Fascin Regulates Prostate Cancer Cell Invasion and Is Associated with Metastasis and Biochemical Failure in Prostate Cancer

Andrew D. Danel,1 Emy Behmoaram,1 Robin T. Vollmer,4 Jacques Corcos,3 Krikor Bijian,1 Kanishka Sircar,1,2 Jie Su,1 Jinsong Jiao,1 Moulay A. Alaoui-Jamali,1, and Tarek A. Bismar1,2

Abstract

Purpose: Prostate cancer metastasis to secondary organs is considered an initial event in the development of hormone refractory disease and remains the major cause of death among prostate cancer patients. In this study, we investigated the role of fascin, a cytoskeleton actin– bundling protein involved in the formation of filopodia and cell migration, in prostate cancer progression.

Experimental Design: Fascin protein expression was examined by immunohistochemistry in a cohort of 196 patients with localized prostate cancer and across several stages of disease progression, including hormone refractory disease. Cellular changes were also assessed in vitro and in vivo in DU145 prostate cancer cell line using fascin gene silencing.

Results: Fascin epithelial expression was significantly up-regulated in localized and hormone refractory prostate cancer compared with benign prostate tissue (P < 0.05). Furthermore, high fascin expression was associated with an increased rate of prostate-specific antigen recurrence following radical prostatectomy (P = 0.075), signifying more aggressive clinical course, thus supporting a function for fascin in prostate cancer progression. In cellular models, fascin gene silencing using small interfering RNA in the androgen-independent prostate cancer cell line DU145 decreased cell motility and invasiveness while increasing cell adhesive properties. In addition, fascin small interfering RNA – expressing DU145 cells implanted orthotopically in mouse prostate showed significantly decreased growth (P < 0.005) and drastically prevented the formation of lymph node metastases (P < 0.001) compared with their matched controls.

Conclusions: Our data show a function of fascin in the regulation of prostate cancer progression and emphasize the importance of fascin as a prognostic marker for aggressive disease and as a potential therapeutic target for advanced androgen independent disease.

Prostate cancer remains one of the most prevalent cancers and a major source for morbidity and mortality in the Western world (1–4). In 2007, ~ 219,000 new patients were diagnosed with prostate cancer causing about 27,000 cancer related deaths (5). Disease progression and the development of hormone refractory disease remain major causes of cancer related death.


to significantly alter the disease course, improved ways to understand, diagnose, and treat aggressive forms of metastatic prostate cancer are needed.

Tumor cell motility is the hallmark of invasion and an essential step in metastasis (6, 7). Understanding the molecular pathways involved in tumor cell motility and how the tumor microenvironment contributes to cell migration and metastasis is critical to developing improved therapeutic targets for the treatment of metastatic prostate cancer. Predicting disease progression is a major and significant step in identifying patients at increased risk for cancer specific death. Therefore, one goal in the diagnosis and treatment of men with prostate cancer is to develop tissue-based molecular tests to distinguish indolent from aggressive forms of prostate cancer before treatment to focus therapeutic interventions on patients more likely to develop disease progression and hormone refractory disease (8).

A crucial early event by which cancer cells switch from localized to invasive phenotype is initiated by the acquisition of autonomous motile properties, a process driven by remodeling of the cellular cytoskeleton, formation of cell protrusions, and dynamic turnover of multiple focal adhesion proteins. These mechanisms mediate cell-matrix attachments and extracellular matrix degradation, and serve as attraction sites for cell motility and cell migration. Fascin, a 55-kDa monomeric globular...
actin-bundling protein, is a key regulator of the actin cytoskeleton and is the leading regulator of filament bundling necessary for the formation of filopodia, which are actin-based protrusive sensory organelles that contribute to the initiation of cell movement and cell migration. Fascin is structurally unique and an evolutionarily conserved member of actin cross-linking proteins that have a regulatory function in the remodeling of cell cytoskeleton, cell-matrix adhesion, and cell migration and invasion (9). At the cellular level, fascin is highly expressed in filopodia-rich cell types such as neurons, mature dendritic cells, and many transformed cells. Fascin acts by organizing F-actin into very tight bundles, a function that is coordinated with actin polymerization and ultimately drives the filopodial life cycle. Moreover, fascin cycles through states of phosphorylation and dephosphorylation of its conserved S39 site, a process shown to regulate the dynamics of actin-bundling activity and filopodial formation in motile cells (10, 11).

Fascin is widely expressed in mesenchymal and stromal tissues, as well as the nervous system, but is low or absent in adult epithelial cells (12, 13). Recent data from several groups have highlighted that fascin is up-regulated in several human carcinomas, and its expression has been correlated with clinically aggressive behavior and patient survival (13–23). For instance, studies conducted on ovarian epithelial tumors reveal increased fascin expression in advanced serous carcinomas as well as metastatic foci, associating fascin with increased tumor aggressiveness and suggesting it as candidate biomarker in gynecologic malignancies (24). In addition, fascin has also been reported to have significant diagnostic potential for thyroid neoplasms because it was shown to be highly up-regulated in a number of thyroid carcinomas and adenomas (25). Furthermore, a recent tissue microarray-based immunohistochemical (IHC) study in breast tumors with basal-like phenotype described the overexpression of fascin coinciding with the up-regulation of markers related to epithelial-mesenchymal transition, a phenomenon often associated with increased metastatic and invasive potential (26). However, to date, fascin expression in prostate cancer and its role in disease progression have yet to be fully investigated.

Translational Relevance

In the current study, fascin epithelial expression was assessed in a cohort of 196 patients with localized prostate cancer. It was found that the expression levels of fascin are highest in hormone refractory disease compared with localized cancer and benign prostate tissue. Moreover, tumors with high fascin expression are associated with increased rate of prostate-specific antigen relapse after radical prostatectomy. Using an androgen refractory prostate cancer cell model, we showed that fascin inhibition by gene silencing decreased cell motility and cell invasive ability, as well as increased cell adhesive property. In mice, down-regulation of fascin prevented prostate cancer progression. Taken together, this study reflects an association between fascin high expression levels and aggressive disease, and highlights the potential utility of this marker as a therapeutic target for aggressive and hormone refractory prostate cancer.

Materials and Methods

Reagents, cell lines, and cell culture

Human prostate carcinoma DU145 cells were originally obtained from the American Type Culture Collection (Manassas, VA) and maintained according to the provider’s instructions. Briefly, these cells were cultured in RPMI medium (Life Technologies) supplemented with 10% fetal bovine serum and penicillin/streptomycin.

Generation of cells expressing stable fascin small interfering RNA

The following small interfering RNA target sequences were used to generate fascin knockdown clones: fascin-2, cctgcattcttgccacagat, and fascin-2, accgctgcttctcaggc. These small interfering RNA target sequences were cloned into a pSuper-retro puromycin vector, as previously described (27). Control retroviral vector pRetro-Super puromycin alone or expressing fascin small interfering RNA was transfected into Phoenix cells using Genejuice (Novagene). Forty-eight hours posttransfection, the supernatant of Phoenix cells was filtered through a 0.45-μm filter and used to infect target cell lines twice, 24 h apart, in the presence of 8 μg/mL polybrene. Forty-eight hours after infection, polyclonal populations were selected for resistance to 1 μg/mL puromycin for 2 wks to generate stable small interfering RNA-expressing cells and matched (bulk) controls. Individual clones with stable expression of fascin small interfering RNA were isolated. Protein expression was examined by standard Western blot analysis.

Western blot analysis

Western blot analysis was carried out as described earlier (28). Briefly, proteins from total cell and prostate tissue (benign and prostate cancer) lysates (50 μg) were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Monoclonal mouse anti-human fascin (clone 55K-2, Dako Corporation) antibody was used as a primary antibody. Enhanced chemiluminescence was used for visualization. Membranes were stripped and reprobed with monoclonal rabbit anti-human glyceraldehyde-3-phosphate dehydrogenase 14C10 (Cell Signaling), which was used as an internal control.

Invasion assay

Cell invasion experiments were done with 8-μm porous chambers coated with Matrigel (Becton Dickinson) according to the manufacturer’s recommendations. Approximately 30,000 serum starved cells were placed into the upper compartment, and the chambers were
placed into 24-well culture dishes containing 400 μL of RPMI supplemented with 0.2% bovine serum albumin with or without 20% serum in the lower compartment. Cells were allowed to invade through the Matrigel membrane for 48 h. The invasive cells underneath the membrane were fixed and stained. Filters were viewed under a bright-field 40× objective and counted in three different fields of view per sample. Each experiment was done at least three times, and results are expressed as mean ± SD.

**Phagokinetic track assay**

Cell motility was examined using a phagokinetic track assay done as described (29). Briefly, sterile coverslips coated with 1% bovine serum albumin and then a uniform carpet of gold colloidal solution containing 150 μmol/L HAuCl₄·4H₂O and 10 mmol/L Na₂CO₃ was applied as described previously (29, 30). Serum-starved cells were plated at low density (0.5 × 10⁴ per coverslip), allowed to attach for 1 h, and then placed in 35-mm tissue culture dishes. Cells were allowed to migrate for 8 h in the presence of serum-supplemented medium. The cells were then fixed in 0.1% formaldehyde, and the areas cleared of gold particles were examined under a microscope.

**Adhesion assay**

Cells were plated at 5 × 10⁴ cells/well in 96-well plates and incubated for 4 h at 37°C in a humidified 5% CO₂ atmosphere. Medium was aspirated, and cells were washed three times with PBS to remove unbound cells. Bound cells were fixed with 4% paraformaldehyde solution for 20 mins. Following washes with PBS, cells were stained with a 0.1% solution of crystal violet for 2 h at room temperature. Excess crystal violet was washed off with distilled water, and the plate was air dried. Cells were then lysed with 1% SDS solution, and the optical density of each well was measured at 580 nm via spectrophotometry, as previously described (31).

**In vivo tumorigenic and invasion studies on mice**

**In vivo** studies were approved by the McGill Animal Care Committee (Protocol number 4101) and were conducted in accordance with institutional and Canadian Federal Guidelines. Male Scid mice were obtained from Charles River Laboratories, St. Zotique, Quebec, Canada. For primary tumors, eight mice were anaesthetized via an i.p. injection of sodium pentobarbital, and 1 × 10⁶ cells of control and fascin small interfering RNA–expressing DU145 cells (at 70% confluence) were implanted in the prostate via a lower midline incision, as previously described (32). Formation of a bulla indicated a satisfactory injection. The incision was then closed with a single layer of surgical clips. After 50 d, animals were sacrificed, prostate tumors were isolated and weighed, and lymph nodes were counted under a stereomicroscope. For data analysis, an unpaired Student’s t test was used to compare tumor growth between control and small interfering RNA–expressing groups. ANOVA was used to compare groups, and significance was set at P < 0.05 level.

**Patient samples and study population**

The study cohort consisted of 196 patients who were treated by retropubic radical prostatectomy for localized prostate cancer between 1992 and 2004 with a mean follow-up of 42 mos (range, 4-142). Clinical and pathologic data were obtained with approval from the institutional review board at Jewish General Hospital, Montreal, Quebec, Canada. Clinical progression was defined as a postoperative serum prostate-specific antigen elevation of >0.2 ng/mL.

![Fig. 2. Fascin regulates cell invasion, motility, and adhesion in DU145 cells. A, the invasion activity of DU145 Fascin small interfering RNA clones and parental DU145 PSR cells were measured using a Matrigel-coated Boyden chamber assay. Statistical analysis was carried out for at least three independent experiments. Down-regulation of fascin expression resulted in a marked reduction in invasiveness, as compared with matched PSR controls. Representative micrographs (right) and quantification (left). B, DU145-cell motility was assessed using a phagokinetic track assay. Cells were grown on a gold particle coat for 48 h. Migrating cells phagocytose and ingest gold particles as they move, leaving behind a clear track. Fascin small interfering RNA–expressing cells remained stagnant and showed a dramatic decrease in their motility (right), as compared with matched control cells (left). C, adhesion of DU145 cells was monitored 4 h after plating. Cells were washed and stained with crystal violet. Cell adhesion was quantified by measuring optical density at 580 nm. Increased absorbance correlates with increased number of adherent cells. Fascin small interfering RNA–expressing cells were significantly more adhesive than PSR control cells. Data are expressed as the average of at least three independent experiments each in triplicate.](https://www.aacrjournals.org/cancerres/article-pdf/15/4/1378/5251891/CancerRes_2009_15_4_1378.pdf)
Notor tumors or only very small tumors were seen in the small interfering RNA group (\( P < 0.005 \)), which was confirmed by pathologic examination of H&E stained sections of the prostate gland and adjacent lymph nodes. A mean number of lymph node metastases \( \pm \) SD from the DU145 control group was significantly higher than in the fascin small interfering RNA DU145 group.

\[ \frac{1}{2} \]

**Tissue microarray construction**

Prostate samples were embedded in three tissue microarray blocks using a manual tissue arrayer (Beecher Instruments). Each block was assembled without previous knowledge of any clinical or pathologic staging information. One to nine cores (average, 3.3) 0.6 mm in diameter were sampled, including benign, high-grade intraepithelial neoplasia and prostate cancer. After construction, 4-μm sections were cut and stained with H&E on the initial slides to verify the histologic diagnosis. Mean patient age was 64.3 (range, 43-80), and 66.2% of the patients had a tumor grade of pT2. A total of 154 patients had available complete follow-up information related to biochemical failure, which was observed in 25.3% (40 of 154) of patients.

**IHC**

To analyze the expression of fascin in prostate cancer tissue microarrays, 4-μm sections were cut, placed on silane-coated slides (Sigma), and dried overnight at 37°C. Tissue microarray sections were deparaffinized in graded alcohols, rehydrated, and boiled (microwave) in 10 mmol/L citrate buffer (pH 6.0) for antigen retrieval. The tissue microarray slides were subsequently incubated for 32 mins at 37°C with primary monoclonal mouse anti–human fascin antibody (55K-2, Dako; 1:50) using the NexES automated immunostainer (Ventana Medical System). The automated Ventana Medical System uses an indirect biotin-avidin system with a universal biotinylated immunoglobulin secondary antibody. Diaminobenzidine was used as a chromogen, and slides were counterstained with hematoxylin before mounting. All staining procedures were done according to the manufacturer’s recommendations. A multislide control tissue microarray slide containing samples of prostate, colon, and breast was used as positive and negative controls, respectively. Negative control was done by omission of the primary antibody and substituting it with normal mouse 1/200 prediluted serum (Ventana).

**Pathologic analysis**

**Manual scoring of fascin epithelial expression.** All tissue microarray cores were assigned a diagnosis (i.e., benign, high-grade intraepithelial neoplasia, or prostate cancer) by the study pathologist (T.A. Bismar). Protein expression was categorized using a four-tiered system (0, negative; 1, weak; 2, moderate; and 3, strong) expression. Moreover, the extent of expression was quantified into four subgroups of 25%, 50%, 75%, and 100%. A final score was obtained by multiplying the intensity and extent of immunostaining within each sample.

**Semi-automated quantitative image analysis of fascin stromal expression.** A semi-automated quantitative image analysis system, ACIS II (Chromavision), was used to evaluate the tissue microarray slides for stromal fascin expression. The ACIS II device consists of a microscope with a computer controlled mechanical stage. Proprietary software was used to detect the brown stain intensity of the chromogen used for IHC analysis and compared this value to the blue background counterstain. Theoretical intensity levels ranged from 0 to 255 chromogen intensity units. The correlation coefficient for the ASCIS II was \( r^2 = 0.973 \), and the reproducibility for the system was previously tested and confirmed by scoring several tissue microarrays on separate occasions and in previous publications (33, 34).

**Statistical methods.** \( \chi^2 \), Kruskal-Wallis nonparametric tests, log-rank tests, logistic regression, and Kaplan-Meier plots were used to test for associations between the status of fascin and other key variables such as specimen diagnosis, pathologic stage, tumor grade, and status of surgical margins. Kaplan-Meier plots, log-rank tests, and the Cox proportional hazard model were used to test for associations between fascin and disease-free survival.

**Results**

Down-regulation of fascin expression in DU145 cells decreased cell motility and invasiveness in fascin small interfering RNA–expressing DU145 cells in vitro. To examine the impact of fascin on prostate cancer cell invasion, we compared cell
motility and cell invasion of DU145 cells stably expressing a pSUPER-retro vector–based fascin small interfering RNA to control cells expressing empty retroviral particles (PSR). Western blot analysis confirmed an efficient reduction in fascin expression (Fig. 1). Transfection of pSUPER-retro vector control (PSR) did not reduce fascin expression levels.

We monitored the impact of fascin down-regulation on DU145 cell invasion, motility, and adhesion using a Boyden chamber assay, phagokinetic track assay, and cell adhesion assay, respectively. As shown in Fig. 2A, cells with reduced fascin expression showed a significant reduction in their invasive capacity compared with control cells. Moreover, fascin small interfering RNA–expressing DU145 cells revealed a significant reduction in cell motility (Fig. 2B) and a 50% increase in cell adhesive capacity (Fig. 2C). These phenotypes were observed in independent experiments using independent small interfering RNA cell clones (not shown).

Fig. 4. Fascin protein expression at various stages of prostate cancer progression. A, fascin immunostaining in benign prostatic tissues versus localized prostate cancer and hormone refractory prostate cancer. Confidence intervals (95%) show normalized mean protein intensity units of fascin, as determined by quantitative evaluation of IHC. Bars, ± SE. B, representative fascin immunostaining in prostatic tissue samples at various stages of prostate cancer progression using tissue microarray. Fascin expression was strongly expressed in hormone refractory (hormone refractory prostate cancer) epithelium whereas moderately present in localized prostate cancer epithelium. Benign prostate epithelium showed negative or very low intensity. C, representative Western blot analysis of fascin expression in DU145 PSR cells, prostate cancer, and benign prostate tissues. Fascin expression is significantly increased in prostate cancer when compared with benign matched control. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. D, disease-free survival versus high fascin intensity (grade 3). Kaplan-Meier analysis of individuals with clinically localized prostate cancer for fascin protein expression. Lower curve, high intensity fascin (grade 3) expression showing lower-probability disease-free survival versus higher curve of samples showing negative, low, or moderate staining intensity (grade 0-2). Log-rank test for significance yielded a P value of 0.075.
**Table 1. Characteristics of study population**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. with assessable stains</td>
<td>196</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>64.3 (43-80)</td>
</tr>
<tr>
<td>Mean initial PSA (range)</td>
<td>9.0 (0.5-51.9)</td>
</tr>
<tr>
<td>Mean Gleason score (range)</td>
<td>6.9 (5-9)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>131</td>
</tr>
<tr>
<td>pT3</td>
<td>64</td>
</tr>
<tr>
<td>pT4</td>
<td>1</td>
</tr>
<tr>
<td>Number with follow-up</td>
<td>154</td>
</tr>
<tr>
<td>Mean of follow-up (mo, range)</td>
<td>42 (0.4-142)</td>
</tr>
<tr>
<td>No. observed with biochemical failure</td>
<td>40</td>
</tr>
<tr>
<td>Average grade of fascin (range)</td>
<td>1.4 (0.2-3)</td>
</tr>
<tr>
<td>Average of cells + for fascin (%, range)</td>
<td>58 (4-100)</td>
</tr>
</tbody>
</table>

Abbreviations: +, positive; PSA, prostate-specific antigen.

**Down-regulation of fascin expression in DU145 cells decreased tumor growth and lymph node metastases in mice.** To examine the preclinical impact of fascin knockdown on prostate cancer progression, we compared tumor growth and the incidence of lymph node metastases induced by control and fascin small interfering RNA–expressing DU145 cells implanted into the prostate. Down-regulation of fascin led to >50% reduction in tumor growth (Fig. 3A-C; \( P < 0.005 \)). In addition, no gross or microscopic lymph nodes were observed with DU145 expressing fascin small interfering RNA, whereas an average of nine nodules per mouse was formed by control cells (Fig. 3B and C, top). This was also confirmed by histologic exam of mouse prostates and the surrounding lymph nodes (Fig. 3B and C, bottom, and Fig. 3D) and by fascin IHC (data not shown).

**Fascin epithelial expression is associated with prostate cancer progression.** To assess epithelial protein expression of fascin in relation to cancer progression, we used two separate tissue microarray blocks composed of benign (19), localized prostate cancer (28), and hormone refractory prostate cancer (49). Error bars and examples of protein expression, as measured by IHC at various stages of prostate cancer and benign prostate tissue, are shown in Fig. 4A and B. The mean epithelial expression of fascin in hormone refractory prostate cancer was 0.98, which was slightly higher than localized prostate cancer (0.86), and both were significantly higher than benign prostate tissue (0.18; \( P = 0.006 \) and 0.045), respectively. Using an outcome tissue microarray of 196 men with localized prostate cancer, only 7% of benign prostate tissue expressed moderate or high intensity fascin levels compared with 72% of high-grade intraepithelial neoplasia and 45% in invasive cancer (\( P \approx 0 \)). Western blot analysis of extracts of DU145 PSR cells, prostate cancer, and benign prostate tissues adjusted for protein concentration showed an increase in the expression of fascin in DU145 PSR and cancer tissues when compared with matched control benign prostate tissue (Fig. 4C).

**Characterization of fascin epithelial and stromal expression in localized prostate cancer, high-grade intraepithelial neoplasia, and benign prostate tissue.** Using high throughput tissue microarray, we assessed the expression of epithelial fascin protein by IHC in a cohort of 196 men with localized prostate cancer. Table 1 summarizes the demographics of the study cohort. Using the methods above, 196 of 240 patient samples were available for analysis. Prostate cancer and high-grade intraepithelial neoplasia were more likely to show increased fascin expression compared with benign prostate tissue (70% and 93% versus 29%), respectively. The mean epithelial fascin expression of high-grade intraepithelial neoplasia was also above that of prostate cancer and benign prostate tissue (\( P \approx 0 \)). Using the ACIS II semi automated image analysis system, the mean fascin stromal expression was significantly different between the three types of prostate tissue analyzed, being highest in prostate cancer versus high-grade intraepithelial neoplasia and benign prostate tissues (\( P = 0.002 \)). Table 2 shows the distribution of epithelial and stromal fascin expression in the three types of prostate tissue.

To investigate fascin expression as a marker for prostate cancer, we investigated the level at which fascin epithelial expression is best able to differentiate between malignant and benign prostate tissue, using cut points of intensity level (negative, weak, moderate, and high) and the percentage of cells showing epithelial fascin expression. An intensity grade of three showed a 97% probability of that sample being cancer, with 79% certainty, which was highest among other intensity levels and percentages of epithelial cell staining. Complete analysis with several tested cut points is summarized in Table 3.

**Fascin epithelial expression is associated with biochemical failure in patients with localized prostate cancer.** We investigated the epithelial expression of fascin and its association with pathologic parameters, including pathologic stage and surgical margin. There was no statistically significant association between the epithelial expression of fascin and either Gleason score, pathologic stage, or surgical margins. In this cohort of patients, presurgical serum prostate-specific antigen was the only parameter significantly associated with \( pT \) stage > pT2. However, we noticed that the maximum intensity of epithelial fascin was most strongly associated (although at a marginal statistical level) with pathologic stage (> pT2; \( P = 0.07 \)), along with:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Benign</th>
<th>HGPIN</th>
<th>Cancer</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. specimens</td>
<td>251</td>
<td>83</td>
<td>872</td>
<td>—</td>
</tr>
<tr>
<td>Grade &gt; 0 (%)</td>
<td>29</td>
<td>93</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>Mean cells + (%), range</td>
<td>17 (0-100)</td>
<td>67 (0-100)</td>
<td>50 (0-100)</td>
<td>0</td>
</tr>
<tr>
<td>Mean stromal fascin</td>
<td>183.5 (136-211)</td>
<td>181.5 (155-205)</td>
<td>185.6 (131-214)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Abbreviation: HGPIN, high-grade intraepithelial neoplasia.

\( P^* \) value for grade was evaluated by \( \chi^2 \) test, and the \( P \) value for percent epithelium was analyzed via a Kruskal-Wallis test. \( P \) value for stromal fascin expression was evaluated with a log-rank test.
with an average percentage of cells positive \((P = 0.06)\) for fascin expression.

Because our in vitro studies showed that a reduction of fascin expression caused a decrease in the invasive ability of prostate cancer cells, we then investigated if tumors with high level epithelial fascin expression (intensity, 3) are associated with aggressive behavior compared with tumors showing moderate or less epithelial fascin expression. Patients with high levels of epithelial fascin expression showed lower probability of disease-free survival compared with patients with moderate, low, or no expression \((P = 0.075)\). Figure 4D is a Kaplan-Meier curve showing the association between fascin epithelial expression and probability of disease-free survival of patients with localized prostate cancer. Fascin stromal expression at mid point levels of 188, as measured by the ASCIS II image analysis system, showed an association with lower disease-free survival, but it did not reach statistical significance \((P = 0.75);\) data not shown.

### Discussion

This is the first study to investigate and characterize fascin expression as a biomarker in prostate cancer using high-throughput tissue microarrays of localized and hormone refractory prostate cancer combined with preclinical in vitro and in vivo studies in an androgen-independent prostate model.

Herein, we investigated fascin expression in relation to disease progression and biochemical failure in patients with localized prostate cancer. The observations noted in patient clinical samples are in line with our in vitro and in vivo findings. Fascin is a known stromal marker that has also been reported to be expressed in several epithelial malignancies, including the pancreas, ovary, colon, and breast. Furthermore, fascin expression has been noted to be associated with more aggressive behavior \((14)\). The current study strengthens these observations by showing an association between fascin protein expression and prostate cancer progression. Fascin epithelial expression was significantly elevated in high-grade intraepithelial neoplasia lesions and invasive prostate cancer relative to benign prostate tissue. Moreover, fascin expression was found to be highest in hormone refractory prostate cancer, which represents the final stage of disease progression and is associated with subsequent high mortality rate among patients. Furthermore, we showed a strong association between high epithelial fascin expression and biochemical failure in patients with localized prostate cancer, although at a statistical level that was marginally expressed. This in turn reflects our patient sample size used in this study.

Recent observations may argue that using serum prostate-specific antigen as a marker for disease progression may not be as significant as cancer-specific death \((34–37)\). However, given the fact that our in vitro experiments using small interfering RNA knockdown approach to inhibit fascin expression in DU145 prostate cancer cells clearly confirms a reduction in cell motility and invasion while showing an increase in cell adhesive properties is supportive of our tissue microarrays results. Equally important was the inhibition of prostate cancer growth and metastases noted in a preclinical mouse model wherein control and small interfering RNA–expressing DU145 cells were implanted orthotopically into the prostate. Together, our experimental data support the hypothesis that fascin is a significant player in prostate cancer disease progression. Our data are in agreement with a previous study by Xie et al. \((38)\) wherein down-regulation of fascin in esophageal squamous cell carcinoma cell lines inhibited cell proliferation and invasion while increasing matrix metalloprotease activity. However, the manner by which fascin regulates cell invasion still remains to be determined \((9)\). A study by Yamashiro et al. \((36, 39)\) reported that overexpression of fascin resulted in remodeling of cytoskeleton architecture and increased membrane protrusions rich in fascin. Moreover, a recent study by Hashimoto et al. \((40)\) showed that fascin down-regulation in colorectal carcinomas decreased Rac-dependent migration and focal adhesion turnover. These findings along with the fact that fascin binds to β-catenin, a cell-cell adhesion molecule involved in the Wnt signaling pathway and previously showed to influence prostate cancer progression and the development of bone metastasis \((22)\), suggest that fascin may contribute to prostate cancer invasiveness and progression via these pathways \((41, 42)\). Ongoing studies will help to establish these connections to prostate cancer progression at the molecular level.

In summary, our study shows that fascin expression in prostate cancer cells promotes cell motility and invasion, confirming its increased expression in aggressive prostate cancer phenotypes. Potential drugs targeting fascin and/or intracellular signaling pathways modulated by fascin may prove to be effective in the prevention or treatment of metastatic prostate cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Acknowledgments

We thank Naciba Benlimame for her technical assistance and photography.

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**Table 3. Cut points in fascin and discrimination between benign and malignant**

<table>
<thead>
<tr>
<th>Cut point</th>
<th>Probability of cancer</th>
<th>1-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>0.89</td>
<td>0.51</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0.96</td>
<td>0.74</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0.97</td>
<td>0.79</td>
</tr>
<tr>
<td>% &gt; 30</td>
<td>0.91</td>
<td>0.58</td>
</tr>
<tr>
<td>% &gt; 60</td>
<td>0.93</td>
<td>0.62</td>
</tr>
</tbody>
</table>

NOTE: Tissue microarray specimens with high-grade intraepithelial neoplasia were excluded from this analysis. A cut point of grade 1 implies that all tumors with grade \(<1\) were considered benign and all those with \(\geq 1\) were considered malignant, and cut points at grades 2 and 3 were done in an analogous fashion. A cut point of \(\% > 30\) implies that all tumors with \(\%\) positive cells \(\leq 30\) were considered benign and all those with \(\%\) positive cells \(> 30\) were considered malignant, and cut point at \(\% > 60\) was done in an analogous fashion. 1-S is the information content provided by the cut point about the presence of cancer versus benign result. A value of 0 for 1-S implies that the information provided is similar to a coin toss, and a value of 1 implies that the information provided is equivalent to certainty.
Regulation of Prostate Cancer Progression by Fascin

References

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