The Expression of Fascin, an Actin-Bundling Motility Protein, Correlates with Hormone Receptor–Negative Breast Cancer and a More Aggressive Clinical Course

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ABSTRACT

The invasion and metastasis of tumor cells is a major cause of mortality in cancer patients. In the current study, we investigated the expression of fascin, an actin-bundling motility-associated protein, in 210 invasive breast carcinomas with corresponding 5-year clinical follow-up. Fascin expression was compared with hormone receptor (ER/PR) status, HER2 status, cancer grade, cancer stage, metastasis pattern, disease-free survival, and overall survival. Fascin expression was seen in 16% (33/210) of the cases and correlated with ER negativity (22/33, P < 0.001), PR negativity (21/33, P < 0.001), Bloom-Richardson grade 3 (19/29, P < 0.001), and advanced stage (stage 3 or 4, P = 0.04). There was no correlation between fascin expression and HER2 status or pattern of metastases. Patients whose tumors were positive for fascin showed both a decreased mean disease-free survival (74.44 versus 100.52 months, P = 0.002) and mean overall survival (77.58 versus 98.98 months, P = 0.002), independent of tumor stage and HER2 status, but not independent of ER/PR status or cancer grade. Given fascin’s role in altering cell motility, overexpression may contribute to a more aggressive clinical course in ER/PR-negative breast cancers. If so, then fascin may represent a new molecular target for therapeutic intervention in patients with ER-negative breast cancer.

INTRODUCTION

Breast cancer is one of the leading causes of cancer mortality for women in the United States today. The American Cancer Society estimates that over 200,000 new cases and 40,000 cancer deaths will occur annually (1). In general, most individuals with cancer die not from the tumor in the primary site, but rather from local invasion and/or distant metastasis (2, 3). Metastasis is a complex process involving neovascularization, stromal invasion by cancer cells, and infiltration into vascular and lymphatic spaces, survival in the circulation, extravasation, and growth at a secondary site (2–7). The cellular mechanisms, which mediate these processes, are poorly understood, but one requirement is enhancement of cell motility. Augmented movement of cancer cells has been reported to correlate with greater metastatic potential in animal models and a poor prognosis in human cancer (2). Multiple cellular and extracellular factors regulate cell motility, through actions on the assembly of the actin cytoskeleton. Fascin, a cytoplasmic protein that functions to bundle cytoplasmic actin filaments, a process which is critical in determining the distribution and activity of the actin cytoskeleton (8). Recently, fascin has emerged as a key bundling protein in diverse forms of actin-based motility-structures (8). Fascin, is normally expressed in neuronal and mesenchymal cells and is low or absent in epithelia (8, 9). However, striking up-regulation of fascin has been reported in several human epithelial tumors including breast, colon, lung, and ovarian carcinomas (10–15). Initial findings implicate fascin as a potentially significant mediator of tumor cell invasion, and an aggressive clinical course.

A preliminary study of 58 patients with breast cancer showed that fascin expression correlated significantly with tumor grade, DNA ploidy, and correlated inversely with estrogen receptor (ER) and progesterone receptor (PR) expression (10). These observations suggest that fascin may contribute to the disease progression of some breast cancers. It has been well established in the literature that hormone receptor (ER/PR)–negative breast cancers have a more aggressive clinical course with decreased disease-free and overall survival, compared with hormone receptor–positive tumors (16–18). Furthermore, in vitro experiments have shown that hormone receptor–negative breast cancer cells have increased cell motility and increased invasiveness (19, 20). These published observations suggest a possible relationship between hormone receptor negativity, enhanced cell motility, and fascin expression in invasive human breast carcinomas.

Studies of breast cancer cells in culture have suggested that fascin expression may be regulated, in part, through the overexpression of the membrane receptor tyrosine kinase HER2. MDA-MB435 tumor cells stably transected with HER2 show a marked increase in mRNA and protein levels of fascin. In correlation, cell motility was increased (21). Other studies have shown that the motility responses of fascin-positive breast cancer cells to insulin-like growth factor I depend on fascin protrusions (22). The current study sought to further investigate the relationship between fascin expression and ER, PR, and HER2 status as well as explore possible relationships between fascin expression and patient prognosis and patterns of metastasis.
MATERIALS AND METHODS

Tissue Microarrays. A series of tissue microarrays (TMA) were constructed containing over 210 consecutive primary invasive breast carcinomas diagnosed at The Cleveland Clinic Foundation between 1995 and 1996. The constructed TMA series consisted of various 8 × 12 arrays of 1.5 mm tissue cores from archived formalin-fixed, paraffin-embedded surgical blocks. Two separate tissue cores of invasive carcinoma, totaling a surface area of 3.5 mm², represented each surgical case in the TMA series. Each separate tissue core was assigned a unique TMA location number, which was subsequently linked to an Institutional Review Board–approved database containing corresponding to a 5-year clinical follow-up.

Immunohistochemistry. Fascin, ER, PR, and HER2 expression was assessed via immunohistochemistry. Immunohistochemistry was carried out using the fully automated Ventana Benchmark system. Briefly, a 4-μm-thick unstained section of each TMA was placed on electrostatically charged glass slides and baked to allow for tissue adherence. The glass slides were pretreated with the recommended pretreatment solution provided by Ventana for tissue deparaffinization and antigen retrieval. After primary antibody incubation, antigen detection was done via peroxidase/3,3′-diaminobenzidine after a secondary biotinylated antibody/streptavidin amplification step. For double-labeled sections, alkaline phosphatase red was used for secondary antigen detection. Lastly, a hematoxylin counterstain was applied. Immunohistochemistry scoring was done by two independent observers (B.Y., D.H.) completely blinded to the clinical outcome data.

The expression of fascin (DAKO, clone 55K-2) was first tested on positive control tissue (cytoplasmic staining of Reed-Sternberg cells in Hodgkin’s disease) using serial dilutions to determine the optimal antibody concentration (1:50). Each separate tissue core was scored on a 0 to 3+ intensity scale (1+ = weak cytoplasmic and membrane staining, 2+ = moderately intense staining, and 3+ = strong staining), and the results entered into the research database. An individual case was considered to be positive if at least one of the two tissue cores contained at least 10% of invasive tumor cells with 1 to 3+ cytoplasmic and membranous staining.

ER and PR status was re-tested on the TMA series and the results compared with the reported values on the surgical report of each case. Immunohistochemistry of primary antibodies for ER (Ventana, clone 6F11) and PR (Ventana, clone 16) were prediluted by the manufacturer for optimal antigen detection. For double-labeled sections, alkaline phosphatase red was used for secondary antigen detection. Lastly, a hematoxylin counterstain was applied. Immunohistochemistry scoring was done by two independent observers (B.Y., D.H.) completely blinded to the clinical outcome data.

The expression of HER2 (Ventana, clone CB11) was prediluted by the manufacturer for optimal antigen detection. HER2 scoring (0 to 3+) was based upon previously published scoring schemes (22, 23), and the score of each separate tissue core was entered into the research database. A case was considered to be HER2-amplified if at least one of the two tissue cores contained at least 5% of invasive tumor cells with 2+ or 3+ membranous staining.

Statistics. Bivariate analysis was done via χ² analysis (fascin positivity versus ER positivity, PR positivity, HER2 status, tumor stage, and tumor grade) or via one-way ANOVA with post hoc analysis (percentage of ER-positive or PR-positive cells versus intensity of fascin staining). Survival data was calculated via the generation of Kaplan-Meier curves. Deaths due to causes other than breast cancer were treated as censored observations. Subsequent multivariate analysis (death due to breast cancer versus fascin positivity and either ER, PR, HER2, tumor stage, or tumor grade) was done via Cox’s proportional hazards model. All statistics were carried out using Statistical Package for the Social Sciences software. Statistical significance was assumed if P < 0.05. Mean follow-up of the study population was 67 months (1-106 months).

RESULTS

Characteristics of the Study Population and TMA Validation. A summary of the study population is listed in Table 1. There were 210 consecutive, newly diagnosed patients with adequate formalin-fixed, paraffin-embedded tissue at our institution from 1995 through 1996. The majority of these (168)
were infiltrating ductal carcinoma. The remaining 42 cases consisted of 32 infiltrating lobular carcinomas, 4 cancers with mixed ductal and lobular features, 3 mucinous carcinomas, and 3 medullary carcinomas. The majority were T1 (128) or T2 (56) in size and ER/PR-positive (76% and 60%, respectively). HER2 amplification was detected via immunohistochemistry in 19 (9%) cases.

The traditional clinicopathologic prognostic variables, including tumor stage, grade, ER/PR status, and HER2 status of the study population correlated well with survival, as displayed in Fig. 1. The mean survival for ER-positive patients was 110.42 ± 1.56 months versus 76.56 ± 4.01 months for ER-negative patients ($P = 0.002$). PR-positive patients likewise had an increased mean survival of 95.45 ± 1.50 months versus 91.13 ± 3.36 months for PR-negative patients ($P = 0.007$, data not shown). HER2 nonamplified patients showed an increased mean survival of 98.28 ± 1.65 months versus 79.69 ± 7.80 months for HER2-amplified patients ($P = 0.023$). Finally, the mean survival time inversely correlated with both Bloom-Richardson grade and cancer stage (grade 1 = 94.78 ± 3.16 months, grade 2 = 92.70 ± 2.24 months, grade 3 = 85.12 ± 3.26 months, stage 1 = 102.54 ± 1.53 months, stage 2 = 96.30 ± 1.21 months, stage 3 = 72.38 ± 4.85 months, and stage 4 = 39.63 ± 11.27 months).

There was an excellent concordance between the reported ER/PR results done on the full histologic sections from 1995 and 1996 and the re-tested ER/PR status done on the TMA series ($k = 0.91, P < 0.001$). Additional steps to reduce sampling error in the TMA series included the use of larger 1.5 mm cores, as well as utilizing two separate tissue cores per case, each tissue core from a different area of the primary tumor. Using two separate 1.5 mm cores from each case allows for a total surface area of 3.5 mm$^2$, which in our experience, is more than twice the area necessary for adequate tumor sampling (24) and more than 10 times the surface area necessary reported by others (25).

**Fascin Expression Correlates With Hormone Receptor–Negative Breast Cancers.** Normal breast ductal epithelium was negative for fascin, although supporting myoepithelium frequently shows weak to moderate staining (Fig. 2A). Fascin expression was observed in a cytoplasmic and membranous pattern in 33 (16%) of the 210 primary invasive carcinomas. Fig. 2B displays typical fascin-positive cells in one of the tissue cores from the TMA series. Fascin expression correlated with both ER-negative (22/33, $P < 0.001$) and PR-negative (21/33, $P < 0.001$) tumors. Fig. 3A graphically shows the disproportionate distribution of fascin-positive tumors between hormone receptor–positive and hormone receptor–negative tumors. There was no correlation between fascin expression and HER2 status ($P = 0.189$). Fascin-positive tumors tended to be Bloom-Richardson grade 3 (19/29, $P < 0.001$) and of advanced stage (stage 3 or 4, $P = 0.046$). The correlative findings with fascin expression are summarized in Table 2.

To further characterize the relationship between fascin and the hormone receptor status, the percentage of ER-positive cells per case was plotted against the intensity of fascin expression. In addition, selected slides from the TMA series were concurrently double-labeled for both ER (brown) expression and fascin (red) expression. Fig. 3B graphically exhibits the existence of an inverse relationship between the intensity of fascin expression and the percentage of ER-positive cells ($P < 0.001$, ANOVA).

![Fig. 1 Kaplan-Meier curves of the study population showing that the traditional clinicopathologic prognostic variables correlated well with survival, including ER status (A), HER2 status (B), tumor grade (C), and tumor stage (D). All $P$ values were statistically significant ($< 0.01$), including PR status (data not shown).](clincancerres.aacrjournals.org)
Double labeling for ER and fascin revealed that although 11 cases were dual-positive for both fascin and ER, all of the individual ER-positive cells were fascin-negative, and all of the individual fascin-positive cells were ER-negative (Fig. 4). Tumors which failed to express (< 5%) either fascin or ER were rare (data not shown).

**Fascin-Positive Breast Cancers Have Worse Prognosis.** Five-year clinical follow-up was available for all 210 patients in the study, including time of recurrence, sites of metastases, and cause of death. Fascin-positive tumors show both a decreased disease-free and overall survival compared with fascin-negative tumors (Fig. 5). The mean disease-free survival for fascin-positive tumors was 73.85 months versus 100.42 months for fascin-negative tumors ($P < 0.001$). The mean overall survival for fascin-positive tumors was 77.98 months versus 98.82 months for fascin-negative tumors ($P = 0.002$). Subsequent multivariate analysis revealed that fascin expression is an independent prognostic predictor from PR ($P = 0.028$), HER2 ($P = 0.0010$), and stage ($P = 0.0235$), but not independent of ER or grade. There was no correlation between fascin expression and tumor size (T1, T2, T3, or T4), lymph node status (N1, N2, or N3), the occurrence of metastases, the site of metastases (lung, liver, bone, central nervous system, and soft tissue), or the pattern of metastases (visceral dominate, bone dominate, and local recurrence).

**DISCUSSION**

In the current study, fascin expression was seen in 16% of invasive human breast cancers and correlated with hormone receptor-negative cases and a more aggressive clinical course for these patients. Fascin plays an important role in the assembly of cell-motility structures, which have been shown to be critical in cancer invasion and metastasis (2). Therefore, it is possible that fascin expression in cancer cells may lead to a more clinically aggressive course through augmented cell motility and enhanced metastatic potential, a finding supported by in vitro observations (11, 26).

**Evidence for the Up-regulation of Fascin in Human Carcinomas.** Fascin is typically expressed at very low levels in normal epithelia (10–15). Likewise, in the current study, normal breast ductal epithelium was negative for fascin, whereas supporting myoepithelium frequently showed weak to moderate staining. Reports in the literature have documented high levels of
The expression of fascin in lung cancer correlated with a shorter survival and was an independent prognostic predictor of unfavorable clinical course of the disease (13, 14). Overall, in ovarian tumors, the expression of fascin in cultured tumor cells was significantly associated with the ability of these cells to grow intraperitoneally in a xenograft animal model (15). Similarly, in the current study, we have shown that a subset of invasive breast cancers reveal a marked overexpression of fascin, which was associated with an aggressive clinical course and poor disease-free and overall survival.

**Fascin Expression and Hormone Receptor Status in Breast Cancer.** Hormone receptor–negative breast cancers traditionally have a worse prognosis and fewer available treatment options (ineffectiveness of hormonal therapy) compared with hormone receptor–positive tumors (16–18). It is interesting that hormone receptor–negative breast cancers also display increased cell motility in vitro (19, 20). In a study examining the ability of breast cancer cell lines to penetrate into a collagen-fibroblast matrix, cells expressing mRNA for estrogen receptor showed a noninvasive phenotype, whereas cells lacking estrogen receptor mRNA were shown to be highly invasive (20). Similarly, in a modified Boyden chamber assay for invasion, estradiol induced inhibition of breast cancer cell invasion and motility, and a similar inhibitory effect for estradiol was found when the wild-type estrogen receptor-α was stably transfected into the ER-negative MDA-MB231 and 3Y1-Ad12 cells (19). These authors state that the mechanism of the inhibitory effect of estrogen receptor on cell motility is unknown. However, the results presented here suggest that regulatory interactions between the estrogen receptor and fascin gene expression could explain, in part, the alterations of in vitro invasion and cell motility. These findings, along with the data presented here, suggest a connection between the expression of fascin and the absence of hormone receptors, increased cell motility, and decreased survival in human breast cancers. This notion is supported by fascin expression being a poorer prognostic factor, which was not independent of ER negativity or high tumor grade.
in the current study. Furthermore, whereas 11 of the 33 fascin-positive tumors were also ER-positive, these tumors tended to show a lower level of ER expression and double-labeling studies (simultaneous staining for ER and fascin in the same section) showed that on an individual tumor cell basis, fascin and ER were never coexpressed in the same tumor cell. Clearly, more studies need to be done to further elaborate the regulatory relationship between fascin expression and hormone receptor expression in human breast cancer. If the expression of fascin in human breast cancers proves similar in prospective studies, it may represent a potential therapeutic target for patients with hormone receptor–negative breast cancer.

**Current Molecular Understanding of the Role of Fascin in Cell Motility.** Fascin is a 55-kDa globular actin-bundling protein that is principally expressed in normal mesenchymal, endothelial, dendritic, and neuronal cells (8). In vitro, fascin bundles F-actin into unipolar bundles that have high stability and mechanical rigidity. In intact cells, fascin localizes to protrusive, actin-based structures that are formed at the leading edge of migrating cells or in response to specific extracellular matrix components, where it imparts structural rigidity to actin bundles to facilitate the outward extension of membrane edges (8). Experiments have shown that fascin-actin bundling is required for cell migration on thrombospondin-1 and contributes to cell migration on fibronectin (28). The formation of fascin and actin bundles in response to thrombospondin-1 depends on the transmembrane proteoglycan syndecan-1 and signaling by GTPases Cdc42 and Rac (26, 29). Elevated syndecan-1 expression has been shown in a subset of breast cancers and correlated with high histologic grade, large tumor size, ER/PR-negative status, and was related to an aggressive phenotype and poor clinical behavior (30, 31). Thus, fascin and syndecan-1 may act in concert to mediate a more aggressive clinical course for this subset of hormone receptor–negative breast cancers, through enhanced tumor cell motility.

**Fascin Expression and HER2 Status in Breast Cancer.** In vitro studies have suggested that fascin expression in breast cancer may be regulated, in part, through the overexpression of HER2 (21). MDA-MB435 tumor cells stably transfected with HER2 show a marked increase in mRNA and protein levels of fascin in cell culture. In correlation, cell motility was increased (21). The data presented in this report failed to reveal any association between fascin with HER2 status in tissue samples. This could be due to limited population size or an institutional bias. Alternatively, the forced overexpression of this receptor in cell cultures by transfection may represent an artificial system, which may well not reflect the biological complexity of HER2 gene amplification and protein overexpression occurring in vivo.

**Summary.** Current prognostic factors do not provide sufficient information to allow accurate individual risk assessment for patients with node-negative breast cancer, emphasizing the need for new prognostic and therapeutic strategies for early stage breast cancer. In this regard, a greater understanding of the molecular and cellular basis of breast cancer phenotypes, and how these mechanisms pertain to clinical behavior and aggressiveness of disease, are important first steps to identifying and evaluating potential new molecular targets for their suitability in clinical practice. Given fascin’s role in enhancing cell motility, the data presented here suggests that fascin expression may contribute to a more aggressive clinical course and thus an enhanced metastatic potential in ER/PR-negative breast cancer. If fascin is a downstream mediator contributing to a more aggressive clinical course through enhanced cell motility and metastatic potential, then this protein may represent a new molecular target for therapeutic intervention in this subset of patients with hormone receptor–negative breast cancer.

**REFERENCES**


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