Fascin expression predicts an aggressive clinical course in patients with advanced breast cancer

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Abstract. Fascin is an actin cross-linking protein, which regulates actin dynamics and filopodia or spike formation, as well as the epithelial-mesenchymal transition, and has been implicated in cell motility. Although, fascin is pivotal in mediating the aggressive behaviour of various types of cancer, its prognostic significance according to tumour stage has yet to be evaluated. Therefore, the present study investigated fascin expression in 194 patients diagnosed with invasive ductal carcinoma of the breast between 2000 and 2005. Fascin protein expression levels were evaluated by immunostaining on a tissue microarray, and the association between fascin expression and various clinicopathological parameters was analysed. Fascin expression was significantly correlated with various clinicopathological parameters, including high histological grade, tumour necrosis, resistance to adjuvant therapy, high expression of p53 and Ki-67 and specific therapeutic markers (oestrogen and progesterone receptor negativity; all P<0.05). Furthermore, univariate and multivariate analyses identified a significant association between fascin expression, and poor disease-free and overall survival, in late-stage breast cancer (all P<0.05). Therefore, fascin may be crucial in predicting aggressive tumour behaviour, particularly in patients with advanced-stage disease that has acquired the properties of migration and invasion.

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Introduction

Breast cancer is the most commonly diagnosed neoplasm and the third leading cause of cancer-associated mortality in the United States, with 22.2 mortalities per 100,000 women associated with breast cancer each year. The five-year relative survival rate for breast cancer has gradually increased since the early 1990s and between 2007 and 2011 it was ~89.2%. However, the prognosis of patients with breast cancer is dependent on the disease stage at the time of diagnosis. In particular, the survival rates of patients with localised disease and regional lymph node metastasis at diagnosis are higher than those of patients presenting with distant metastasis (1). A number of studies have established molecular markers, which are associated with distinct histopathological features, the response to adjuvant therapy and/or the clinical outcome of breast cancer (2-7). Furthermore, the following clinicopathological factors are considered to be useful markers for predicting prognosis and identifying therapeutic targets in patients with advanced breast cancer: American Joint Committee on Cancer (AJCC) stage, histological grade, oestrogen receptor (ER) and progesterone receptor (PR) expression, human epithelial growth factor receptor 2 (HER2) amplification, p53 expression and Ki-67 labelling index (2-6). Based on data obtained from molecular or immunohistochemical (IHC) analyses, breast cancer is classified into four major subtypes: Luminal A, luminal B, basal-like and HER2-positive (7). More recently, the luminal B subtype has been subdivided according to HER2 status and Ki-67 labelling index (8).

Despite improvements in the treatment of breast cancer, the high mortality rate of patients with direct invasion of adjacent organs or distant metastases remains a problem (9). Therefore, improved understanding of the molecular and cellular mechanisms of tumour invasion and metastasis is required for the development of more effective treatment strategies. The multi-step process of metastasis involves numerous cellular events, including neovascularisation, stromal invasion, lymphovascular invasion and growth at a secondary site (10,11). Furthermore, increased tumour cell motility, combined with extracellular matrix degradation at the invasive front of the tumour, are critical early processes in metastasis. (12)

Fascin-1 is a 55-kDa cytoskeletal actin-binding protein that packages actin filaments into tertiary structures, including microspikes, stress fibres and membrane ruffles, within dynamic cellular structures, resulting in the enhancement of cell motility, migration and adhesion (13,14). Fascin-1 (also known as fascin) is primarily expressed during embryonic development, while its expression in adults is highly restricted to neurons, glial cells, endothelial cells and antigen-presenting dendritic cells (15). The additional forms of fascin, fascin-2 and -3, are expressed in retinal photoreceptor cells and the testes, respectively (16). Published data has demonstrated that fascin is upregulated or highly expressed in the human cancer of various organs, including the oesophagus (17), breast (18), colon (19), lung (20), stomach (21) and urinary bladder (22), as well as in individual tissues, and that the expression of fascin is associated with aggressive behaviour (23). Previous studies of breast cancer have identified that fascin overexpression is associated with factors representing aggressive tumor behaviour, for example hormonal receptor negativity, a triple-negative subtype and/or a basal-like phenotype (24-26). However, to the best of our knowledge, no reports have thus far identified a correlation between fascin expression and disease-free or overall survival rates, according to the AJCC tumor node metastasis (TNM) stage.

Therefore, the aim of the present study was to investigate fascin expression in a large cohort of patients with invasive ductal carcinoma (IDC) of the breast, and to assess any statistical correlations between fascin expression, and clinicopathologic parameters, molecular subtypes and patient survival according to the AJCC stage of breast cancer.

Patients and methods

Patient selection. The present study included 194 Korean women diagnosed with IDC at Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine (Seoul, Republic of Korea) between 2000 and 2005. Various clinicopathological variables were established by reviewing patient records and haematoxylin and eosin-stained slides. For example, histological grade was determined using the modified Bloom-Richardson-Elston grading system (27). Additionally, tumours were staged with reference to the size and/or extent of the tumour, regional lymph node involvement and metastasis, using the seventh edition of the AJCC TNM classification system (28).

Ethical Statement. The study was performed according to the Declaration of Helsinki and approved by the local Ethics Committee of the Kangbuk Samsung Hospital (KBSMC14011, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea).

Tissue microarray (TMA) construction. A series of 194 tumour TMA specimens were assembled using a tissue-array instrument (AccuMax™ Array; ISU ABXIS Co. Ltd., Seoul, Korea). The TMAs consisted of 10x6 arrays of IDC tissue cores measuring 2.0-mm in diameter, and the cores were obtained from well-preserved, morphologically representative tumour tissue samples in archived, formalin-fixed, paraffin-embedded blocks. The assembled array was held

in an X-Y position guide with a 1-mm increment between the individual samples, a 2-mm depth puncture prevention device and semi-automatic micrometers. Considering the limitations associated with obtaining representative areas of a tumour, the present study used duplicate 2.0-mm diameter tissue cores from each donor block. The percentage of tumour in each tissue core was >70%.

Immunohistochemistry. For IHC staining, the TMA slides were deparaffinised with heat at 55˚C for 30 min, followed by three 5-min washes with xylene (Duksan Pure Chemicals Co., Ltd., Ansan, Korea). The sections were then rehydrated by a series of successive 5-min washes in 100, 90 and 70% ethanol (Duksan Pure Chemicals Co., Ltd.). Antigens were retrieved by microwaving the samples for 4 min 20 sec in 250 ml of 10 mM sodium citrate (pH 6.0, Duksan Pure Chemicals Co., Ltd.). Furthermore, endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxidase (Duksan Pure Chemicals Co., Ltd.) for 20 min. Immunostaining for PR, ER, HER2, p53 and Ki-67 was performed using a DakoCytomation Autostainer with a universal staining system and a ChemMate™ Dako EnVision™ Detection kit (Dako North America, Inc., Carpinteria, CA, USA). The primary antibodies used were as follows: Anti-PR (1:200 dilution; Dako, Glostrup, Denmark), anti-ER (1:200 dilution; Lab Vision Corporation, Fremont, CA, USA), anti-p53 (1:5,000 dilution; Sigma-Aldrich, St. Louis, MO, USA), anti-HER2 (1:200 dilution; Dako) and anti-Ki-67 (1:200 dilution; Dako). For fascin, affinity-purified rabbit anti-human-fascin polyclonal antibody (1:500 dilution; Leica Microsystems Ltd., Milton Keynes, UK) was used, and detection (4 min incubation at room temperature) was performed with the UltraTech horseradish-peroxidase streptavidin-biotin detection system (Beckman Coulter, Inc., Marseille, France) using an automatic staining machine from the Bond™ Intense R Detection kit (Leica Microsystems Ltd., Milton Keynes, UK).

Interpretation of IHC staining. The sections were observed using an Olympus BX51 light microscope (Olympus Corporation, Tokyo, Japan). The IHC results were used to classify the tumours into the following five molecular subtypes: Luminal A (ER- and/or PR-positive, HER2-negative, Ki-67 low), luminal B HER2-positive (ER- and/or PR-positive, HER2-positive), luminal B HER2-negative (ER- and/or PR-positive, HER2-negative, Ki-67 high), HER2-positive (ER- and PR-negative, HER2-positive) and triple-negative (ER-, PR- and HER2-negative) (9,29-31).

Positive fascin immunostaining was defined as exclusive cytoplasmic staining with no nuclear staining. Normal tissue composed of endothelial cells was used as the positive control tissue. Staining intensity of tumour cells was graded on a scale of 0-3, as follows: No staining, 0; weak 1; moderate, 2; or strong, 3 (Fig. 1). In addition, the extent of tumour staining was scored based on the percentage of tumour cells exhibiting staining, using the following scoring system: 1-25% staining, 1; 26-50% staining, 2; 51-75% staining, 3; or 76-100% staining, 4. In cases with a discrepancy between the duplicated cores, the highest score of the two tissue cores was used as the final score. To calculate the combined immunoreactive score, the staining intensity and extent of tumour staining scores were multiplied (9,32).
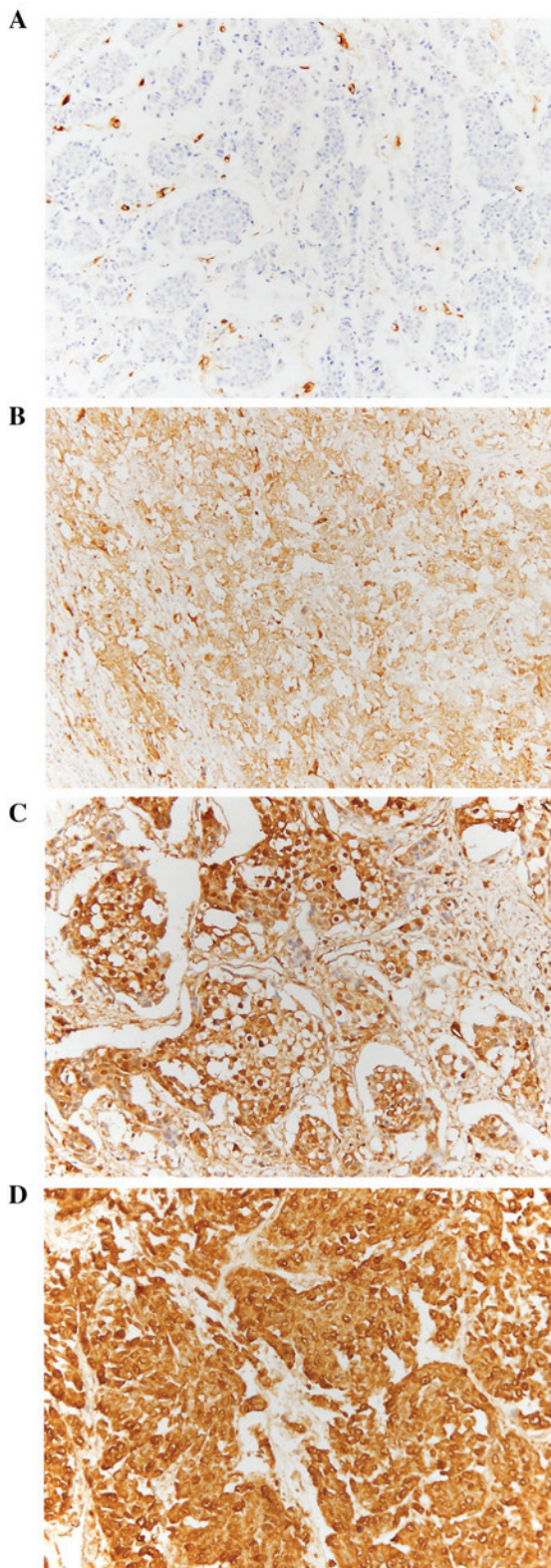
Figure 1. Representative microphotographs revealing (A) negative, (B) weak, (C) moderate and (D) strong intensity fascin expression using immunohistochemical staining (original magnification X200).
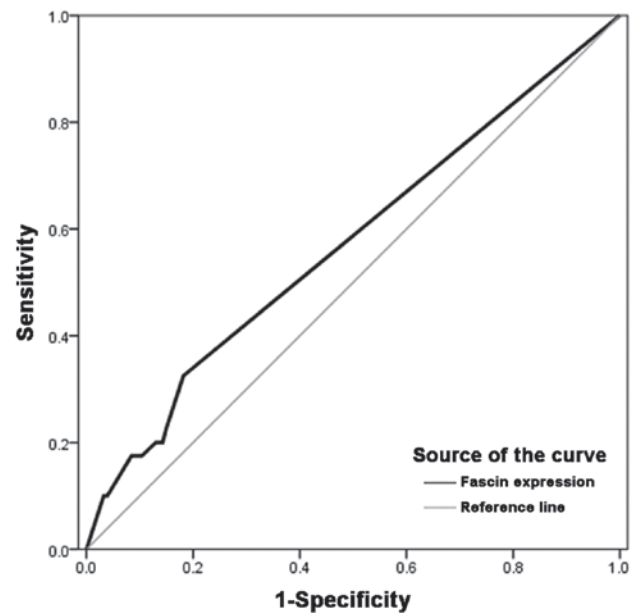

Figure 2. ROC curve for determination of the optimal cut-off value for fascin expression correlated with overall survival rate in patients with invasive ductal carcinoma of the breast (area under the ROC, 0.572). ROC, receiver operating curve.

The ROC curves revealed effective discriminatory power for the correlation between overall survival and fascin expression in the tumour samples (area under the ROC curve, 0.572; Fig. 2). Using the ROC curve, fascin expression was classified as negative (intensity score, <1) or positive (intensity score, ≥1).

Statistical analysis. Correlations between specific clinicopathological parameters and fascin expression were analysed by performing the χ^2 test, the linear by linear association test and Fisher's exact test. Furthermore, a Student's t-test was used to examine the association between fascin protein expression and continuous variables, including p53 expression and Ki-67 labelling index. Disease-free survival was defined as the time from the date of diagnosis to the date of recurrence or development of novel distant metastasis. Similarly, overall survival was defined as the time from the date of treatment to the final follow-up visit or cancer-associated mortality. Survival curves were generated using the Kaplan-Meier method and were compared by performing the Tarone-Ware test. Additionally, multivariate analysis was performed to identify independent prognostic markers for disease-free and overall survival using a Cox multistep regression model. P<0.05 was considered to indicate a statistically significant difference, and all statistical analyses were performed using SPSS statistical software for Windows (version 18.0; SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics. The mean and median ages of the patients were 48.2 and 47.0 years, respectively (range, 26-79 years). Treatment strategies for these cases of breast cancer included modified radical mastectomy with axillary lymph node dissection (151 patients; 77.8%), modified

The optimal cut-off values for fascin expression were calculated by plotting receiver operating characteristic (ROC) curves of sensitivity versus 1-specificity. The cut-off value calculated from the ROC curve was then used to evaluate the association between patient mortality and fascin expression.
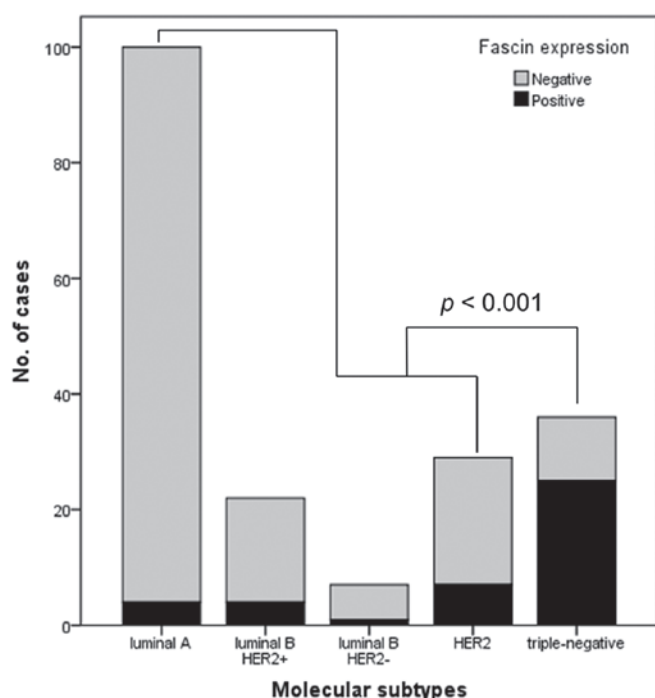
Figure 3. Fascin expression is more frequently observed in triple-negative cases of breast cancer compared with other subtypes (P<0.001). HER2, human epithelial growth factor receptor 2.

radical mastectomy (25 patients; 12.9%), breast-conserving surgery with axillary lymph node dissection (8 patients; 4.1%) and breast-conserving surgery without axillary lymph node dissection (10 patients; 5.2%). At the time of surgery, the T and N classifications of the current cohort were as follows: T1, 66 patients (34%); T2, 114 patients (58.8%); T3, 14 patients (7.2%); and N0, 86 patients (44.3%); N1, 63 patients (32.5%); N2, 22 patients (11.3%); N3, 23 patients (11.9%). Furthermore, the distribution of AJCC staging was as follows: Stage I, 38 patients (19.6%); stage II, 109 patients (56.2%); and stage III, 47 patients (24.2%). Overall, 149 patients received combination chemotherapy with trastuzumab and tamoxifen, 36 received trastuzumab chemotherapy alone and 7 received tamoxifen chemotherapy alone. The remaining 2 patients, who did not receive further treatment, were diagnosed with tubular carcinoma and a 2-mm IDC, respectively. Following surgery, 49 (25.3%) patients developed local recurrence or novel distant metastases, and 40 (20.6%) patients succumbed during the mean follow-up period of 74.2 months.

Fascin expression is correlated with certain clinicopathological parameters and molecular subtype. In the present study, fascin expression was positive in 41/194 (21.1%) and negative in 153/194 (78.9%) cases of IDC. Fascin expression was significantly associated with local recurrence, a high histological grade, tumour necrosis, ER- and PR-negativity, and high expression of p53 and Ki-67 (all P<0.05), but was not significantly associated with HER2 positivity (Table I). Notably, fascin expression was significantly correlated with resistance to adjuvant therapy (P<0.001).

Compared with the other subtypes investigated, triple-negative breast cancer was associated with old age and vascular invasion. HER2 and triple-negative breast cancer were frequently observed in patients with a high histological grade and tumour necrosis (all P<0.05; data not shown). Furthermore, luminal B HER2-negative and HER2 breast cancer were correlated with lymphatic invasion, and patients with luminal B HER2-negative breast cancer exhibited a good response to adjuvant therapy compared with that of other subtypes (all P<0.05; data not shown).

Fascin expression is associated with triple-negative breast cancer and correlated with patient survival. With respect to molecular subtype, the distribution of fascin expression was as follows: Four cases of luminal A (4%), 1 case of luminal B HER2-negative (14.3%), 4 cases of luminal B HER2-positive (18.2%), 25 cases of triple-negative (69.4%) and 7 cases of HER2 (24.1%) breast cancer. A comparison of the various molecular subtypes revealed significantly greater occurrence of fascin expression in the triple-negative subtype than in the luminal A, luminal B or HER2 subtypes (P<0.001; Fig. 3).

Analysis of patient survival using the Kaplan-Meier method identified that fascin expression was significantly correlated with poor disease-free and overall survival rates (P<0.05; Fig. 4). Other prognostic factors, including advanced tumour stage, high histological grade and lymphatic and perineural invasions, were also correlated with worse disease-free and overall survival in univariate analysis (all P<0.05; Table II). In multivariate analyses, the significant correlation between fascin and overall survival persisted (P=0.013), whereas fascin expression only exhibited a marginal association with decreased disease-free survival (P<0.069).

Fascin expression is correlated with patient survival, according to AJCC TNM staging and response to adjuvant therapy. Fascin expression was associated with poor disease-free and overall survival in patients with an advanced tumour stage, nodal metastasis or advanced AJCC stage (all P<0.05; Fig. 5). However, no significant correlation was observed between fascin and survival rate for patients with early stage disease. In multivariate analyses, the significance of the association between fascin expression and disease-free or overall survival was retained for patients with an advanced AJCC stage, and the association between fascin expression and disease-free survival was retained for patients with an advanced T classification (all P<0.05; Table III).

Analysis according to the adjuvant treatment response, identified that fascin expression was significantly associated with disease-free and overall survival in patients with resistance to adjuvant therapy, however, no significance was observed following adjustment for the aforementioned potential confounders (data not shown).

Discussion

During cancer progression, an increase in cell motility is essential for tumour invasion and subsequent dissemination or metastasis. This increase in motility occurs via the modulation of actin filaments to form finger-like plasma membrane protrusions termed invadopodia, (33,34). Such dynamic rearrangement of the actin cytoskeleton is regulated by numerous actin-binding proteins (35), including fascin.
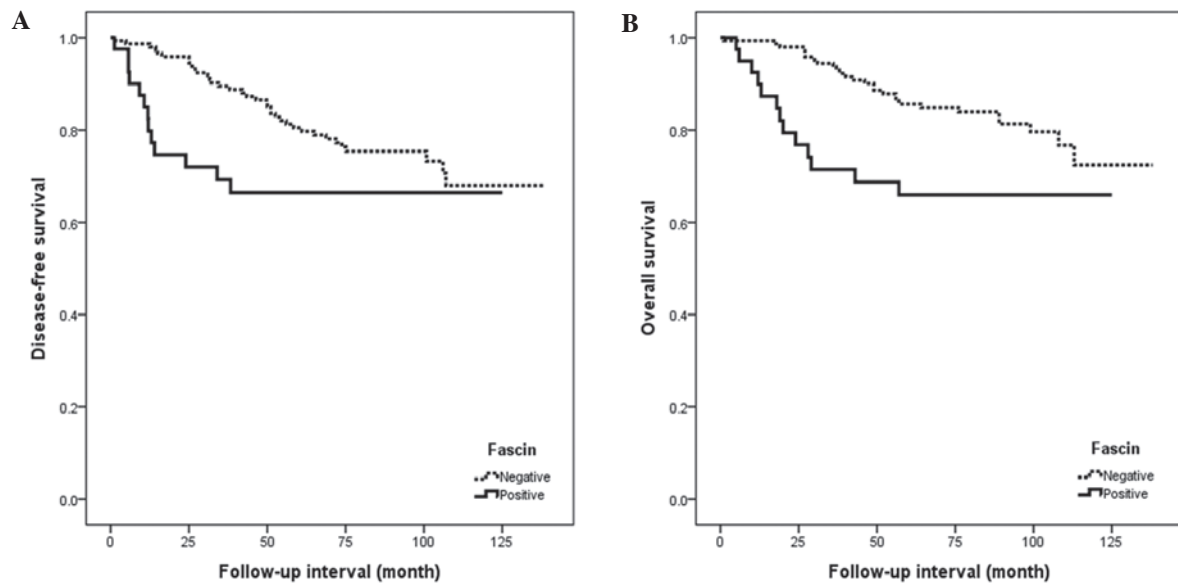
Figure 4. Fascin expression is correlated with poor disease-free and overall survival rates in patients with invasive duct carcinoma of the breast. (A) Disease-free and (B) overall survival curves derived using the Kaplan-Meier method, demonstrating significant correlation with fascin expression. Disease-free survival, P=0.045; overall survival, P=0.004.
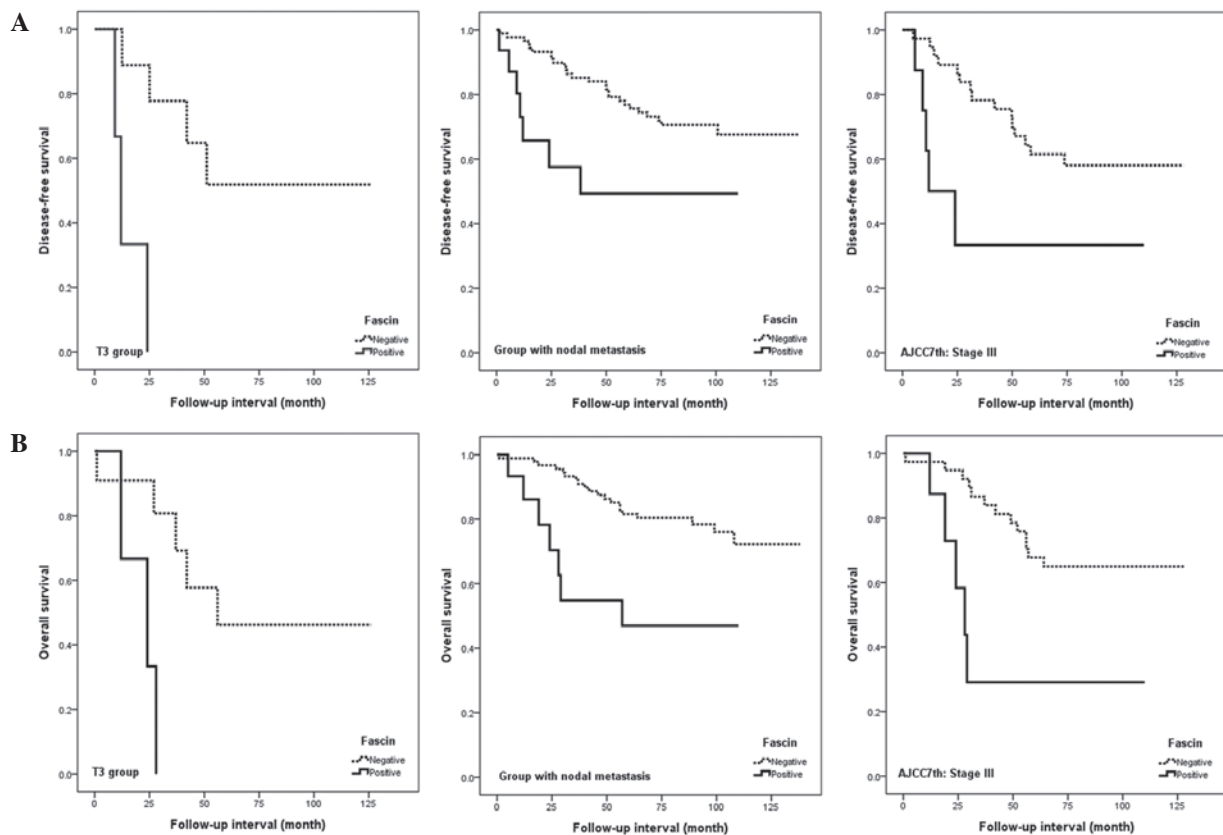

Figure 5. Fascin expression is correlated with disease-free and overall survival rates, according to T3, nodal metastasis and AJCC stage III in invasive duct carcinoma of the breast. (A) Disease-free and (B) overall survival curves derived using the Kaplan-Meier method, demonstrating significant correlation with fascin expression according to T3, nodal metastasis and AJCC stage III advanced breast cancer. Disease-free survival, P=0.001, 0.009 and 0.007; overall survival P=0.013, 0.001 and 0.004, respectively. AJCC, American Joint Committee on Cancer.

Fascin is an actin cross-linking protein, which localises to filopodia at the leading edge of migratory cells. Enhanced fascin expression is associated with increased cell migration and invasion (36). Studies have demonstrated that fascin expression is significantly associated with triple-negativity, as well as poor clinical outcome, in hormone receptor-negative or triple-negative breast cancer (25,26). However, to the best of our knowledge, no previous studies have, thus

Table I. Correlation between clinicopathological parameters and fascin expression in invasive ductal carcinoma.

Parameter	Patients, n (n=194)	Fascin expression, n (%)		P-value
		Negative (n=153)	Positive (n=41)	
Age, years				
≤47	104	82 (53.6)	22 (53.7)	0.994[a]
>47	90	71 (46.4)	19 (46.3)	
AJCC stage				
I	38	29 (19.0)	9 (22.0)	0.441[b]
II	109	85 (55.6)	24 (58.5)	
III	47	39 (25.5)	8 (19.5)	
T category				
T1	66	55 (35.9)	11 (26.8)	0.369[b]
T2	114	87 (56.9)	27 (65.9)	
T3	14	11 (7.2)	3 (7.3)	
N category				
N0	86	61 (39.9)	25 (61.0)	0.155[b]
N1	63	54 (35.3)	9 (22.0)	
N2	22	21 (13.7)	1 (2.4)	
N3	23	17 (11.1)	6 (14.6)	
Local recurrence				
Absence	188	151 (98.7)	37 (90.2)	0.019[c,d]
Presence	6	2 (1.3)	4 (9.8)	
Distant metastasis				
Absence	147	118 (77.1)	29 (70.7)	0.396[a]
Presence	47	35 (22.9)	12 (29.3)	
Tumour size, cm				
≤2	69	58 (37.9)	11 (26.8)	0.188[a]
>2	125	95 (62.1)	30 (73.2)	
Tumour border				
Well-defined	40	30 (19.6)	10 (24.4)	0.501[a]
Ill-defined	154	123 (80.4)	31 (75.6)	
Number of tumours				
Single	181	142 (92.8)	39 (95.1)	0.599[a]
Multiple	13	11 (7.2)	2 (4.9)	
Paget's disease				
Absence	187	146 (95.4)	41 (100)	0.349[a]
Presence	7	7 (4.6)	0 (0.0)	
Histological grade				
1	27	26 (17.0)	1 (2.4)	<0.001[b,d]
2	89	76 (49.7)	13 (31.7)	
3	78	51 (33.3)	27 (65.9)	
Lymphatic invasion				
Negative	91	72 (47.1)	19 (46.3)	0.935[a]
Positive	103	81 (52.9)	22 (53.7)	
Vascular invasion				
Negative	179	144 (94.1)	35 (85.4)	0.093[c]
Positive	15	9 (5.9)	6 (14.6)	
Perineural invasion				
Negative	163	127 (83.0)	36 (87.8)	0.456[a]
Positive	31	26 (17.0)	5 (12.2)	
Tumour necrosis				
Absence	109	95 (62.1)	14 (34.1)	0.001[d]
Presence	85	58 (37.9)	27 (65.9)	

Table I. Continued.

Parameter	Patients, n (n=194)	Fascin expression in tumour, n (%)		P-value[a]
		Negative (n=153)	Positive (n=41)	
Central tumour fibrosis				
Absence	158	122 (79.7)	36 (87.8)	0.238[a]
Presence	36	31 (20.3)	5 (12.2)	
EIC				
Absence	163	125 (81.7)	38 (92.7)	0.088[a]
Presence	31	28 (18.3)	3 (7.3)	
Oestrogen receptor				
Negative	65	33 (21.6)	32 (78)	<0.001[f]
Positive	129	120 (78.4)	9 (22)	
Progesterone receptor				
Negative	92	56 (36.6)	36 (87.8)	<0.001[f]
Positive	102	97 (63.4)	5 (12.2)	
HER2				
Negative	143	113 (73.9)	30 (73.2)	0.929[a]
Positive	51	40 (26.1)	11 (26.8)	
Adjuvant therapy[f]				
Responder	175	145 (96)	30 (73.2)	<0.001[d]
Non-responder	17	6 (4.0)	11 (26.8)	
p53 expression		20.84	63.33	<0.001[d,e]
Ki-67 labelling index		2.56	7.37	<0.001[d,e]

[a]χ^2 test; [b]linear by linear association test; [c]Fisher's exact test; [d]$P<0.05$; [e]Student's t test; [f]excludes two patients that did not undergo adjuvant therapy. AJCC, American Joint Committee on Cancer; EIC, extensive intraductal component; HER2, human epidermal growth factor receptor 2.

Table II. Disease-free and overall survival analyses (n=194).

Survival	Univariate significance[a]	Multivariate significance[b]	Hazard ratio	95% CI
Disease-free				
Fascin expression (negative vs. positive)	0.045	0.069	1.878	0.952-3.705
AJCC stage (I or II vs. III)	<0.001[c]	0.047[c]	1.898	1.01-3.568
Histological grade (1 or 2 vs. 3)	0.003[c]	0.182	1.555	0.813-2.975
Lymphatic invasion (absence vs. presence)	0.002[c]	0.206	1.600	0.772-3.316
Perineural invasion (absence vs. presence)	<0.001[c]	<0.001[c]	3.594	1.92-6.726
Overall				
Fascin expression (negative vs. positive)	0.004[c]	0.013	2.475	1.214-5.042
AJCC stage (I or II vs. III)	<0.001	0.059	1.954	0.976-3.912
Histological grade (1 or 2 vs. 3)	<0.001[c]	0.205	1.616	0.769-3.393
Lymphatic invasion (absence vs. presence)	0.001[c]	0.127	1.936	0.829-4.519
Perineural invasion (absence vs. presence)	<0.001[c]	0.001[c]	3.148	1.577-6.283

[a]Tarone-Ware test; [b]Cox proportional hazard model; [c]$P<0.05$. CI, confidence interval; AJCC, American Joint Committee on Cancer.

far, identified an association between fascin expression and survival in patients with late-stage disease or resistance to adjuvant therapy.

Previous studies have demonstrated that fascin expression is associated with reduced survival in various types of cancer (20-23,26,37). The expression of fascin in tumours was initially considered to be important in promoting tumour progression, and numerous studies of colon cancer have proposed that fascin is more important in advanced stage disease compared with early stage disease (20,37,38).

Table III. Correlations between disease-free and overall survival, and fascin expression according to the size and/or extent of the tumour, nodal metastasis and AJCC stage group.

A, Disease-free survival

Survival	Recurrence/total	Univariate significance[a]	Multivariate significance[b]	Hazard ratio	95% CI
T stage					
T1	14/66	0.969	0.502	1.757	0.338-9.126
T2	28/114	0.177	0.491	1.363	0.565-3.291
T3	7/14	0.001[c]	0.044[c]	15.512	1.08-222.759
Nodal metastasis					
No	16/86	0.217	0.456	1.511	0.51-4.479
Yes	33/108	0.009[c]	0.186	1.842	0.745-4.556
AJCC stage					
I	7/38	0.605	0.491	1.920	0.301-12.26
II	22/109	0.203	0.661	1.251	0.459-3.41
III	20/47	0.007[c]	0.029[c]	3.800	1.148-12.58

B, Overall survival

Survival	Mortality/total	Univariate significance[a]	Multivariate significance[b]	Hazard ratio	95% CI
T stage					
T1	12/66	0.701	0.283	2.562	0.461-14.245
T2	20/114	0.013[c]	0.065	2.551	0.942-6.904
T3	8/14	0.013[c]	0.067	9.952	0.848-116.797
Nodal metastasis					
No	13/86	0.660	0.204	2.143	0.66-6.958
Yes	27/108	0.001[c]	0.101	2.185	0.858-5.566
AJCC stage					
I	5/38	0.361	0.507	1.880	0.292-12.125
II	17/109	0.024	0.098	2.554	0.84-7.767
III	18/47	0.004[c]	0.048[c]	3.239	1.012-10.366

[a]Tarone-Ware test and [b]Cox proportional hazard model, adjusted for histological grade, and lymphatic and perineural invasion. [c]$P<0.05$. AJCC, American Joint Committee on Cancer; CI, confidence interval.

However, a study based on lung cancer reported that fascin expression may be associated with shorter survival in patients with early stage disease (22), although an alternative study of lung cancer did not observe a significant association between fascin expression and survival in early stage disease (39). Thus, there is controversy regarding the association between fascin expression and the clinical outcomes of patients with cancer. In the present study, fascin expression was significantly associated with poor disease-free and overall survival in patients with advanced stage breast cancer. Additionally, fascin expression was significantly associated with worse disease-free and overall survival in patients who were resistant to adjuvant therapy in univariate analyses, although this significance did not remain following multivariate analyses.

Fascin may enhance tumour cell motility and invasion in addition to accelerating tumour cell proliferation, thereby enhancing cancer cell survival and contributing to the development of additional distant metastases (40,41). In the present study, fascin expression was significantly associated with local recurrence and the survival of patients with late stage disease. This may be due to the role of fascin in upregulating other proteins known to be critical for the execution of metastasis, for example urokinase-type plasminogen activator, and matrix metalloproteinases-2 and -9 (19). However, the precise mechanisms by which fascin promotes cancer development and progression are not fully understood, and the potential prognostic value of fascin may vary according to biological factors, the degree of cancer progression and therapeutic tolerance.

There were a number of limitations to the present study. Firstly, it shares similarities with a previous retrospective study that did not identify continuous associations over time (42). Therefore, the current results did not clarify whether associations with fascin are maintained throughout invasive

tumour growth or whether they contribute only to the initiation of invasive growth. This is a significant consideration when determining therapeutic strategies based on the control of fascin activity. In addition, the number of patients with late-stage disease or resistance to adjuvant therapy was relatively small, potentially limiting the statistical power of the study in future analyses.

In conclusion, the results of the present study demonstrated that fascin expression was significantly correlated with breast cancer patient survival, particularly for those with late stage disease. In addition, fascin expression was significantly correlated with known predictors of poor survival, including high histological grade, tumour necrosis, ER- and PR-negativity, resistance to adjuvant therapy and elevated expression of p53 and Ki-67. Thus, fascin may be considered as a key protein in the promotion of tumour progression, and may facilitate the prediction of outcomes and improvement of prognostic models in breast cancer. Furthermore, fascin may present a promising therapeutic target for the inhibition of metastasis, particularly in patients with advanced breast cancer.

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