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Short Communication

Fascin, an actin-bundling protein associated with cell motility, is upregulated in hormone receptor negative breast cancer

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Summary Loss of hormone receptor (HR) status in breast carcinomas is associated with increased tumour cell motility and invasiveness. In an immunohistological study of 58 primary breast cancers, oestrogen (ER) and progesterone (PR) receptor levels were inversely correlated with the expression of fascin, an actin-bundling protein associated with cell motility ($P < 0.0001$ and $P = 0.0019$, respectively). In addition, fascin was preferentially expressed in non-diploid tumours ($P = 0.03$). In summary, the upregulation of fascin in HR-negative breast cancers may contribute to their more aggressive behaviour. © 2000 Cancer Research Campaign

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The presence of oestrogen and progesterone receptors (ER and PR) is an important prognostic and predictive factor in human breast cancer. Patients with tumours that express ER and PR display a less aggressive phenotype with longer disease-free and overall survival than patients with tumours with no or minimal ER/PR expression (Knight et al, 1977; Early Breast Cancer Trialists' Collaborative Group, 1992). In addition, tumours bearing hormone receptors (HRs) are more likely to respond to hormonal therapy (Jordan et al, 1988). In vitro experiments demonstrated that HR-negative breast cancer cell lines show increased motility and invasiveness and that invasion and metastasis of ER-positive cells can be blocked by ER-antagonists such as tamoxifen (Kantor and Zetter, 1996; Rochefort et al, 1998).

Significantly, the precise molecular mechanisms responsible for the association of increased motility and invasiveness of HR-negative breast cancer cells in vitro, and the more aggressive phenotypes observed in the clinic, are unknown. In this regard, however, increased attention has recently been directed in various cell systems towards proteins having the capacity to modulate actin cytoskeleton dynamics (Keely et al, 1997; Carmeci et al, 1998; Honda et al, 1998).

In this study we analysed the expression of fascin, an actin-bundling protein associated with cell motility, in immunohistochemical sections of breast cancer tissue derived from HR-negative and HR-positive tumours. Our results demonstrate that fascin is significantly upregulated in ER- and PR-negative breast cancer, conceivably contributing to the more malignant phenotype of HR-negative tumours via effects upon actin based structures required in cell motility and/or invasion processes.

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MATERIAL AND METHODS

Tissue specimens were obtained from 58 female patients (14 pre-, 44 postmenopausal, median age 61 yrs, range 33–85 yrs) with primary invasive breast cancer. None of the patients had received preoperative irradiation or chemotherapy. The 58 tumours were histologically categorized as 38 ductal, 7 lobular, 7 medullar, 2 mucinous, and 4 tubular carcinomas according to the World Health Organization classification. Normal, non-malignant breast tissue taken from sites adjacent to cancerous lesions were also collected for immunostaining. Patient and tumour characteristics are listed in Table 1.

Formalin-fixed, paraffin-embedded specimens were cut, dewaxed and blocked with 1% (w/v) bovine serum albumin for 30 minutes. Sections were then incubated with a mouse monoclonal anti-human fascin antibody (1:20 dilution; Dako Corp, Carpinteria, CA) for 16 h at 4°C and immunostained using the alkaline phosphatase anti-alkaline phosphatase (APAAP) immune complex method (Universal APAAP kit, Dako) (Pinkus et al, 1997). The binding products were visualized with alkaline phosphatase substrate containing naphthol AS-MX phosphate, Fast Red TR reagents and levamisole as chromogen. Negative controls were carried out by replacing the primary antibody with normal mouse lgG1. Slides were counterstained with haematoxylin and mounted with glycergel (Dako).

Immunohistochemical staining was independently scored by two observers without the knowledge of all other clinicopathologic features. Discrepant cases were reviewed to achieve a consensus. The extent and pattern of the staining were each evaluated. Cases were scored as positive when more than 5% of the tumour cells showed positive staining. Presence of immunoreactivity in more than 50% of cells was scored as diffuse positive. Borderline staining was defined as positive staining confined to the outer edges of the tumour clusters. Non-borderline staining was defined as presence of diffuse staining without apparent spatial localization.

Table 1 Patient and tumour characteristics

	Number of patients	
Total	58	
Median age (range)	61 yrs	(33–85)
Premenopausal	14	
Postmenopausal	44	
Histology (fascin positive tumours)		
ductal	38	(8)
lobular	7	(1)
medullary	7	(2)
mucinous	2	(0)
tubular	4	(1)
Stage		
T1	22	
T2	27	
T3	2	
T4	7	
N0	29	
N1	21	
N2	7	
NX	1	
M0	51	
M1	7	
Receptor status		
ER positive	48	
ER negative	10	
PR positive	44	
PR negative	14	

Oestrogen and progesterone receptor levels were determined using a routine enzyme immunoassay (Leclercq et al, 1986). Tumours were classified as ER- or PR-positive if receptor levels were ≥10 fmol/mg (Sigurdsson et al, 1990). Ploidy of tumour cells was determined by flow cytometry using a method described elsewhere (Vindelow et al, 1983).

Statistical analysis was carried out using the Chi-Square-Test unless otherwise mentioned. Statistical significance was assumed if $P < 0.05$.

RESULTS

Forty-eight of 58 breast cancers had ER levels ≥ 10 fmol/mg (= ER-positive), 43 of which (93%) were negative for fascin. In contrast, in 7 of 10 ER-negative tumours (70%) fascin expression could be readily demonstrated via immunohistochemical staining. Statistically, this difference was highly significant ($P = 0.0002$, Fisher's exact test). An inverse correlation between PR and fascin expression was likewise observed as 39 of 44 (88.6%) PR-positive and only 7 of 14 (50%) PR-negative tumours showed a negative staining reaction for fascin ($P = 0.0047$, Fisher's exact test) (Figure 1A). All 6 tumours negative for ER and PR were fascin positive in contrast to only 4 of 40 (10%) tumours positive for ER and PR (Figure 1B). As expected, ER and PR levels were well correlated ($P = 0.0032$, Spearman test). Fascin expression showed no significant correlation with menopause status, tumour stage, histology, grading, number of lymph nodes involved, presence of metastasis at time of surgery, CEA and CA15–3 levels. However, flow cytometry analysis revealed that fascin negative tumors were more likely diploid (26 of 43 (60.5%)) than non-diploid (17 of 43 (39.5%)), a difference that reached statistical significance ($P = 0.03$). No significant correlation was found between histological tumour grading and ploidy ($P = 0.12$).

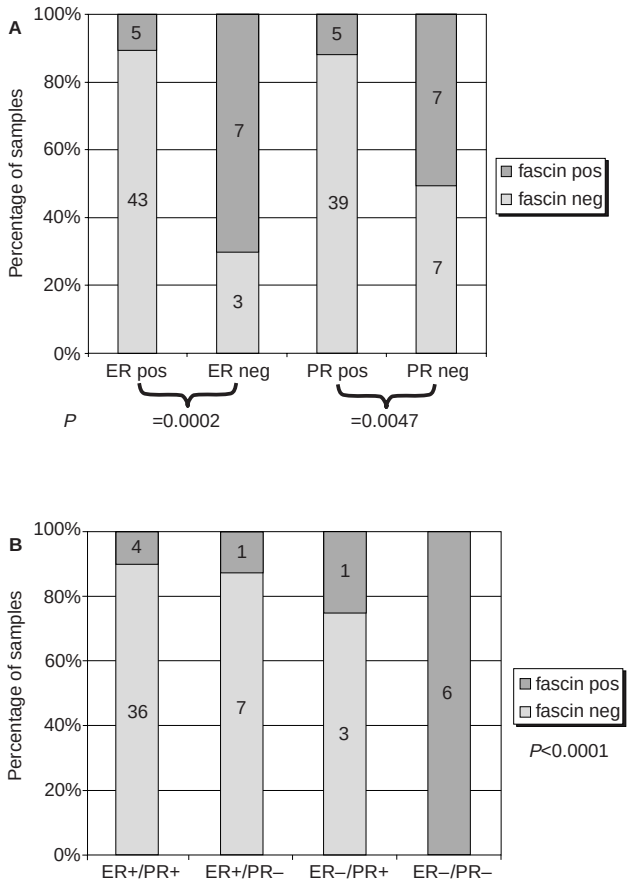

Figure 1 Differential expression of fascin in ER- or PR-positive and -negative breast cancers. Numbers in bars reflect the absolute number of cases. Statistical evaluation by Fisher's exact test (panel A) or Chi-square test (panel B)

Immunohistochemically, fascin positivity appeared as cytoplasmic staining with a marked enhancement in areas of tumour–host interaction in most samples (Figure 2).

DISCUSSION

It is well-known that ER-negative breast cancers display a more aggressive behaviour than ER-positive tumours. As regulation of the actin cytoskeleton plays a crucial role in cell motility and cancer invasion, molecular modulators of actin dynamics might be anticipated to contribute to the malignant phenotype of cancer cells. In the context of normal cells, for example, it is well accepted that the plasticity of the cytoskeleton is modulated via the activities of actin-associated proteins (Mitchison and Cramer, 1996).

Proteins that are capable of bundling or binding actin filaments are numerous and include fimbrin, various tropomyosin isoforms, gelsolin, α-actinin, and α-catenin (Otto, 1994). Early studies identified changes in the organization of the cytoskeleton and junctional proteins in cancer cells, largely indicating a reduction in the expression of actin associated proteins (Ben-Ze'ev, 1985; Asch et al, 1996). In addition, it has been shown that restoration of vinculin and α-actinin expression in cancer cells results in decreased tumorigenicity and metastatic properties (Rodriguez Fernandez et al, 1992; Gluck et al, 1993).

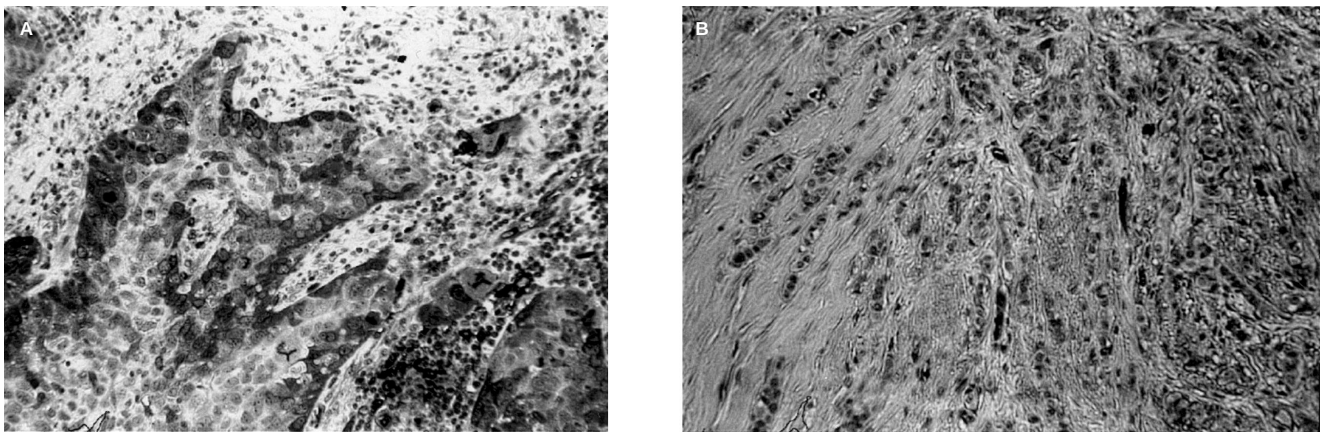

Figure 2 Immunohistological expression of fascin in two different invasive ductal carcinomas (APAAP method, 40×). Note the cytoplasmic staining and enhanced reaction at the tumour–host border in the fascin-positive carcinoma (panel A). A tumour with negative staining for fascin is shown in panel B. Note that endothelia of small vessels are fascin-positive, which validates the immunohistochemistry reaction in fascin-negative tumours

More recently, however, various proteins that modulate dynamic properties of the actin cytoskeleton have been associated with an increased malignant potential in tumour cells, namely the small G-proteins Rac, Rho, and cdc42, the guanine nucleotide exchange factor Tiam-1, EMS1, and actinin-4 (del Peso et al, 1997; Hordijk et al, 1997; Hui et al, 1997; Keely et al, 1997; Honda et al, 1998). Further, the actin-binding protein moesin, a member of the talin-4.1 superfamily was found to be associated with the ER-negative breast cancer phenotype (Carmeci et al, 1998). Similar to fascin, moesin is localized in filopodia and other subcellular structures engaged in active cell motility processes (Amieva and Furthmayr, 1995).

Fascin possess two actin-binding domains within a single molecule, permitting tight packing of filamentous actin (Tilney et al, 1995). Fascin has been found in highly motile and dynamic subcellular structures such as microspikes, lamellipodia, and filopodia (Edwards and Bryan, 1995). The overexpression of native human fascin in a pig epithelial cell line (LLC-PK1) was associated with reduced cell–cell junction integrity, the development of a fibroblastic phenotype, and increased cell motility (Yamashiro et al, 1998), which has recently been correlated with phosphorylation of fascin on serine 39 (Adams et al, 1999). Reduced junctional integrity has likewise been associated with increased fascin expression in the absence of glucocorticoids (Wong et al, 1997), again consistent with reduced adhesion and increased motility in cells expressing relatively greater levels of fascin. Our own experiments, to date published in abstract form, have revealed that fascin exhibits highly increased levels in breast cancer cell lines over-expressing the receptor tyrosine kinase and prognostic indicator c-erbB-2/HER-2, and that such cells exhibit dramatically increased cell dynamics and in vitro motility (Grothey et al, in press).

In this study we demonstrate that the expression of fascin is clearly associated with the absence of ER and PR in invasive breast carcinomas. Moreover, fascin is preferentially expressed in non-diploid tumours although no correlation with the histological grading could be observed. Histologically, fascin staining is often enhanced at the leading edges of infiltrating tumours, which indicates its role as a pathogenic factor for tumour cell invasion. The molecular mechanism leading to the increased expression of fascin in HR-negative breast carcinomas is presently unknown, and work is in progress to analyse the effect of steroid hormones on fascin's transcriptional regulation (GenBank accession number U90355, submitted February 24, 1997, by Tubb BE, Lee R, and Bryan J).

In conclusion, ER-and PR-negative breast cancers are characterized by an increased expression of the actin-bundling, motility-associated protein fascin. It is conceivable that fascin may serve as a downstream cytoskeletal effector contributing to the more aggressive/malignant phenotype of HR-negative breast cancer.

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