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Expression of Actin-bundling Protein Fascin and its Relationship with Altered E-cadherin and β-catenin Expressions in Ovarian Serous Neoplasms

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Background : Fascin, an actin-bundling protein, has been found in specialized normal cells, including the neuronal, endothelial and dendritic cells, and its expression is known to be greatly increased in various human neoplasms. **Methods :** Immunohistochemical stainings for fascin, β-catenin, and E-cadherin were performed in normal ovary tissue (n=13), and in benign (n=14), borderline (n=32), and malignant (n=74) ovarian serous neoplasms. We evaluated the fascin expression, and its relationship with the β-catenin and E-cadherin expressions, as well as the clinicopathologic factors. **Results :** Fascin expression was detected in the majority of the borderline (100%, 32/32) and malignant tumors (90.5%, 67/74), but it was not seen in the normal ovarian surface epithelial cells and the benign tumors ($p<0.001$). Fascin expression was significantly correlated with the occurrence of peritoneal metastases in the carcinomas ($p=0.043$). A significant relationship between the expressions of fascin and β-catenin ($p=0.046$), as well as E-cadherin ($p=0.035$) was noted. There was no significant correlation with the tumor grade of carcinoma, the FIGO stage, tumor recurrence, tumor-related death and the survival rate. **Conclusions :** In ovarian serous neoplasms, the fascin expression may be closely linked with tumor progression and metastasis, and it was associated with the up-regulation of β-catenin and E-cadherin.

Key Words : Ovary, Serous neoplasms; Fascin; Metastasis

Ovarian carcinoma is one of the leading causes of cancer-related deaths from gynecological cancer and it is the 7th most common cancer worldwide.[1] Because epithelial ovarian cancer (EOC) lacks any specific early disease symptoms, more than two thirds of these patients present with an advanced stage of disease, and the prognosis is extremely poor. Despite that these patients are frequently diagnosed at an advanced stage, dissemination of their disease is often confined to the peritoneal cavity,[2] and cancer spreading outside the abdomen is a rare and late event. Arriving at a better understanding of the biology of the peritoneal dissemination for serous neoplasms, the most prevalent tumor of all the EOCs (60% of all EOCs), can be helpful to further investigate the clinical potential of cancers and for finding new and effective therapeutic strategies.

Invasive tumor cells are characterized by their acquisition of motility and the loss of cell-cell adhesion, and this has been associated with several types of actin cross-linking proteins. Among these molecules, fascin is 55-kDa actin-bundling protein that plays an important role in the organization of several types of actin-based structures such as filopodia, spikes, lamellipodial ribs, dendrites and microvilli, and its overexpression in epithelial cells induces membrane protrusions and it also increases cell motility.[3] Fascin is normally expressed at high levels in specialized normal cells such as neuronal, endothelial, and antigen-presenting dendritic cells,[4-6] and in many transformed cells.[6-9] The fascin expression level is usually low in epithelial cells, but is often up-regu-

lated in transformed cells and several types of human neoplasms, such as breast,[7] ovary,[6] pancreas,[8] and non-small cell lung carcinomas.[9] The high expression of fascin in transformed cells suggests that fascin may play an important role in tumor pathophysiology. In breast cancer, the expression of fascin is restricted to the high-grade tumors that are more proliferative and metastatic.[7] Thus, fascin is likely to be involved in the metastasis of breast cancer, and fascin could be an important marker for breast cancer pathology.

In a study conducted on cell lines, a functional relationship between E-cadherin and fascin has been documented in transformed epithelial cell lines.[10] Fascin has been shown to bind to the Armadillo repeat domain of β-catenin and it competes with the cytoplasmic domain of E-cadherin for an association with β-catenin *in vitro.* To investigate whether fascin might be associated with the disruption of cell-to-cell adhesion systems, and also to determine whether it could be used as a predictive marker for the progression of ovarian cancer, we examined the expression of fascin protein and its relationship with the expression of β-catenin and E-cadherin in benign, borderline and malignant ovarian serous neoplasms.

MATERIALS AND METHODS

Study material

Tissue samples were obtained from the surgical pathology archives of the Department of Pathology at the Kangbuk Samsung Hospital and Samsung Medical Center, Sungkyunkwan University School of Medicine. We retrieved the formalin-fixed, paraffin-embedded blocks from 120 patients who had undergone oophorectomy for serous ovarian neoplasm from 1992 to 2002. We also included 13 cases of normal ovarian tissue for comparison. Clinical information, including the follow-up data, the pathology reports, paraffin blocks and slides was available to review all the tumors. The tumors were surgically staged according to the International Federation of Gynecology and Obstetrics (FIGO) Staging Systems. The grading of the tumors was also assigned according to the FIGO system. For statistical purposes, the tumors were divided into well-differentiated, moderately differentiated and poorly differentiated groups of carcinomas, and FIGO stage I and II carcinomas were grouped as early stage carcinomas, while FIGO stage III and IV carcinomas were grouped as advanced disease.

Of the 120 tumors, 14 were benign, 32 were borderline, and 74 were malignant. Thirty of the borderline tumors were stage I (93.8%), and 2 were stage III (6.2%), while 7 of the malignant tumors were stage I (9.5%), 2 were stage II (2.7%), 55 were stage III (74.3%), and 10 were stage IV (13.5%). There was peritoneal involvement noted in 54 cases, i.e., 4 borderline tumors and 50 carcinomas. The mean age of the patients was 49 years for the benign tumor group, and it was 43 years for the borderline tumor group and 55 years for the carcinoma group.

The follow-up period ranged from 1 to 112 months with average of 31.5 months (median period: 25 months). There were tumor recurrences in 26 cases, and these were all carcinomas. Tumor-related death occurred in 18 cases and all of them showed the histologic features of serous adenocarcinoma.

The clinicopathologic parameters such as the FIGO staging, histologic grade, peritoneal metastasis and the follow up data, including tumor recurrence, tumor-related death and the duration of survival were compared. The clinical characteristics are summarized in Table 1.

Construction of tissue microarray (TMA)

After reviewing all of the hematoxylin and eosin stained slides from the cases, one representative slide was selected from each case, and one area of the tumor was circled on the slide. The corresponding paraffin-embedded tissue blocks were then retrieved. The selected area was circled on the block with a marker pen for construction of the TMA. The TMA was constructed using a manual tissue puncher. The selected area in the donor block was cored with a 4 mm diameter needle, and the tissue was transferred to a recipient paraffin block.

Table 1. Clinical characteristics of ovarian serous neoplasms

Histologic classfication	No. (cases)	Mean age (range) (years)	Peritoneal metastasis	Stage				Recurrence	Tumor related death
				I	II	III	IV		
Benign	14	49 (33-83)	0					0	0
Borderline	32	43 (18-73)	4 (12.5%)	30 (93.8%)	0	2 (6.2%)	0	0	0
Malignant	74	54.6 (13-83)	50 (67.6%)	7 (9.5%)	2 (2.7%)	55 (74.3%)	10 (13.5%)	34 (36.2%)	23 (24.5%)
Total	120	50.3 (13-83)	54 (44.6%)	44 (36.4%)	3 (2.5%)	71 (58.7%)	17 (14%)	35 (28.9%)	23 (19%)

Immunohistochemistry

The tissue sections in glass slides were deparaffinized with xylene, hydrated in series of diluted alcohol solution and then immersed in 3% H_2O_2 in order to quench the endogenous peroxidase activity. The sections were then microwaved in 10 mM sodium citrate (pH 6.0) for 15 min for performing antigen retrieval. After the antigen retrieval, avidin and biotin were applied consecutively to the slides to eliminate any endogenous biotin-related background staining. The sections were then incubated with primary antibodies for 60 min, and after rinsing them three successive times with washing buffer, they were further incubated with biotinylated goat anti-mouse antibodies (DiNonA, Seoul, Korea) for 20 min. After rinsing, the tissue sections were incubated with the HRP-conjugated streptavidin for 20 min at room temperature. The slides were washed and the chromogen was developed for 5 min with using liquid 3,3′-diaminobenzidine; the slides was then counter-stained with Meyer's hematoxylin, dehydrated, and mounted with Canada balsam for examination. Distilled water containing 0.1% Tween 20 was used as a rinsing solution. The list of primary antibodies used, along with their respective dilutions and the staining patterns of the antibodies, is given in Table 2.

Interpretation of immunohistochemistry

All the slides were evaluated by light microscopy with performing a semiquantitative estimation for the membranous and cytoplasmic immunoexpression of the tumor cells, and this was done by two independent observers. Immunoreactivity was assessed without the observers having any previous knowledge of the clinicopathologic features.

The membranous immunoexpression for E-cadherin and β-catenin, and the membranous and cytoplasmic staining for fascin were analyzed, and these were scored according to the presence and extent of the staining, using the following scale: 0 (negative) was staining of 0-5% of the tumor cells, 1 was staining of 6-25% of the tumor cells, 2 was staining of 26-75% of the tumor cells, and 3 was staining of 76-100% of tumor cells. Staining of more than 5% of the tumor cells was recorded as positive immunoreactivity. The staining intensity was subclassified as: 1, weak; 2, moderate; and 3, strong. Each lesion was examined and scored separately by the two pathologists, and those cases with discrepant scores were discussed until agreement was achieved. The scores for the extent of positivity of the tumor cells and the staining intensity scores were multiplied to produce an immunoreactive score (IS) for each tumor specimen.

Table 2. Primary antibodies used in this study

Antibodies	Clone	Source	Dilution	Staining pattern
Fascin	55K-2	DAKO	1:200	Membrane and cytoplasmic
β-catenin	17C2	DiNonA, Seoul, Korea	1:150	Membrane and nuclear
E-Cadherin	36B5	DiNonA, Seoul, Korea	1:25	Membrane

Statistical analysis

Statistical analyses for the significant differences of the immunohistochemical expression were performed with using a two-tailed Fisher's exact test, Pearson's χ^2 test, Kruskal-Wallis test, and the Mann-Whitney U Test, and p-values <0.05 were considered statistically significant. Logistic regression analysis was performed to compare the explanatory variables with the occurrence of peritoneal metastases. All the statistical comparisons were performed using SPSS, version 11.0 software (SPSS, Chicago, USA).

RESULTS

Fascin expression

For the normal ovary, fascin immunoreactivity was observed in the endothelial cells of the networking small vessels and dendritic cells. The ovarian stromal cells showed variable, weak immunoreactivity for fascin. However, the normal ovarian surface epithelial cells and the epithelial inclusion glands did not show any fascin immunoreactivity (0/13). Fascin expression was recognized in the majority of the borderline (100%: 32/32) and malignant tumors (90.5%: 67/74), but it was not seen in the benign tumors (0%: 0/14) (p<0.001) (Table 3). A fine granular to strong diffuse cytoplasmic staining pattern and an occasional membranous staining pattern were observed. The fascin expression was often markedly enhanced at the edge of the tumor and

Table 3. Fascin immunoreactivity in normal ovarian surface epithelium, benign, borderline, and malignant serous ovarian neoplasms

Histologic diagnosis	Fascin expression				p value
	0	1+	2+	3+	
Normal (13)	13	0	0	0	<0.05
Benign (14)	14	0	0	0	
Borderline (32)	0	3	9	20	
Malignant (74)	7	18	10	39	

at the highly proliferating areas that showed papillary features.

Table 4. Relationship between fascin expression in serous carcinomas and clinicopathologic parameters

Characteristics	Fascin-negative	Fascin-positive	IS (mean±SD)	p-value
Case (n)	7	67		
Clinical Stage				NS
I	2 (28.6%)	5 (7.5%)	3±4.12	
II	0	2 (3%)	6±0	
III	3 (42.9%)	52 (77.6%)	4.65±2.95	
IV	2 (28.6%)	8 (11.9%)	2.8±2.94	
Tumor grade				NS
G1	0	6 (9%)	5.6±3.03	
G2	2 (28.6%)	15 (22.4%)	4.35±2.96	
G3	5 (71.4%)	45 (67.2%)	4.04±3.14	
Recurrence	2 (28.6%)	24 (35.8%)	5.12±3.27	NS
Tumor related death	2 (28.6%)	16 (23.9%)	4.28±3.10	NS
Peritoneal metastasis	3 (42.9%)	47 (70.1%)	4.78±2.99	0.043

IS, immunoreactive score; SD, standard deviation.

The surrounding ovarian stroma sometimes showed weak to strong fascin immunoreactivity even without there being any fascin expression in the tumor cells. The fascin expression was significantly correlated with the occurrence of peritoneal metastases in the carcinomas (p=0.043) and with invasive growth, including microinvasion, in the borderline tumors (p=0.043). The expression of fascin did not correlate with the tumor grade of carcinoma, the FIGO stage, tumor recurrence, tumor-related death and the survival rate (Table 4).

Table 5. Relationship between fascin expression and β-catenin or E-cadherin expression

Fascin expression	E-cadherin expression		p-value	β-catenin expression		p-value
	(−)	(+)		(−)	(+)	
(-)	5/21	16/21		9/21	12/21	
(+)	7/99	92/99	0.035	20/99	79/99	0.046

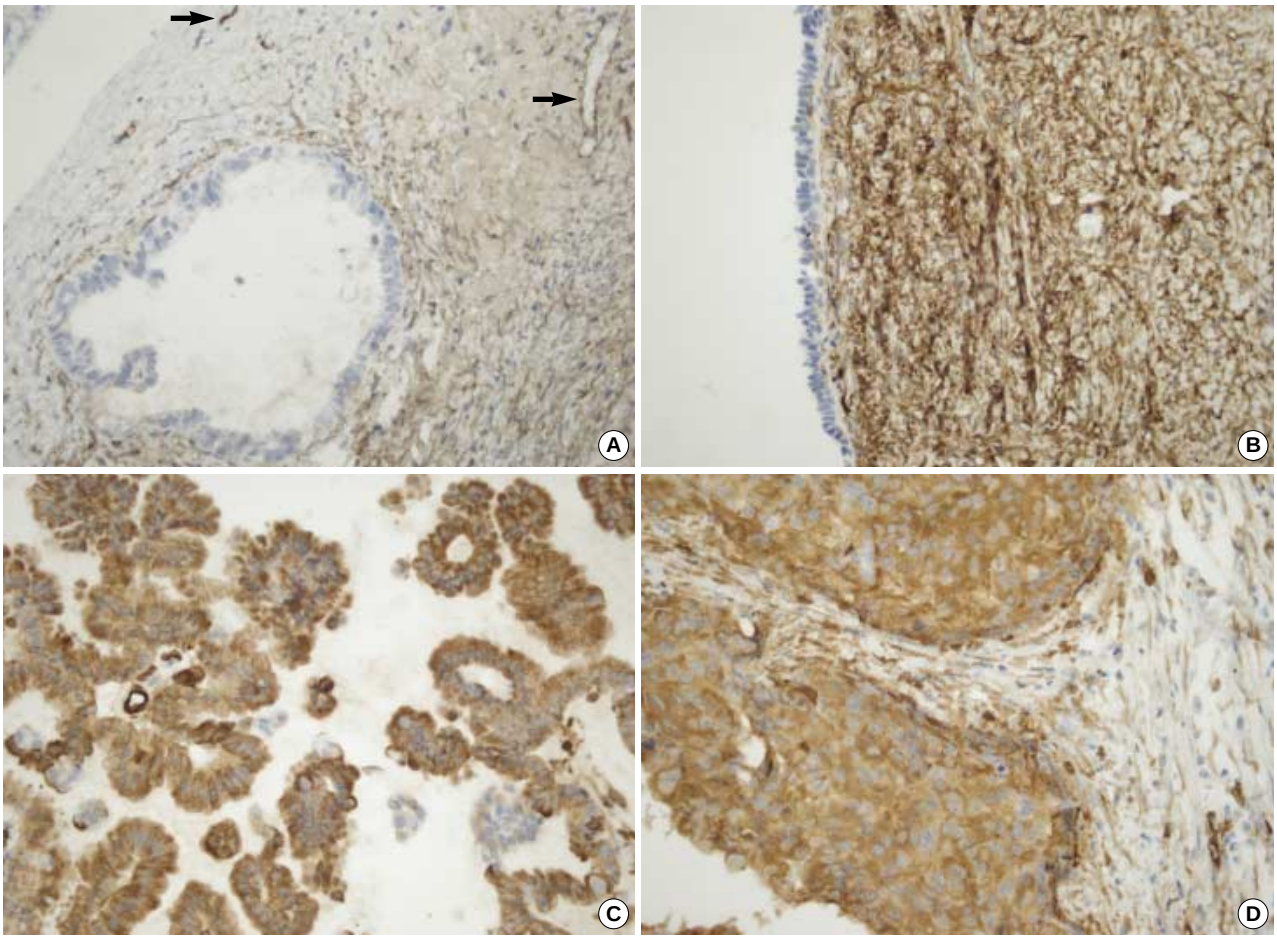

Fig. 1. Immunohistochemical stains for fascin. The epithelial cells of inclusion cyst in normal ovary (A) and benign serous neoplasm (B) show no immunoreactivity for fascin in contrary to endothelial cells of blood vessels (arrow). In both borderline (C) and malignant serous ovarian tumor (D), immunohistochemistry for fascin reveals cytoplasmic and membranous staining.

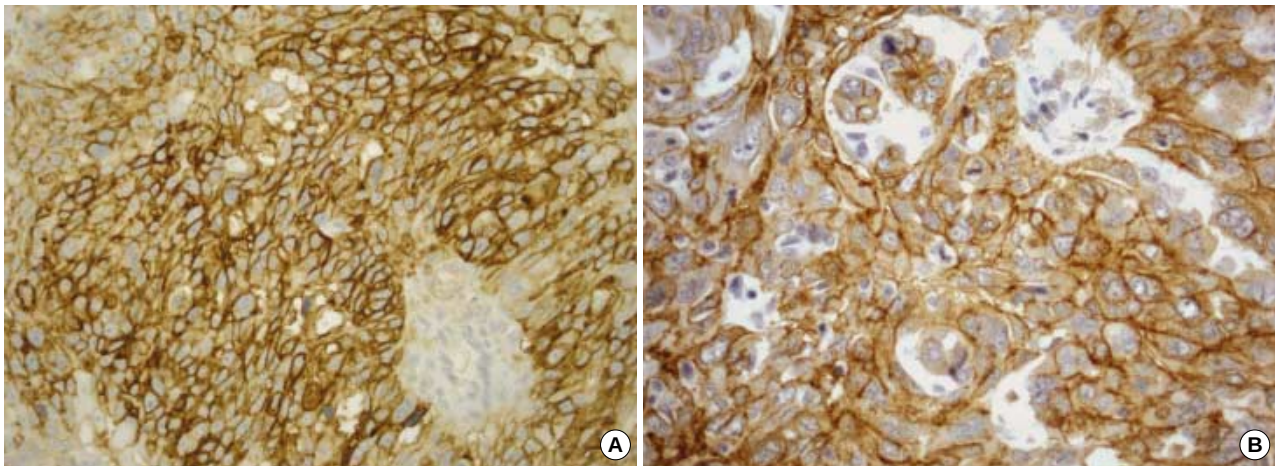

Fig. 2. Immunohisthochemical stains reveal membranous immunoreactivity for E-cadherin (A) and β-catenin (B) in serous carcinoma.

E-cadherin and β-catenin expressions, and their relationship with fascin expression

Membranous E-cadherin expression was recognized in the majority of the benign (100%: 14/14) and borderline tumors (96.9%: 31/32), but it was less frequently seen in the adenocarcinomas (85.1%: 63/74) (p=0.015), and a reduced E-cadherin immunoexpression was demonstrated in 5 of the 21 fascin negative cases (23.8%) and in 7 of the 99 fascin positive cases (7.1%).

Membranous staining for β-catenin was found in 91 cases and this was not different among the benign (9/14), borderline (28/32), and malignant neoplasms (54/74) (p=0.11, 0.61). β-catenin expression was found in 12 of the 21 fascin negative cases (57.1%) and in 79 of the 99 fascin positive cases (79.8%).

A significant relationship between the expression of β-catenin and fascin (p=0.046), and between the expression of the E-cadherin and fascin (p=0.035) was noted (Table 5).

On the multivariate study, the fascin score and the E-cadherin immunoreactivity were independent predictors of peritoneal metastases (p=0.005 and p=0.049).

The typical images of fascin immunohistochemistry in the normal ovary and in the benign, borderline and malignant serous ovarian neoplasms, and the immunohistochemical staining for β-catenin and E-cadherin are shown in Fig. 1, 2.

DISCUSSION

For tumor cells to break loose from a primary mass, enter the blood vessels or lymphatics, and then produce a secondary growth at a distant site, they must first go through a series of steps such as the reduced adhesion of tumor cells and the acquisition of the cell motility function. The tumor cells' high motility and invasive properties allow them to penetrate the basement membrane and spread to the surrounding tissues; these properties are thought to result from a rearrangement of the cytoskeletal microfilaments by the action of actin cross-linking proteins. Among these actin cross-linking proteins, this study focused on fascin, which is present in membrane ruffles, microspikes, and other motility-associated cell fibers.[3] The expression of fascin in epithelial neoplasms has been reported for several types of human neoplasms, such as ovarian,[6] breast,[7] pancreatic,[8] colon,[11] lung,[9] and skin tumors,[12] in contrast to the usually absent or low fascin expression in the normal epithelial cells. We suspected that fascin plays a role in the invasion of borderline and malignant serous ovarian tumors, and so we examined the expression of fascin in normal ovary tissue and, in benign, borderline, and malignant ovarian serous tumors, and we also examined the relation between the expression of fascin and the clinicopathological parameters. This study has demonstrated that the expression of fascin is significantly high in borderline and malignant tumors compared with benign tumors. Our data indicate that fascin expression may be closely linked with the progression of tumor in both the ovarian serous borderline and malignant neoplasms, although the serous borderline and malignant tumors frequently display very different molecular genetic and immunohistochemical alterations. We also found that fascin up-regulation was significantly associated with peritoneal metastasis, and it was independent of the overall survival, and this suggests a role for this molecule in the metastasis of ovarian serous neoplasms.

Jawhari *et al.*,[11] have reported that there was an apparent increase in cell proliferation after fascin transfection of the colonic epithe-

lial cell lines. Fascin immunoreactivity was also significantly associated with an increased proliferative activity in the typical and atypical carcinoid tumors.[13] In the present study, the fascin expression was increased in the proliferative foci with papillary growth, budding and tufting being seen. These results may suggest that fascin has some important role in local tumor progression for ovarian serous neoplasms.

The cadherin family of transmembrane glycoproteins is of particular importance for cellular adhesion. E-cadherins mediate the homotypic adhesions in epithelial tissues; thus, E-cadherins serve to keep the epithelial cells together and they relay signals between the cells. E-cadherins are linked to the cytoskeleton by the catenins, and these catenins are proteins that lie under the plasma membrane.[14,15] Overexpression of fascin in epithelial cells has been shown to correlate with disorganization of the adherens junctions and with decreased cell-to-cell attachment activity.[10,16,17] The mechanism of these effects is believed to involve an interaction between fascin and β-catenin in the cadherin- and occludin-dependent adhesion complexes.[10,16] Tao *et al*.,[10] have reported that yeast two-hybrid analysis revealed the binding of fascin to the Armadillo repeat region of β-catenin in a noncadherin complex. They also demonstrated that fascin and E-cadherin utilize a similar binding site within β-catenin, such that they form mutually exclusive complexes with β-catenin. However, when Jawhari *et al*.,[11] examined both the effects of fascin transfection on the expression of the major proteins of the E-cadherin-catenin complex and also the possible association of fascin with this complex, fascin transfectant cells showed no alteration of E-cadherin, or the α-, β-, or γ-catenin levels, and There was no change in the amount of E-cadherin at the biochemical level. The fascin overexpressing cells were not affected for their ability to assemble the appropriately located adherens junctions or their ability to localize E-cadherin and β-catenin to the cell-cell margins on the transmission electron microscopy and immunofluorescent staining studies. This suggests that fascin up-regulation does not affect the E-cadherin/catenin complex.[11] To further investigate the mechanisms of invasion in ovarian serous neoplasms, we evaluated the association between the fascin expression and the E-cadherin expression and β-catenin expression in serous ovarian neoplasms. In the present study, a reduced expression of E-cadherin was more frequently seen in malignant tumors than in benign or borderline tumors, and it was significantly higher for the fascin negative cases than for the fascin positive cases. Similar to E-cadherin, β-catenin immunoexpression was also correlated with fascin immunoreactivity. Based on these findings, we conclude that the effects of fascin on cell organization and cell behavior may be mediated by fascin's effects on the organization of the E-cadherin/catenin cell-to-cell adhesion complexes.

In conclusion, our results suggest that fascin expression may be closely linked with tumor progression and metastasis in serous neoplasms. Moreover, because the fascin expression was associated with up-regulation of β-catenin and E-cadherin, fascin seems to play a role for adhesion as well as motility.

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