carcinoma progression was investigated. Several growth factors and various mediators such as extracellular matrix metalloproteinase inducer (EMMPRIN) with a molecular weight of 58 kDa, secreted by the microenvironment of the tumor cells and surrounding fibroblasts, induce production of proteinases and this results in the degradation of extracellular matrix [2-4]. In living cells, actin filaments are involved in cell locomotion, cytokinesis, vesicular and organelle transport, cell-cell and cell-matrix interactions, and the establishment and maintenance of cell morphology. Actin regulatory proteins provide mechanical support to cellular protrusions and stress fibers. The bundling of actin filaments in many cells is controlled by several different actin binding and bundling proteins. Fascin, one of the actin bundling proteins with a molecular weight of 55-58 kDa, crosslinks actin filaments via two discrete F-actin-binding sites into tight bundles. β-catenin, the cytoplasmic partner of E-cadherin, links cadherins to α-catenin and the actin cytoskeleton and mediates strong cell-cell adhesion in the adherence junction [5-8]. Polarity of nucleus and peripheral actin cytoskeleton alterations are fundamental changes in tumor cells which involve cell morphology, cell locomotion, cell-cell adhesion and cell-matrix interactions. Recently, the role of actin bundling protein fascin in tumor progression has been shown in malignant epithelial tumors including ovarian carcinoma [8-10].

In this study, the first objective was to investigate the role of fascin in tumor progression and invasiveness and the second objective was to assess the role of fascin in endothelial cell migration and angiogenesis in epithelial ovarian neoplasms.

Materials and Methods

Tissue samples of 94 malign epithelial ovarian neoplasms, 13 borderline epithelial neoplasms, 25 serous and mucinous cystadenomas and four normal ovarian tissues obtained between 1990 and 2000 were retrieved from the files of the Pathology

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Department. All samples were surgically staged according to International Federation of Gynecology and Obstetrics (FIGO) criteria. Patient characteristics and clinicopathologic findings were obtained from hospital records. All tumors were microscopically reevaluated for grade, presence or absence of vascular invasion, calcification and local cellular immune response. Paraffin sections which were selected from blocks of the most representative areas of invasive tumor were included in the study. The paraffin blocks were cut 4 μm and immunohistochemical assays were performed for the expression of fascin using liquid mouse monoclonal antihuman fascin antibody, (Novocastra, NCL-L-FASCIN, USA). Tissue sections were deparaffinized in xylene, rehydrated in alcohol solutions, and placed in 0.5% hydrogen peroxide in methanol for 10 min to block the endogenous peroxidase activity. Rehydration was completed by placing them in absolute alcohol and finally in water. The slides were treated with a boiling solution of freshly prepared 0.05 M-citrate buffer, pH 6.0 for 5 min in a pressure cooker. The sections were reacted overnight with the primary antibodies at a dilution of 1:200 in buffer. They were rinsed in phosphate buffered saline (PBS) before being treated with biotinylated universal secondary antibody for 10 min. After further rinsing, the slides were treated with avidin-biotin-peroxidase complex (Novocastra, Novostain Universal Detection Kit) and rinsed again. Immunostaining was accomplished by incubating them in 3amino-9-ethylcarbozole (AEC) for 7 min and then the slides were rinsed in distilled water and counterstained with Mayer's hematoxylin. Sections of human tonsil were used as positive controls. Capillary endothelium was also used as an endogenous positive control. As a negative control, the primary antibody was replaced by PBS.

The epithelial fascin score for each tumor specimen was the sum of the percentage score and the intensity score, and was defined as 'total epithelial fascin score'. Tissue samples were divided into three groups according to percentage of stained cells; ≤ 10% as 1, 11-50% as 2 and 51-100% as 3. The intensity of immunostaining was scored on a three point scale: 1 = weak; 2 = moderate; 3 = intense. Stromal staining was scored on a three point scale: focal weak staining as 1; regional moderate staining as 2; diffuse and moderate to intense staining as 3. The role of fascin in endothelial cell migration and angiogenesis was investigated by counting intratumoral microvessels in areas with the highest vascularity. The microvessel count was evaluated with a six inch monitor of a digital camera system (Nikon Digital Sight, DS-5M-L1) on each selected area at a X200 field (X20 objective and X10 ocular). The frame area for each captured image was the same. From the peripheral part of each tumor an approximately equal area of tumor and stroma was selected. The homogeneous or heterogeneous labelling pattern of fascin in microvessels was assessed in benign, borderline and invasive epithelial ovarian tumors.

All statistical analyses were performed using SPSS (Statistical Package of Social Services, Chicago, IL, USA) for Windows version 11.5. Data were analyzed according to the Kruskal Wallis test, Pearson chi-square test and Fisher's exact chi-square test, Exact Pearson chi square test, Pearson correlation test, t test and ANOVA. Probability values less than 0.05 were considered statistically significant.

Results

Mean ages of patients with normal ovaries, cystadenomas, borderline ovarian tumors and malign ovarian tumors were 46, 47, 35 and 55, respectively. Of the 94 malign epithelial ovarian carcinoma patients, 18 were early (Stage I-II) and 76 were late (Stage III-IV) stages. Table 1 shows the distribution of stages, clinical and histopathologic features of biopsies after pathologic review.

Table 1. — *Stage and histologic distribution of carcinomas, borderline tumors and cystadenomas.*

Variable	Carcinomas n = 94	Borderline tumors n = 13	Cystadenomas n = 25
Stage			
IA	6	7	
IB	4	3	
IC	4	2	
IIA	2		
IIC	2		
IIIA	5		
IIIB	1	1	
IIIC	57		
IV	11		
Unstaged	2		
Grade			
1	23		
2	58		
3	13		
Histologic subtype			
Serous	64	4	11
Endometrioid	15	1	
Clear cell	4		
Mucinous	4	8	14
Malignant Brenner	2		
Mixed	5		

Fascin immunohistochemistry results

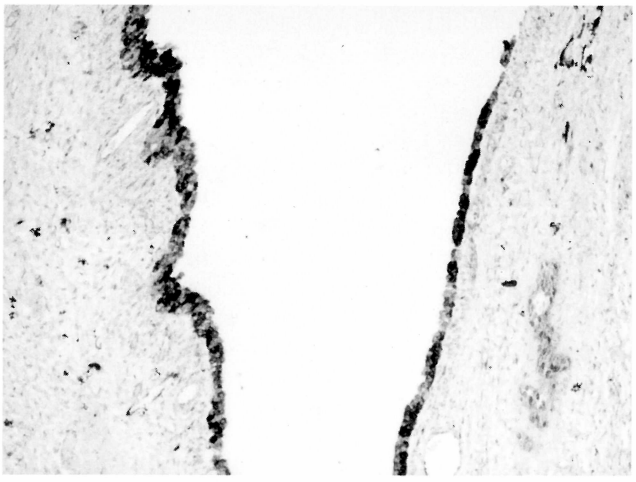
Normal ovarian tissue

Moderate fascin staining in normal ovaries was marked in every developmental stage of follicular epithelium and corpus luteum (Figure 1). Cortical stromal tissue was stained with fascin in focal areas. Microvessels stained homogeneously and mean microvessel count was 40.75 ± 5.92 in the peripheral portion of the follicules. Microvessel count ranged from 26 to 53.

Cystadenoma

Epithelium of mucinous cystadenomas was not stained with fascin. Stromal fibroblasts stained weak to moderate intensity in focal areas in all samples. Microvessels stained homogeneously in 13 of 14 cases. Mean microvessel count was 68.63 ± 17.60. Weak epithelial fascin staining score ranging from two to three was observed in five of 11 serous cystadenomas. Stromal fibroblasts stained weak to intense in ten of 11 samples. Microvessels stained homogeneously in all cases. Mean microvessel count was 48.66 ± 7.69.

Microvessel count ranged from 14 to188. Mean microvessel count for all cystadenomas of the ovary was 54.32 ± 8.75.



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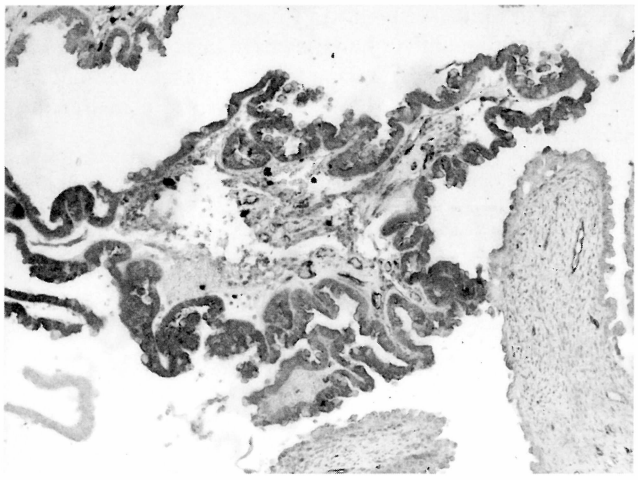
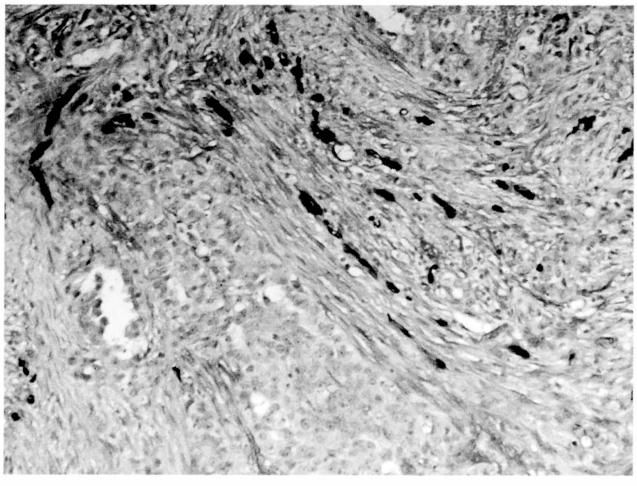


Fig. 2



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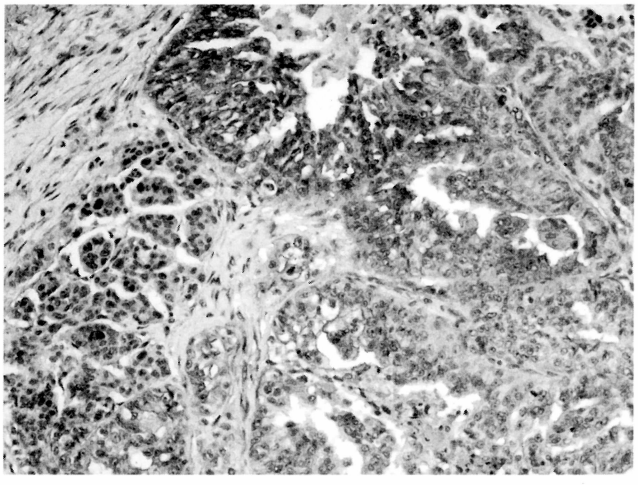

Fig. 4

Figure 1. — Follicular cyst epithelium stained homogeneously with fascin (fascin immunohistochemistry x 200).
Figure 2. — Atypically proliferated serous tumor. Epithelium stained for fascin (fascin immunohistochemistry x 200).
Figure 3. — Moderately differentiated serous carcinoma. Dense fascin staining of stroma (fascin immunohistochemistry x 200).
Figure 4. — Epithelial fascin staining in a moderately differentiated serous ovarian carcinoma (fascin immunohistochemistry x 400).

Borderline epithelial ovarian neoplasms

In mucinous borderline tumors total epithelial fascin score was two in five of eight samples. Fascin score of stromal fibroblasts ranged from one to three in all samples. Microvessels stained homogenously in seven of eight samples. Epithelium of serous borderline tumors was stained with fascin in three of four samples with the score of 2 to 6 (Figure 2). Fascin score of stromal fibroblast ranged from one to three in four samples. Microvessels stained homogeneously in two of four samples. Epithelium and stromal fibroblasts stained weakly in one sample of endometrioid borderline tumor. Microvessel count ranged from 27 to165 for borderline tumors of the ovary. Microvessels stained homogeneously and mean microvessel count was 57.84 ± 11.30.

Malignant ovarian carcinoma

Fascin staining in stroma was observed in 92 of 94 samples (Figure 3). The stromal fascin scores ranged from 1 to 3. The epithelial portion of the tumor was stained with fascin in 61 (64%) of 94 samples (Figure 4). The epithelial fascin scores ranged from 2 to 5. Serous carcinomas containing dense psammomatous classifications did not show any epithelial or stromal fascin staining. The areas of squamous metaplasia in endometrioid carcinomas showed intense fascin labelling. Microvessel count ranged from 10 to 140. Mean microvessel count was 40.73 ± 4.29.

Intensity of local cellular immune response was weak in 17 (65%) cystadenomas, in 11 (84%) borderline ovarian tumors and in 57 (60%) ovarian carcinomas. Moderate to intense local cellular immune response was observed in one (4%) cystadenoma, in two (15%) borderline ovarian tumors and in 35 (37%) malign ovarian tumors.

Vascular invasion was observed in 81 (86%) of 94 malign epitelial ovarian tumors. Psammomatous calcifications were observed in three (12%) of 25 cystadenomas, in three (23%) of 13 borderline tumors and in 43 (45%) of 94 malign ovarian tumors.

Comparison of clinical and pathologic findings and scores in malignant epithelial ovarian tumors are given in Table 2. Table 3 shows a comparison of fascin immunoreactivities and Table 4 shows angiogenesis in malignant, borderline tumors and cystadenomas.

Table 2. — *Comparison of clinical and pathologic findings and scores in malignant epithelial ovarian tumors.*

Carcinoma	Variable			p	r	Test
Epithelial fascin scores	Grade	n	Mean rank	.966		Kruskal
(range 0 to 6)	1	23	46.48			Wallis
	2	58	46.15			
	3	10	44.05			
Disease-free survival		n	Mean months	.206		t test
Lymphovascular	absence	11	38.36 ± 9.91			
space involvement	presence	57	27.96 ± 3.04			
Overall survival		DOD*	Alive	.646		Fisher's exact
Lymphovascular space involvement	absence	3	4			chi square
	presence	6	15			
Stage	Epithelial fascin scores			.462	.078	
Pearson correlation	Disease-free survival			.067	-.225	
	Overall survival			.321	-.195	

*DOD, died of disease.

Table 3. — *Comparison of fascin immunoreactivity in malign epithelial ovarian tumors and borderline tumors with normal ovarian tissue samples and cystadenomas.*

	Variable				p	Test
Stromal fascin scores		n		Mean rank	.000**	Kruskal
(range 0 to 3)	Benign*	29		39.81		Wallis
	Borderline	13		51.38		
	Malign	94		79.72		
Epithelial fascin scores		n		Mean rank	.080	Kruskal
(range 0 to 6)	Benign*	29		54.84		Wallis
	Borderline	13		67.81		
	Malign	94		72.81		
Local cellular immune		Absence	Mild	≤ Moderate	.000**	Pearson
response	Benign*	11	17	1		chi square
	Borderline	0	11	2		
	Malign	2	57	35		
Calcification		Absence	Presence		.001**	Pearson chi square
	Benign*	26	3			
	Borderline	10	3			
	Malign	51	43			
Lympho-vascular space		Absence	Presence		.000**	Pearson
involvement	Benign*	29	0			chi square
	Borderline	12	1			
	Malign	14	80			

*Benign group consisted of 25 cystadenoma samples and 4 normal ovarian tissue samples.
**Statistically significant.

Table 4. — *Comparison of fascin staining of microvessels and angiogenesis malign epithelial ovarian tumors, borderline tumors and cystadenomas.*

	Variable				p	Test
Fascin staining of microvessel		Benign*	Borderline	Malign	.143	Exact Pearson
endothelium	Homogeneous	28	10	79		
	Heterogeneous	1	3	12		
Mean micro-	Benign*	52.44 ± 7.61			.228	ANOVA
vessel count	Borderline	57.84 ± 11.30				
	Malign	40.73 ± 4.29				
Overall survival		Mean microvessel count			.717	t test
	DOD* (n = 8)	43.50 ± 8.95				
	Alive (n = 19)	39.73 ± 4.68				

*DOD, died of disease.

Total epithelial fascin scores were not significantly different in the groups ($p = .080$, $p > 0.05$). Stromal fascin scores of invasive and borderline epithelial ovarian tumors were significantly higher than the benign group (.000, $p < 0.001$). Presence of psammomatous calcifications ($p = 0.001$), local cellular immune response (.000, $p < 0.001$) and vascular invasion (.000, $p < 0.001$) were significantly high in malign neoplasms. There was no significant difference between total epithelial fascin scores and grade in malignant ovarian tumors (.966, $p > 0.05$). Presence or absence of lymphatic vessel invasion was not associated with disease-free survival (.206, $p > 0.05$) and overall survival (.646, $p > 0.05$). There was no significant correlation between the stage and epithelial fascin scores ($r = .078$, $p = .462$), stage and disease-free survival ($r = -.225$, $p = .067$) and overall survival ($r = -.195$, $p = .321$). Mean microvessel count of normal ovaries, cystadenomas, borderline tumors, and invasive tumors were 40.75 + 5.92, 54.32 + 8.75, 57.84 + 11.30 and 40.73 + 4.29, respectively. There were no significant differences in terms of mean microvessel count (.228, $p > 0.05$), and homogeneous or heterogeneous fascin staining of microvessels (.143, $p > 0.05$) between the groups. The length of follow-up ranged from one month to 105 (mean = 30.81 ± 3.01) months.

Discussion

Fascin is expressed at high levels in specialized normal cells such as neural cells, antigen-presenting dendritic cells, endothelial cells, squamous epithelium and many transformed cells. Fascin is localized in membrane ruffles, microspikes, stress fibers and lamellipodia and its overexpression in epithelial cells induces membrane protrusions and other motility-associated structures. Fascin containing actin bundles provide mechanical support in adherence junctions of epithelial cells [5-7]. Down-regulation or up-regulation of fascin expression is demonstrated in several types of human neoplasms, such as skin tumors including melanomas, ovarian neoplasms, breast cancers, colon carcinomas, pancreas carcinomas, lung carcinomas, anaplastic large cell lymphomas and Hodgkin's disease. These studies supported the role of fascin in generating and maintaining tumorigenic phenotype [8-16]. In studies, fascin expression in breast cancer was restricted to high grade tumors. The up-regulation of fascin in estrogen and progesterone receptor-negative breast cancers suggested that fascin may have a fundamental role in acquisition of malignant tumorigenic phenotype [11, 12]. In this study we observed various degrees of epithelial fascin staining in 20% of cystadenomas, 62% of borderline epithelial tumors and 64% of invasive epithelial ovarian tumors, and epithelial fascin staining was not associated with grade, stage of tumor or survival in malignant epithelial ovarian tumors. These results can be explained in part by the difference in etiologic factors and involved pathogenetic pathways in ovarian carcinoma compared with breast cancers. Hu *et al.* [9], demonstrated increased fascin expression in tissue

samples and cell cultures derived from ovarian cancer and in tissues of borderline and carcinomatous ovarian neoplasms, and suggested that fascin could serve as an important prognostic factor for abnormal ovarian epithelial pathology. In the study negatively stained ovarian surface epithelium was used as a normal control group. In our study we observed that moderate fascin staining in normal ovaries was marked in every developmental stage of follicular epithelium, corpus luteum and cortical stromal tissue stained with fascin in focal areas whereas ovarian surface epithelium never expressed fascin. Because hyperplastic lesions are rarely observed in the ovary, we used normal ovaries and cystadenomas as control groups in this study. Comparison of malignant epithelial ovarian neoplasms with cystadenomas and normal ovaries demonstrated that scores of stromal fascin expression, presence or absence of local cellular immune response, vascular invasion and psammomatous calcifications were significantly higher in malignant epithelial ovarian tumors.

Overexpression of fascin in neoplastic epithelium and tumoral stroma are closely associated with invasive phenotype [8, 11]. It was demonstrated that β-catenin is associated with fascin in a non-cadherin complex in various tumors such as cervix carcinoma [17, 18]. In ovarian carcinoma progression the TCF/β-catenin pathway is rarely activated [19]. Actin filaments are directly involved in cell motion, cell shape and cell polarity. Up-regulation or down-regulation of fascin is associated with rearrangement of metabolic, motogenic, mitogenic and morphogenic functions of tumor cells which are regulated by various cytokins and growth factors. Recently, some of the regulatory mechanisms of fascin have been supported by the following studies. Wong [20] and Guan *et al.* [21] demonstrated that glucocorticoids down-regulate fascin and TGF-α reverses the steroid induced down-regulation of fascin and abrogates the glucocorticoid stimulation of tight junction formation in rat mammary epithelial tumor cells. Jawhari *et al.* [10] showed that in colonic carcinoma cell-lines up-regulation of fascin is associated with aggregation of growing cells which results in glandular differentiation as manifested by polarization of nuclei toward the basal surface of cells and the organization of cells around a central lumen. Adams *et al.* [22] demonstrated that within the extracellular matrix, syndecan-1 promotes cell spreading and formation of fascin spikes by adhesive glycoprotein trombospondin-1. Recently, Maitra *et al.* [13] investigated fascin and heat-shock protein 47 (HSP47) expression in pancreatic ductal adenocarcinoma. The authors demonstrated that 95% of pancreatic ductal adenocarcinomas express fascin in neoplastic epithelium and 65% of them also express HSP47 in the neoplastic cells, whereas desmoplastic stroma labelled intensely with HSP47 in all samples. Goncharuk *et al.* [8] demonstrated that local aggressive and rarely metastatic skin cancers such as basal cell carcinoma were associated with diffuse and intense fascin immunoreactivity, whereas those cancers with risk of metastasis such as melanoma were associated with weak or down-regulated fascin expression. Shonukan *et al.* [23] demonstrated that there were high levels of neurotrophin expression in the normal tissue adjacent to brain metastases of melanoma. It was suggested that interaction between fascin and neurotrophin provides a direct link between the NGF signaling pathway and neurotrophin-mediated melanoma cell movement by down-regulation (dephosphorilation) of fascin. The TNF/NGF pathway is associated with alterations in the regulation of apoptosis and resistance of tumor cells to therapy [24-27]. These studies and our data suggested that up-regulation of fascin in tumoral tissue may promote invasion of ovarian carcinoma by cell-matrix adhesion.

The second aim of the study was to investigate the role of fascin in endothelial cell migration in benign and malign ovarian tumors. Neovascularization is an important factor in ovarian carcinoma progression [28, 29]. We used fascin immunohistochemistry to investigate angiogenenesis which is normally homogeneously stained by fascin. Since both cystadenomas and malignant ovarian tumors have a wide range of microvessel count, we could not find any significant difference in terms of microvessel count. Down-regulation of fascin in microvessel endothelium also was not significantly different between benign and malign epithelial ovarian tumors.

Our study reveals that the mean-rank value of stromal fascin score is smallest in benign tumors and increases with the degree of malignancy ($p < 0.001$). This result emphasizes the importance of actin bundling protein fascin in the progression of ovarian neoplasms. We could not find a significant difference in microvessel count which may be related to the wide range of vascular count distribution in ovarian neoplasms and also to the limited number of our cases. Further studies are necessary in larger series to reveal the prognostic importance of microvessel count and regulatory molecular mechanisms of fascin in ovarian carcinoma.

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