

Fascin in ovarian epithelial tumors

**Evanthia Kostopoulou¹, Alexandros Daponte², Anastasios Terzis¹, Maria Nakou¹, Ioanna Chiotoglou³,
Dimitrios Theodosiou¹, Christos Chatzichristodoulou⁴, Ioannis E. Messinis² and George Koukoulis¹**

¹Department of Pathology, ²Department of Obstetrics and Gynecology, ³Laboratory of Cytogenetics and Molecular Genetics and

⁴Department of Hygiene and Epidemiology, University Hospital of Larissa, Medical School of Thessalia, Larissa, Greece

Summary. Fascin contributes to the formation of actin-based protrusions involved in cell migration. Fascin has emerged as a prognostic marker in some carcinomas. We examined ovarian neoplasms to check any correlation between fascin expression and established clinicopathologic parameters.

Fascin immunoreactivity was semiquantitatively scored in 100 ovarian tumors (62 carcinomas, 15 borderline tumors and 23 cystadenomas). Double staining for fascin and Ki-67 was performed in selected carcinomas. Western Blotting was done in frozen samples.

Fascin immunoreactivity was highest in carcinomas, lowest in cystadenomas and intermediate in borderline tumors; these results were in accordance with those from Western blotting analysis. Fascin was statistically increased in carcinomas of advanced stage and in serous carcinomas. It was also increased in metastatic foci and in tumor foci with lower Ki-67 labeling.

We conclude that in ovarian tumors fascin is associated with certain features of increased tumor aggressiveness. Future studies could determine if fascin may become a routinely helpful marker in gynecological pathology or clinical oncology.

Key words: Fascin, Ovary, Ki-67, Carcinoma, Invasion

Introduction

Fascin is a 55kDa globular protein that belongs to a unique family of actin-bundling proteins (Duh et al., 1994; Kureishy et al., 2002). In vertebrates there are three forms of fascin: fascin-1, which is expressed by mesenchymal tissues and in nervous system, fascin-2, which is expressed by retinal photoreceptor cells and

fascin-3, which is testis specific (Tubb et al., 2002; Adams, 2004a).

Fascin-1 (also known as fascin) contributes to the formation of various actin-based cellular structures (Jawhari et al., 2003; Adams, 2004b; Tseng et al., 2005; Vignjevic et al., 2006). Among those, and critical in cancer cell biology, appear to be the cellular surface protrusions that mediate cell movement. In vitro studies, based on transfection experiments, have shown that elevated levels of fascin increased the speed of cell migration and emphasized the association between fascin expression and motility of transformed cells (Jawhari et al., 2003).

In human carcinoma samples, preliminary studies of fascin expression reveal increased expression in comparison to that of non-neoplastic epithelial tissues, whereas recent reports suggest the emergence of fascin as a new prognostic indicator (Grothey et al., 2000; Pelosi et al., 2003a,b; Goncharuk et al., 2003; Hashimoto et al., 2004, 2005a,b, 2006; Yoder et al., 2005; Tong et al., 2005; Choi et al., 2006; Jin et al., 2006; Zigeuner et al., 2006).

Cancer cell invasion, in part a reflection of increased cellular motility (Wang et al., 2005), remains a critical parameter of the biologic behavior of all carcinomas in general and of ovarian carcinomas in particular. There are already two reports, from one team of investigators, concerning fascin expression in ovarian carcinoma (Kabukcuoglu et al., 2006a,b). In contrast to some other carcinoma types, there was not a clear cut relation between fascin immunohistochemical staining and established clinicopathologic markers generally considered to reflect the biological aggression of carcinomas.

The aim of our study was to look at another well studied group of cases for a possible relationship of fascin expression with stage and grade of ovarian carcinomas. The proliferative activity was additionally examined, since some studies have suggested a relationship between Ki-67 and fascin immuno-

reactivities (Pelosi et al., 2003a; Hashimoto et al., 2004, 2006).

Materials and methods

Patients and surgical specimens

We assessed the immunostaining of fascin in 100 specimens of ovarian tumors from 98 patients, who underwent tumor resections in conjunction with complete surgical staging when indicated, between 2001 and 2006. The material was retrieved from the files of the Pathology Department of the University Hospital of Thessaly. There were 9 cases with a history of preoperative treatment. The tumors were classified according to the World Health Organization classification and staged according to F.I.G.O. (W.H.O. Classification of Tumours, 2003). A three-tiered grading system was used (Silverberg, 2000).

The carcinoma group (62 cases) represented almost all types of ovarian carcinomas thought to be derived from the surface epithelium (Table 1). Forty-five were serous carcinomas (6 stage IV, 31 stage III, 7 stage I). Tumor grade was estimated to be 3 in 35 of these cases. Additionally, there were 9 serous tumors of low malignant potential. There were also 15 serous cystadenomas. Few clear cell carcinomas were present, only 3 cases. In addition, there were 9 endometrioid carcinomas of variable stage (I-III) and grade (1-3). The study tumor group included also one mucinous carcinoma and 5 mucinous tumors of low malignant potential.

Non-neoplastic samples included five non-neoplastic ovaries removed at hysterectomy for leiomyomas or cervical lesions.

The samples were fixed in 10% buffered formalin solution, embedded in paraffin blocks and cut at 4 μ m sections.

Immunohistochemical procedures

Two different primary monoclonal antibodies were used in every case: clone IM20 (dilution 1:300, 20min at room temperature (RT), Novocastra, Newcastle upon

Tyne, U.K.) and 55k-2 (dilution 1:100, 30min RT, Cellmarque, Hot Springs, U.S.A.). Immunohistochemical staining for the first antibody was performed in a commercially available automated immunostainer (Bond Max, Vision Biosystems, Australia). Immunohistochemical staining for 55k-2 was performed manually. For antigen retrieval Bond Epitope Retrieval Solution 2 (30min, Vision BioSystems, Mount Waverley, Australia) was used for clone IM20 and Trilogy (15min, 600W, Cellmarque, Rocklin, U.S.A.) for 55k-2. Binding of the primary antibodies was assessed by the Bond Polymer Refine Detection (Vision Biosystems, Newcastle upon Tyne, U.K.) and Envision Detection System (Dako, Denmark), respectively, with DAB as a chromogen.

For double immunohistochemical staining the slides were pretreated in target retrieval solution high pH (DakoCytomation, Glostrup, Denmark) and stained manually with the double Envision Detection System (Dako, Denmark), with MIB-1 (dilution 1:80, 25min RT, DakoCytomation, Glostrup, Denmark) being the first antibody, stained with Envision /HRP with DAB as a chromogen (nuclear stain), and fascin (clone IM20, dilution 1:300, 20min RT, Novocastra, Newcastle upon Tyne, U.K.) being the second antibody, stained with Envision /AP with Fast Red as a chromogen (cytoplasmic stain).

Assessment of fascin immunohistochemical staining

All slides were initially evaluated by two pathologists (GK, EK), blindly and independently. During a subsequent joint evaluation a final consensus immunoreactivity score was obtained and used for statistical analysis. Cytoplasmic immunoreactivity of tumor cells was assessed in comparison to the immunoreactivity of endothelial cells, which were used as internal positive controls. Two aspects of immunoreactivity were semiquantitatively evaluated, extent and intensity. The extent of immunoreactivity (EI) was categorized into 4 groups according to the percentage of immunostained neoplastic cells: <25% (1+), 25%-50% (2+), 51%-75% (3+) and >75% (4+). The intensity of immunoreactivity was also semiquantitatively graded by comparing it to the staining intensity of endothelial cells present in the same histopathologic slide. Intensity was considered as "weak to moderate" when it was less intense than that of endothelial cells, "and "intense" when it was of similar intensity to that of the endothelial cells.

After preliminary analysis of the findings, the pathologists involved in the evaluation of the immunohistochemical staining realized that the visualized differences in the immunoreactivity among various cases were appreciated best by counting only the cellular subpopulation showing "intense" immunohistochemical staining, and expressing it as HIES (Highest Immunohistochemical Expression Score). In order to calculate HIES a value was assigned for the

Table 1. Ovarian tumors included in the study.

Tumor type	Malignant	Borderline	Benign
Serous	45	9	15
Endometrioid	9	1	-
Mucinous	1	5	7
Clear cell	3	-	-
Other	4 ^a	-	1 ^b

^a: Including undifferentiated carcinomas and malignant mixed epithelial tumors (The score was evaluated in the total area examined); ^b: Brenner tumor.

percentage of the said subpopulation (0, 1: <25%, 2: 25-50%, 3: 50-75%, 4: >75%), with HIES ranging from 0 to 4.

Statistical evaluation

Statistical analyses were performed using the Statistical Package SPSS 11.0 for Windows (Chicago, U.S.A.). The Chi-square test or Fisher exact test was used for qualitative data. The Mann-Whitney test was used for quantitative data. A *p* value of 0.05 or less was considered statistically significant.

Western blotting

Western Blotting experiments were performed on proteins isolated from sections of tumor tissue kept at -80°C. Cells were lysed with NET-Triton Lysis Buffer (0.01 M Tris-Cl, 0.1 NaCl, 1 mM EDTA pH 7.4, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% Sodium Deoxycholate and a cocktail of protease inhibitors). Aliquots of lysates containing 10 µg of total protein for fascin detection were run on 8-12% NuPAGE Tris-Acetate gel (Invitrogen, Carlsbad, CA, USA) under denaturing and reducing conditions. Proteins were transferred to PVDF membranes (BioRad, USA). Nonspecific binding of antibody to the membrane was blocked by one-hour incubation with 5% (w/v) non-fat milk/0.01 (v/v) Tween 20 in PBS.

Immunoblot analysis was performed using mouse monoclonal anti-fascin (1:50 dilution, IM20, Novocastra, Newcastle upon Tyne, U.K.). Human β-actin monoclonal antibody (SIGMA, USA) was used as a protein marker for the quantification of the protein bands. Membranes were then immersed in ECL detection solution (Santa Cruz, USA) and exposed to XAR-5 film (Kodak, USA) for autoradiography. Protein bands were quantified using Epson GT-8000 laser scanner. The ratios of fascin protein band intensity relative to β-actin band intensity were calculated for each sample.

Results

A summary regarding fascin immunoreactivity is presented in Tables 2 and 3. Representative examples of fascin immunostaining are shown in Figures 1 to 6.

HIES was highest in carcinomas regardless of histopathologic type (see table 2). HIES was lower in

borderline tumors but the difference was not statistically significant. Cystadenomas showed the lowest HIES, lower not only than carcinomas, but also than borderline tumors (*p*<0.0001). In addition HIES was found to be significantly elevated in serous as compared to endometrioid ovarian carcinomas (*p*=0.01), see table 3. In borderline serous tumors there was an increase of HIES when compared to mucinous borderline tumors but the difference did not reach statistical significance (*p*=0.06). Only two serous tumors with extensive micropapillary areas were included in the study, with a HIES of 1 and 3, respectively, so no definite conclusions can be drawn concerning fascin positivity in these cases. The exact numbers at the Tables are based on clone IM20 staining, although both antibodies gave similar results, with 55k-2 resulting in slightly higher staining intensity in some cases.

HIES in metastatic foci was increased in comparison to primary tumors, see table 4. The difference was not statistically significant (*p*=0.07). Notably, increased HIES values (mean 2.05) were observed in carcinomas of advanced stages (3 and 4) whereas decreased HIES (mean 0.83) was noted in stage 1 ovarian carcinomas. The observed difference was proven to be statistically significant (*p*=0.005). The corresponding values for serous carcinomas were 2.21 for carcinomas of advanced stage vs 1 for stage I (*p*=0.049).

We also studied another parameter, the extent of immunoreactivity (EI), in a pattern analogous to that of HIES (see table 2). EI was also higher in carcinomas regardless of histopathologic type and it was significantly lower in borderline tumors and

Table 3. Fascin score (HIES) in different tumor groups.

Tumor type	Malignant	Borderline	Benign
Serous	2.10	1.7	0.06
Endometrioid	0.87	0*	-
Mucinous	2*	0.6	0.12
Clear cell	1	-	-
Other	1	-	0*

*one case each

Table 4. Fascin score in metastatic sites.

Number of cases	
Increased	10 ^a
Unchanged	10 ^b
Decreased	3 ^c
Total	23

^a: In one case an increase was observed in 4 lymph nodes tested;

^b: Four of the cases had HIES 4 in both primary tumor and metastasis;

^c: No omental metastases.

Table 2. Fascin score and extent of immunoreactivity.

	HIES	EI
Carcinomas	1.78	57.55
Borderline tumors	1.15	25
Cystadenomas	0.08	6.12

cystadenomas ($p=0.002$ and <0.0001 respectively). Similarly to HIES, the mean value of EI was higher in serous than endometrioid carcinomas, higher in carcinomas of advanced stages when compared to carcinomas of stage 1, and in serous borderline tumors when compared to mucinous tumors, but the statistical evaluation did not reveal a difference of statistical significance. A subset of 19 cases showing more than 80% of fascin immunoreactivity was characterized by markedly increased HIES (mean 3.21), significantly higher than that of all the other cases combined (mean 0.98, $p<0.0001$).

In general, non-neoplastic surface epithelium of the ovary, as well as other epithelial elements, did not show fascin immunoreactivity (Fig. 1a). There was only weak staining of an occasional mesothelial cell (not shown). Exceptionally, a few cells in tubal epithelium showed intense immunostaining (Fig. 1b). Fascin immunostaining was noted in the ovarian stroma and endothelial cells.

Intense immunostaining could be either extensive or very focal (Fig. 2a,b). There were regions with intense staining of the adjacent vasculature in the absence of

immunoreactivity of the tumor (Fig. 3b). The individual tumor cells showed cytoplasmic localization of the immunoreactivity with subtle enhancement at the cellular periphery (Fig. 3a). Focally, there was basal polarization of the reactivity, towards the underlying stroma (Fig. 4b). In two borderline tumors with few foci of microinvasion this phenomenon was particularly prominent in tumor cells adjacent to these foci (Fig. 4b). Some foci of microinvasion themselves showed strong fascin immunoreactivity. In these cases, the extent of immunoreactivity was also increased and it was not restricted to the vicinity of the microinvasion. Overall, serous carcinomas showed more intense and extended immunoreactivity. Interestingly, in two carcinomas with mixed differentiation, serous and endometrioid phenotypes, included in the study, the serous component showed preferential staining. In several cases of serous carcinomas there were also qualitative differences of the immunostaining. Specifically, there was more intense staining of cells localized towards the periphery of the papillae (Fig. 5a) and in these cells there was an almost membrane-like or more accurately “submembranous” distribution of the immunoreactivity (Fig. 5a-inset).

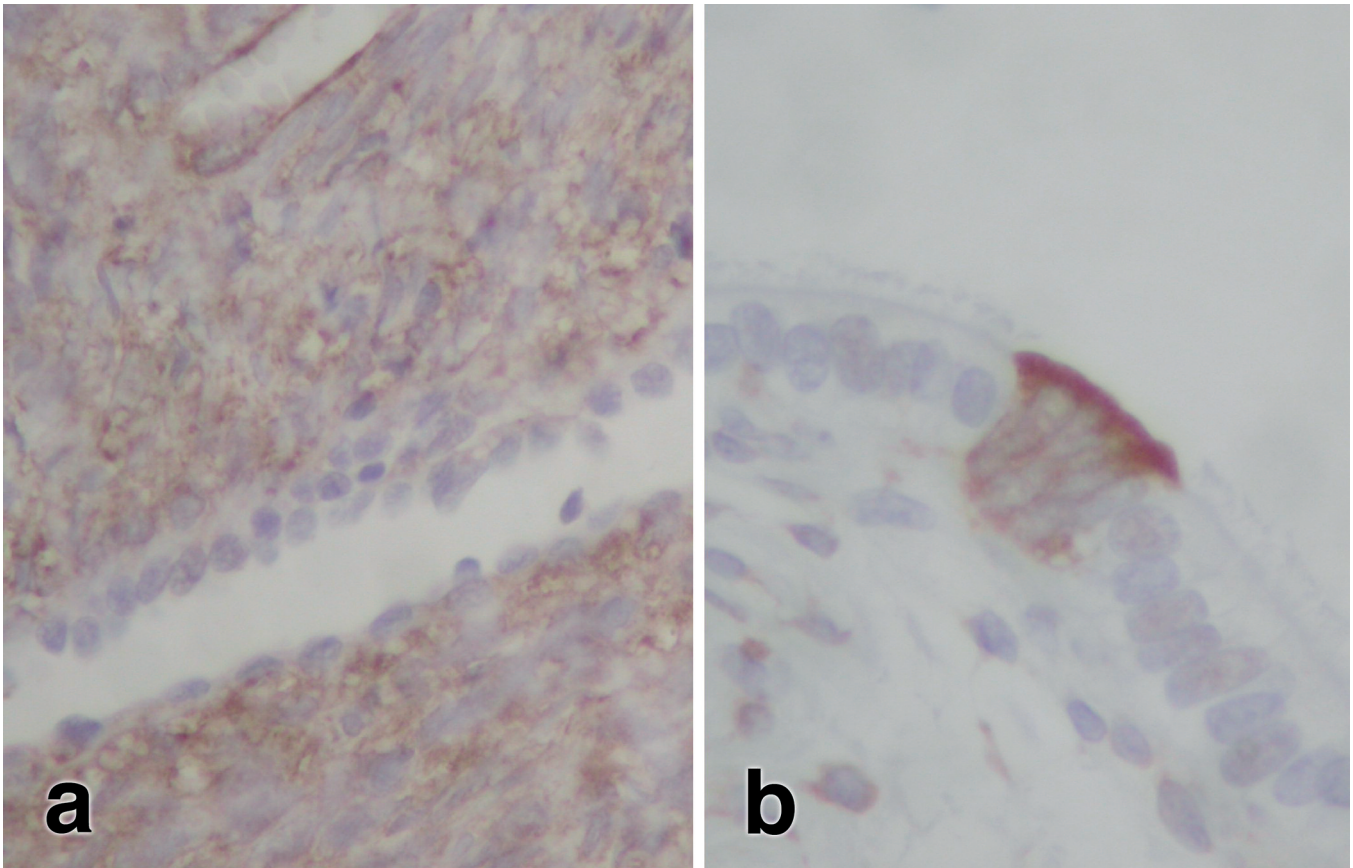


Fig. 1. a. Non-neoplastic surface epithelium of the ovary, in general without fascin immunoreactivity. Fascin immunostaining is noted in the ovarian stromal and endothelial cells. **b.** Few cells in tubal epithelium showed intense immunostaining. x 200

Fascin in ovarian tumors

Fascin expression was also found in the stroma of carcinomas. Often, stromal immunostaining was intensified in the immediate vicinity of tumor nests, an area that for the purpose of the discussion will be designated as “IPS (immediate peritumoral stroma)”. IPS immunoreactivity was noted even in the absence of tumor cell staining and it was localized to fibroblastic/myofibroblastic elements and the endothelial cells (Fig. 6). Note that there was not any morphologic indication that this IPS could be attributed to tumor infiltrating dendritic cells. There was a tumor subgroup consisting of 14 cases that showed high fascin expression in the stroma. We did not observe significant differences between this tumor subgroup and the rest of the tumors. However, we noticed that stromal fascin expression was decreased in a subset of carcinomas showing high fascin expression (HIES above or equal to 2) and we found that this difference was statistically significant ($p=0.033$).

There was not any statistically verified relationship between tumor grade and HIES, although grade 3 carcinomas of all types showed increased HIES

compared to grade 1-2 carcinomas (mean 1.91 vs 1.73). The respective values for serous carcinomas were 2.21 vs 1.75, but the difference was not statistically significant.

Despite no clear association with tumor grade there was an interesting association between HIES and MIB-1 immunoreactivity in the same tumor samples (Fig. 6-double staining). Specifically, MIB-1 labeling index was 55.5% in all carcinomas showing HIES lower than 2 and 37.1% in all carcinomas showing HIES equal to or higher than 2. This decrease of MIB-1 immunoreactivity in tumors showing more intense fascin expression was statistically significant ($p=0.001$). Similar findings were noted when EI and MIB-1 labeling index were compared.

Another interesting finding was the marked decrease of both HIES and EI in a small subgroup of serous ovarian carcinomas that were preoperatively treated by chemotherapy (mean HIES 1.37 vs 2.10). The difference was not proven to be statistically significant ($p=0.08$).

The relative amount of fascin protein in ovarian tumour samples was also visualized by Western blotting

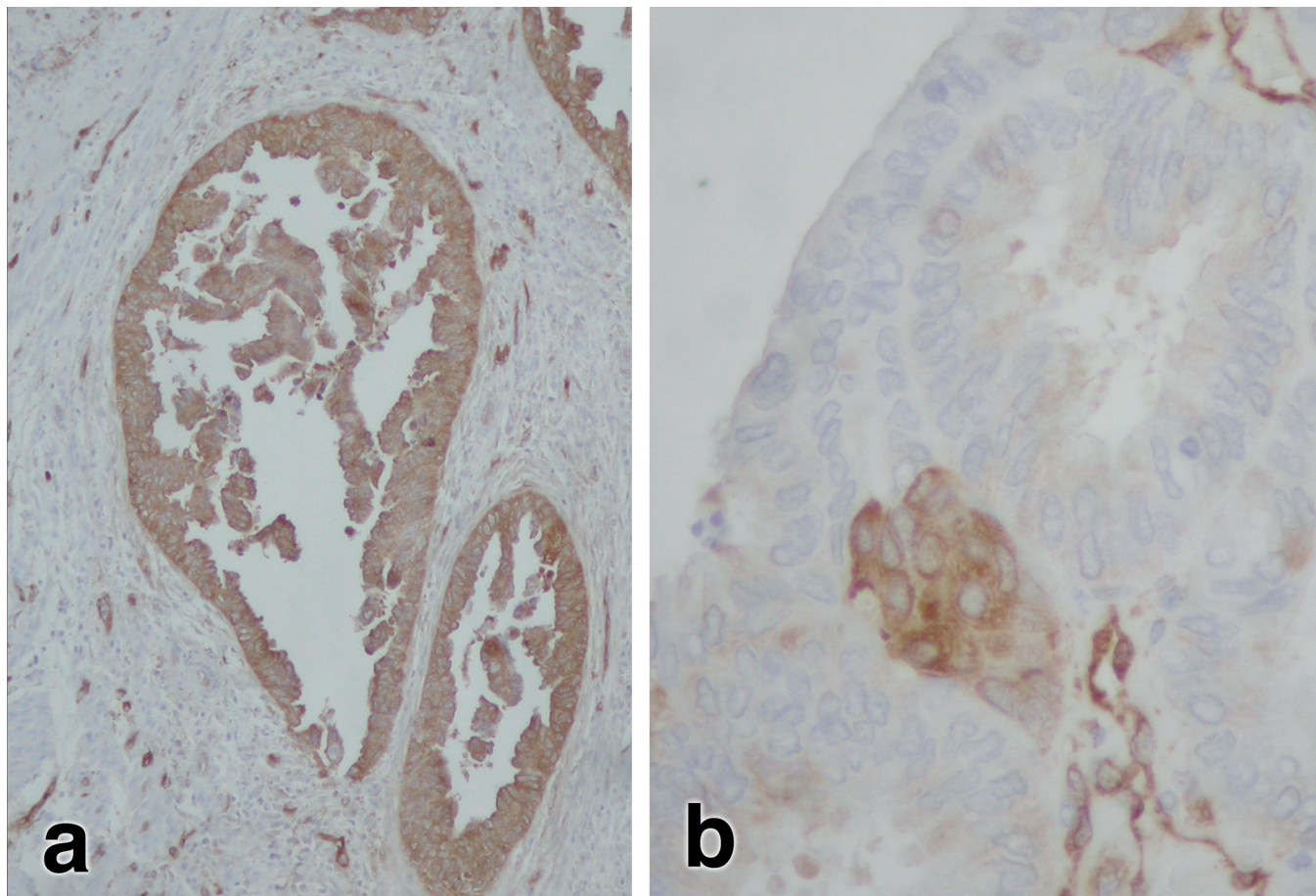


Fig. 2. Intense immunostaining could be either extensive (a) or very focal (b). a, x 100; b, x 200

analysis. In 14 of the cases included in the study tumor tissue kept at -80°C was tested. These included five serous and three endometrioid carcinomas, three serous and three mucinous borderline tumors. The median fascin/ β -actin band intensity ratio was 0.82 (range 0.63-0.98) and 0.67 (range 0.44-0.85) for serous and endometrioid carcinomas, respectively. Fascin protein expression was significantly lower in mucinous than in serous borderline samples: median 0.45 vs 0.66. Although the number of samples examined by Western blotting was lower than that examined by immunohistochemistry, the results were comparable to those obtained by fascin immunostaining. Representative examples are shown in Fig. 7.

Discussion

Tumor cell motility is an important factor in metastasis (Yamaguchi et al., 2005). Fascin is directly involved in mechanisms controlling cellular motility and

indirectly in metastasis (Jawhari et al., 2003; Adams, 2004a; Hashimoto et al., 2005a). Our study showed that fascin immunoreactivity is increased in primary ovarian carcinomas of advanced stage and that in general it is further increased in samples obtained from metastatic sites. We have also shown that fascin immunoreactivity is inversely related to MIB1 immunoreactivity. Possible implications of the aforementioned observations are discussed in the following.

Fascin expression in ovarian carcinomas has been reported previously in cell lines and a limited number of archival cases (Hu et al., 2000). There is overall agreement between the findings by Hu et al. and ours. Specifically, both of us found that fascin expression increased in parallel with tumor progression from borderline to advanced carcinomas. In addition, we confirmed statistically the relationship between increased fascin expression and advanced tumor stage. This relationship was expected, since Hu et al. (2000) found increased fascin expression in cell lines obtained

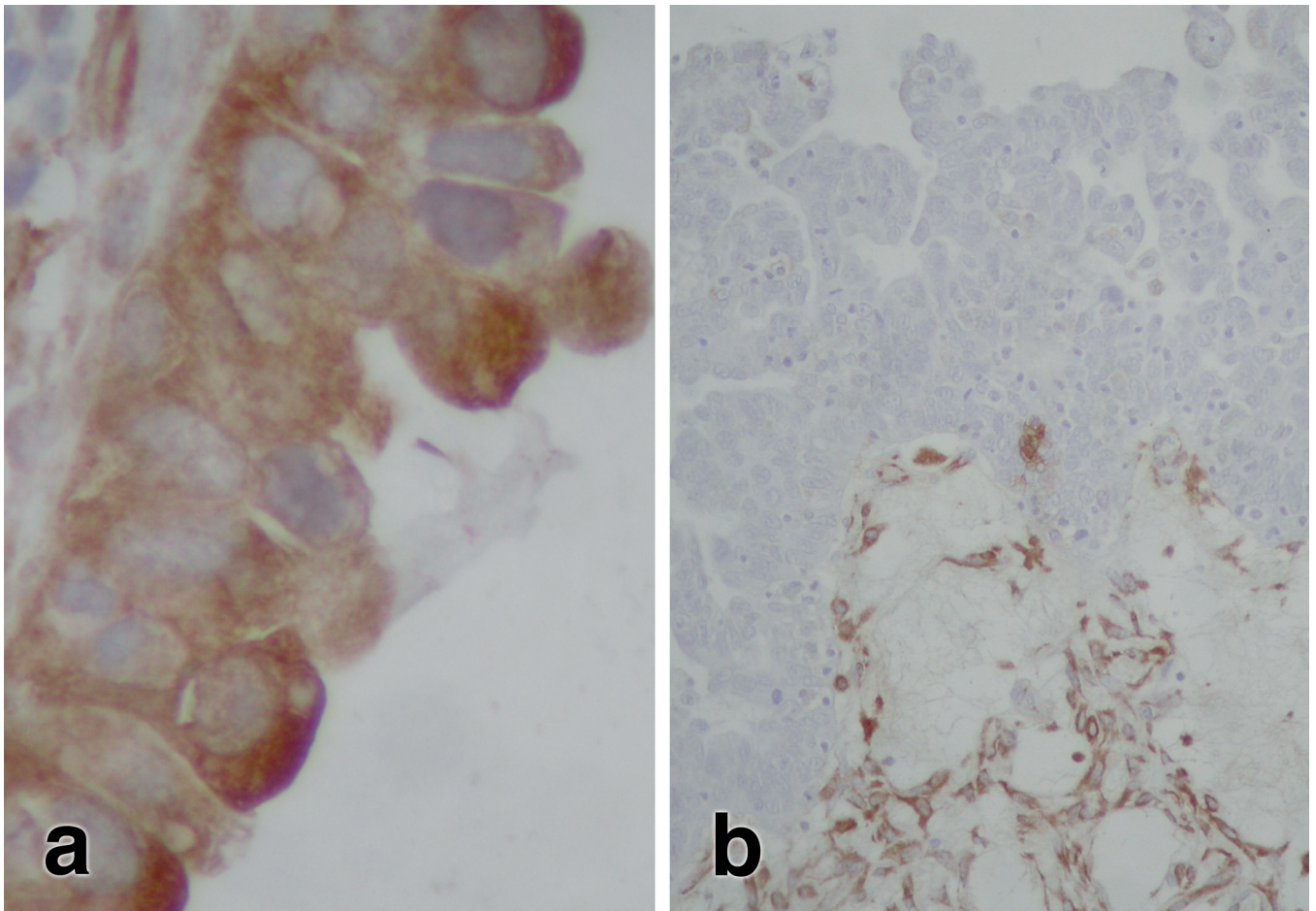


Fig. 3. **a.** Cytoplasmic localization of immunoreactivity with subtle enhancement at the cellular periphery was observed. **b.** Intense staining of the adjacent vasculature in the absence of immunoreactivity of the tumor. **a.**, x 400; **b.**, x 100

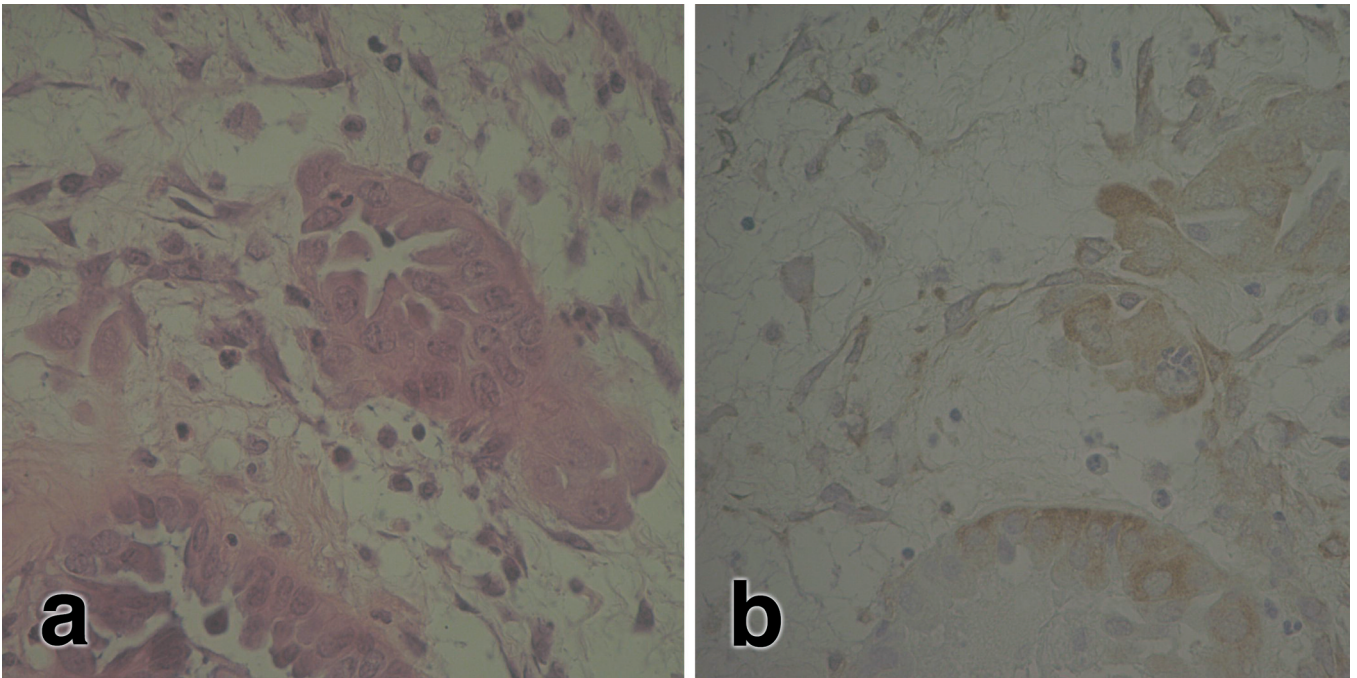
Fascin in ovarian tumors

Fig. 4. A focus of microinvasion in a borderline serous tumor (**a**) showing increased fascin immunoreactivity (**b**). x 200

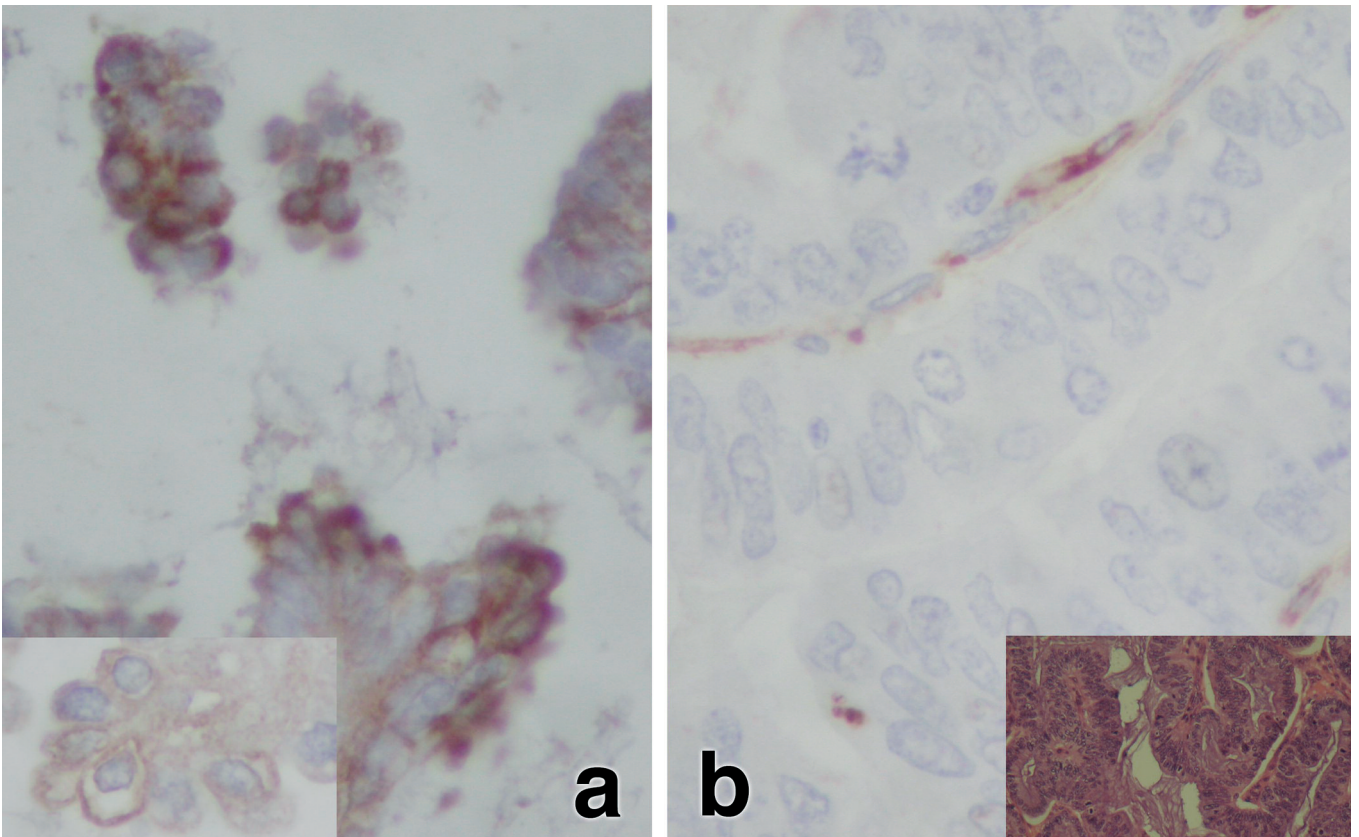


Fig. 5. a. In cases with mixed differentiation, serous and endometrioid phenotypes, the serous component showed preferential staining. x 200. **b.** Area of endometrioid carcinoma, negative for fascin immunostain. Inset: HE stain. x 200, x 100

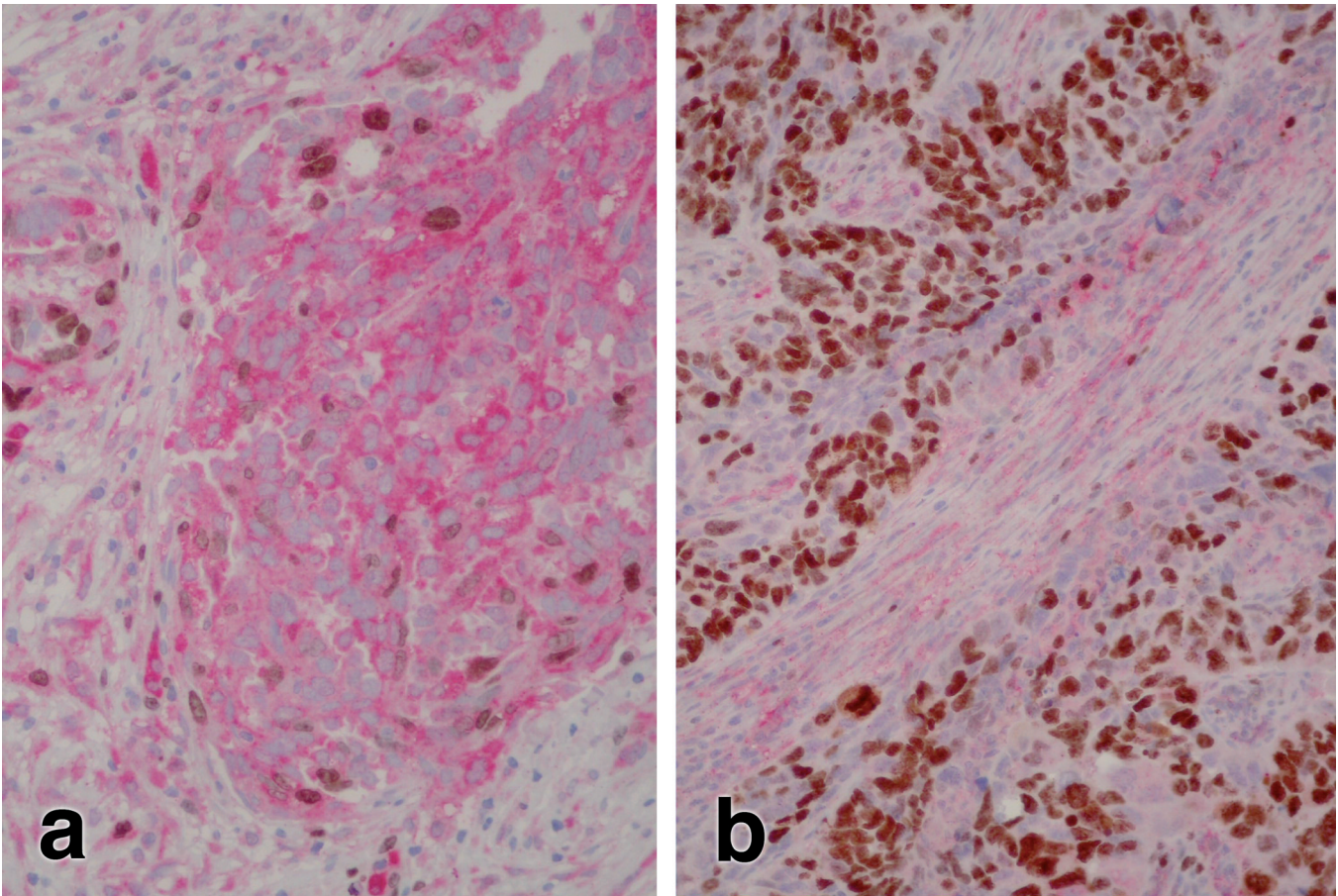


Fig. 6. Double immunohistochemical labelling of fascin and Ki-67: an inverse association was often observed. Note IPS (immediate peritumoral stroma) immunoreactivity. x 100

from advanced stage ovarian carcinomas.

Another study of ovarian carcinomas contained different observations. Specifically, Kabukcuoglu et al. (2006a) reported that epithelial fascin immunoreactivity was not statistically different in cystadenomas vs. borderline tumors or carcinomas. We found significant differences of fascin between borderline tumors or carcinomas vs. cystadenomas. In addition, we found significantly increased fascin in advanced-stage tumors vs. early stage carcinomas and in serous carcinomas vs. endometrioid carcinomas

A careful review of the paper by Kabukcuoglu et al (2006a), studying 132 neoplasms, reveals methodological differences. The immunoreactivity scores were calculated with different methods. They added the score for extent and the score for intensity of immunoreactivity. In addition, they did not specify how the scoring for intensity of the immunoreactivity was derived. We used the endothelial immunostaining as an internal control in each slide, in accordance with previous publications. More importantly, we chose to highlight the neoplastic subpopulation showing the highest intensity of immunoreactivity. The rationale for

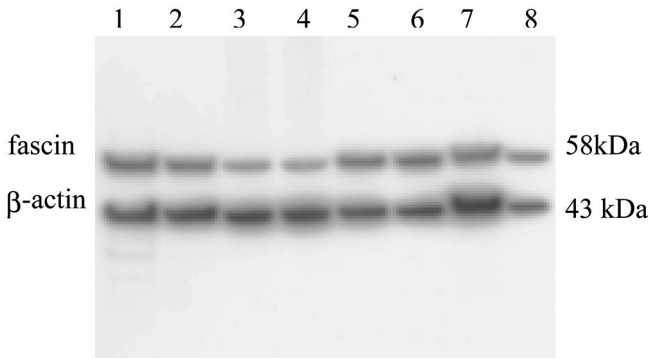


Fig. 7. Western Blotting analysis of fascin protein in cell extracts of frozen tumour samples: columns 1-2 represent samples of an endometrioid carcinoma, 3- 4 of a mucinous borderline tumor, 5-8 of two serous carcinomas.

this selection comes from studies using the so called “in vivo invasion assays” (Wang et al., 2005), showing that, at any given time and location within a tumor, the motile

tumor cells -related to invasion and metastasis-represented only a subset of the entire tumor cell population. Finally, we tried to validate selected immunohistological scores by comparing them to western blotting results. Leaving the aforementioned methodological issues aside, and studying the recent literature one can find that, in other carcinomas, fascin immunoreactivity has been related to tumor aggressiveness estimated by TNM staging or patient survival (Pelosi et al., 2003a; Yoder et al., 2005; Zigeuner et al., 2006; Hashimoto et al., 2006; Choi et al., 2006; Jin et al., 2006). Complete survival data is not yet available for analysis in our cohort of patients but we look forward to examining the role of fascin as a potential prognostic indicator in ovarian carcinomas.

Our findings demonstrate, for the first time in surgical material, that fascin immunoreactivity is increased in samples obtained from metastatic ovarian carcinomas compared to samples obtained from the corresponding primaries. This observation suggests that fascin may be an important factor in ovarian carcinoma metastasis. Moreover, our findings are in agreement with recent observations regarding the expression of *BRMS1* (Zhang et al., 2006), which has been cloned as a metastasis-suppressor gene for human breast cancer and has been mapped to chromosome 11q13.1-q13.2 (Seraj et al., 2000). In metastatic ovarian carcinomas *BRMS1* is downregulated (Zhang et al., 2006). Its transfection in highly metastatic ovarian carcinoma cell lines inhibits metastasis and decreases cellular motility. One of the functional roles assigned to *BRMS1* is downregulation of fascin expression. Fascin overexpression as a result of *BRMS1* downregulation has been proposed as an important metastasis-promoting mechanism (Zhang et al., 2006).

Generally, in surgical pathology, the prediction of tumor behavior depends on the evaluation of several parameters, one of them being the growth fraction. MIB1 immunoreactivity is used frequently to estimate the growth fraction of a tumor. MIB1 and fascin immunoreactivities have been compared with variable results. Some studies have suggested an inverse relationship between MIB1 and fascin immunoreactivities (Pelosi et al., 2003a; Hashimoto et al., 2006). We have confirmed that statistically and by using double immunostaining. The latter showed clearly that tumor cells overexpressing fascin were not cycling. This finding is in agreement with the previously expressed notion that the invading cells are not proliferative (Wang et al., 2005). In addition, it introduces the concept of using various markers to assess tumor cell subpopulations with different characteristics.

Primary mucinous ovarian tumors do not show high fascin immunoreactivity in general (Cao et al., 2004). Especially, borderline mucinous tumors show limited fascin expression. It is well known that various adenocarcinomas metastatic to the ovaries may mimic mucinous primaries, and focally they may simulate a borderline mucinous tumor. Certain types of these

metastatic adenocarcinomas show increased fascin expression (Lu et al., 2004; Hashimoto et al., 2006). Thus, in agreement with the findings by Cao et al. (2004), fascin immunoreactivity might be evaluated in addition to other markers commonly used for the diagnostic work-up of ovarian mucinous tumors, that is cytokeratin 7, cytokeratin 20 and dpc4 (Wauters et al., 1995; Chu and Weiss, 2002; Ji et al., 2002; Hart, 2005).

Our study was based on immunohistochemical data and it could not overcome limitations imposed by the nature of the investigative methodology. It was presumed that enhanced immunostaining reflected overexpression, for several reasons. The regulation of fascin is predominantly transcriptional (Hashimoto et al., 2005a). Fascin protein is in high demand during tumor cell migration when repeated cycles of actin polymerization and bundling take place (Nakagawa et al., 2006; Vignjevic et al., 2006). In addition, fascin mRNA quantification in whole tissue homogenates would include non-neoplastic sources of fascin, (endothelia and myofibroblasts), and it would reflect changes in phenomena other than tumor cell migration, such as neoangiogenesis and desmoplastic reaction.

Increased fascin immunoreactivity in ovarian carcinomas, similarly to other carcinoma types, is associated with certain features of increased tumor aggressiveness. Future studies could determine if fascin may become a routinely helpful marker in surgical pathology or clinical oncology.

References

- Adams J.C. (2004a). Roles of fascin in cell adhesion and motility. *Curr. Opin. Cell Biol.* 16, 590-596.
- Adams J.C. (2004b). Fascin Protrusions in Cell Interactions. *Trends Cardiovasc. Med.* 14, 221-226.
- Cao D., Ji H. and Ronnett B. (2004). Expression of mesothelin, fascin, and prostate stem cell antigen in primary ovarian mucinous tumors and their utility in differentiating primary ovarian mucinous tumors from metastatic pancreatic mucinous carcinomas in the ovary. *Int. J. Gynecol. Pathol.* 24, 67-72.
- Choi P.J., Yang D.K., Son C.H., Lee K.E., Lee J.I. and Roh M.S. (2006). Fascin immunoreactivity for preoperatively predicting lymph node metastases in peripheral adenocarcinoma of the lung 3 cm or less in diameter. *Eur. J. Cardio-thorac.* 30, 538-542.
- Chu P.G. and Weiss L.M. (2002). Keratin expression in human tissues and neoplasms. *Histopathology* 40, 403-439.
- Duh F.M., Latif F., Weng Y., Gei I.L., Modi W., Stackhouse T., Matsumura F., Duan D.R., Linehan W.M. and Lerman M.I. (1994). cDNA cloning and expression of the human homolog of the sea urchin fascin and *Drosophila* singed genes which encodes an actin-bundling protein. *DNA Cell Biol.* 13, 821-827.
- Goncharuk V., Arun B., Middleton L. and Sahin A. (2003). Assessment of actin-binding protein fascin expression in stage I and II invasive breast cancer. A significant correlation with aggressive clinical behaviour. *Mod. Pathol.* 16, 123.
- Grothey A., Hashizume R., Sahin A.A. and McCrea P.D. (2000). Fascin, an actin-bundling protein associated with cell motility, is up-regulated in hormone receptor negative breast cancer. *Br. J. Cancer*

- 83, 870-873.
- Hart W.R. (2005). Diagnostic challenge of secondary (metastatic) ovarian tumors simulating primary endometrioid and mucinous neoplasms. *Pathol. Int.* 55, 231-243.
- Hashimoto Y., Shimada Y., Kawamura J., Yamasaki S. and Imamura M. (2004). The prognostic relevance of fascin expression in human gastric carcinoma. *Oncology* 67, 262-270.
- Hashimoto Y., Skacel M. and Adams J.C. (2005a). Roles of fascin in human carcinoma motility and signaling: Prospects for a novel biomarker? *Int. J. Biochem. Cell Biol.* 37, 1787-1804.
- Hashimoto Y., Ito T., Inoue H., Okumura T., Tanaka E., Tsunoda S., Higashiyama M., Watanabe G., Imamura M. and Shimada Y. (2005b). Prognostic significance of fascin overexpression in human esophageal squamous cell carcinoma. *Clin. Cancer Res.* 11, 2597-2605.
- Hashimoto Y., Skacel M., Lavery I.C., Mukherjee A.L., Casey G. and Adams J.C. (2006). Prognostic significance of fascin expression in advanced colorectal cancer: an immunohistochemical study of colorectal adenomas and adenocarcinomas. *BMC Cancer* 6, 241.
- Hu W., McCrean P.D., Deavers M., Kavanagh J.J., Kudelka A.P. and Verschraegen C.F. (2000). Increased expression of fascin, motility associated protein, in cell cultures derived from ovarian cancer and in borderline and carcinomatous ovarian tumors. *Clin. Exp. Metastasis* 18, 83-88.
- Jawhari A.U., Buda A., Jenkins M., Shehzad K., Sarraf C., Noda M., Farthing M.J., Pignatelli M. and Adams J.C. (2003). Fascin, an actin-bundling protein, modulates colonic epithelial cell invasiveness and differentiation in vitro. *Am. J. Pathol.* 162, 69-80.
- Ji H., Isacson C., Seidman J.D., Kurman R.J. and Ronnett B.M. (2002). Cytokeratins 7 and 20, Dpc4, and MUC5AC in the distinction of metastatic mucinous carcinomas in the ovary from primary ovarian mucinous tumors: Dpc4 assists in identifying metastatic pancreatic carcinomas. *Int. J. Gynecol. Pathol.* 21, 391-400.
- Jin J.S., Yu C.P., Sun G.H., Lin Y.F., Chiang H., Chao T.K., Tsai W.C. and Sheu L.F. (2006). Increasing expression of fascin in renal cell carcinoma associated with clinicopathological parameters of aggressiveness. *Histol. Histopathol.* 21, 1287-1293.
- Kabukcuoglu S., Oner U., Ozalp S., Bildirici K., Yalcin O. and Colak E. (2006a). The role of actin bundling protein fascin in the progression of ovarian neoplasms. *Eur. J. Gynecol. Oncol.* 27, 171-176.
- Kabukcuoglu S., Ozalp S., Oner U., Bildirici K., Yalcin O., Oge T. and Colak E. (2006b). Actin bundling protein fascin expression in ovarian neoplasms: Comparison of histopathologic features of tumors obtained by the first and secondary cytoreduction surgeries. *Eur. J. Gynecol. Oncol.* 27, 123-128.
- Kureishy N., Sapountzi V., Prag S., Anilkumar N. and Adams J.C. (2002). Fascins, and their roles in cell structure and function. *Bioessays* 24, 350-361.
- Lu Z.H., Hu L.P., Evers S., Chen J. and Shen Y. (2004). Differential expression profiling of human pancreatic adenocarcinoma and healthy pancreatic tissue. *Proteomics* 4, 3975-3988.
- Nakagawa H., Terasaki A., Suzuki H., Ohashi K. and Miyamoto S. (2006). Short-term retention of actin filament binding proteins on lamellipodial actin bundles. *FEBS Lett.* 580, 3223-3228.
- Pelosi G., Pastorino U., Pasini F., Maissoneuve P., Fraggetta F., Iannucci A., Sonzogni A., De Manzoni G., Terzi A., Durante E., Bresola E., Pezzella F. and Vialle G. (2003a). Independent prognostic value of fascin immunoreactivity in stage I non-small cell lung cancer. *Br. J. Cancer* 88, 537-547.
- Pelosi G., Pasini F., Fraggetta F., Pastorino U., Iannucci A., Maissoneuve P., Arrigoni G., De Manzoni G., Bresola E. and Viale G. (2003b). Independent value of fascin immunoreactivity for predicting lymph node metastases in typical and atypical pulmonary carcinoids. *Lung Cancer* 42, 203-213.
- Seraj M.J., Samant R.S., Verderame M.F., Welch D.R. (2000). Functional evidence for a novel human breast carcinoma metastasis suppressor, BRMS1, encoded at chromosome 11q13. *Cancer Res.* 60, 2764-2769.
- Silverberg S. (2000). Histopathologic Grading of Ovarian Carcinoma: A Review and Proposal. *Int. J. Gynecol. Pathol.* 19, 7-15.
- Tong G.X., Yee H., Chiriboga L., Hernandez O. and Waisman J. (2005). Fascin-1 expression in papillary and invasive urothelial carcinomas of the urinary bladder. *Hum. Pathol.* 36, 741-746.
- Tseng Y., Kole T., Lee J., Fedorov E., Almo S., Schafer B. and Wirtz D. (2005). How actin crosslinking and bundling proteins cooperate to generate an enhanced cell mechanical response. *Biochem. Biophys. Res. Commun.* 334, 183-192.
- Tubb B., Mulholland D.J., Vogl W., Lan Z.J., Niederberger C., Cooney A. and Bryan J. (2002). Testis fascin (FSCN3): a novel paralog of the actin-bundling protein fascin expressed specifically in the elongate spermatid head. *Exp. Cell Res.* 275, 92-109.
- Vignjevic D., Kojima S., Svitkina T. and Borisy G.G. (2006). Role of fascin in filopodial protrusion. *J. Cell Biol.* 174, 863-875.
- Wang W., Goswami S., Sahai E., Wyckoff J., Segall J. and Condeelis J. (2005). Tumor cells caught in the act of invading: their strategy for enhanced cell motility. *Trends Cell Biol.* 15, 138-145.
- Wauters C., Smedts F., Gerrits L., Bosman F., Ramaekers F. (1995). Keratins 7 and 20 as diagnostic markers of carcinomas metastatic to the ovary. *Hum. Pathol.* 26, 852-855.
- World Health Organization Classification of tumours. Tumours of the breast and female genital organs. (2003). Tavassoli F. and Devilee P. (eds). IARC Press. Lyon.
- Yamaguchi H., Wyckoff J. and Condeelis J. (2005). Cell migration in tumors. *Curr. Opin. Cell Biol.* 17, 559-564.
- Yoder B.J., Tso E., Skacel M., Pettay J., Tarr S., Budd T., Tubbs R.R., Adams J.C. and Hicks D.G. (2005). The expression of fascin, an actin-bundling motility protein, correlates with hormone receptor-negative breast cancer and a more aggressive clinical course. *Clin. Cancer Res.* 11, 186-192.
- Zhang S., Lin Q.-D. and Di W. (2006). Suppression of human ovarian carcinoma metastasis by the metastasis-suppressor gene, BRMS1. *Int. J. Gynecol. Cancer* 16, 522-531.
- Zigeuner R., Droschl N., Tauber V., Rehak P. and Langner C. (2006). Biologic significance of fascin expression in clear cell renal cell carcinoma: Systematic analysis of primary and metastatic tumor tissues using a tissue microarray technique. *Urology* 68, 518-522.