Prognostic Significance of Basal-Like Phenotype and Fascin Expression in Node-Negative Invasive Breast Carcinomas

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Abstract

Purpose: Basal-like phenotype tumors are frequently found among *BRCA1* germ-line mutated breast carcinomas. They are biologically aggressive and have a tendency towards visceral metastasis when untreated. Nevertheless, it has been suggested that they respond to chemotherapy better than other types of tumors. Fascin expression has been associated with lung metastasis in breast cancer. The aim of this study was to determine whether basal-like phenotype and fascin were related in both sporadic and familial tumors and with prognosis in node-negative sporadic breast cancers.

Experimental Design: 230 nonfamilial and 28 hereditary node-negative invasive breast carcinomas were immunohistochemically analyzed using tissue microarrays. Tumors that were estrogen receptor/HER2 negative and cytokeratin 5/6 and/or epidermal growth factor receptor positive were considered to have a basal-like phenotype.

Results: A basal-like phenotype was found in 11.9% of sporadic cancers. Among patients not receiving adjuvant chemotherapy, a basal-like phenotype was associated with poor prognosis ($P = 0.001$, log-rank test) whereas no such association was found in patients receiving it. Tumors with a basal-like phenotype showed local recurrence (17.4%) or visceral metastasis (13%) but not bone metastasis ($P = 0.001$). Fascin expression was observed in 25.1% of sporadic invasive breast carcinomas and was associated with the basal-like phenotype, but not with prognosis or recurrence pattern. Fascin was expressed in 83.3% and 16.7% *BRCA1*- and *BRCA2*-associated carcinomas, respectively ($P = 0.048$).

Conclusions: Basal-like tumors had a tendency towards visceral metastasis and their prognosis was dependent on the use of postoperative chemotherapy. Although fascin expression was associated with the basal-like phenotype, it was not associated with their metastatic behavior. Fascin expression is frequent in *BRCA1*-associated tumors.

Several studies have established that breast carcinomas with expression of basal/myoepithelial markers exhibit specific characteristics of morphology, proliferation, and prognosis. Earlier works reported the existence of a subset of breast carcinomas that expressed basal-cell-type keratins, such as cytokeratin (CK)-5 and CK14 (1–3). Palacios et al. (4) observed that breast carcinomas that expressed the basal-cell cadherin P-cadherin were poorly differentiated, estrogen receptor– and progesterone receptor–negative infiltrating ductal carcinomas with a characteristic growth pattern consisting of a central area of necrosis and/or fibroelastosis surrounded by a ribbon of neoplastic cells growing at the periphery. Subsequently, Tsuda et al. (5, 6) discovered that high-grade infiltrating ductal carcinomas with large, central acellular zones expressed markers of myoepithelial differentiation, such as S-100 and CK14, and had a predisposition to lung and brain metastases. Perou et al. used cDNA studies to define a subgroup of breast cancer characterized by the expression of basal-cell markers, such as keratins CK5 and CK17. They also showed in a subsequent tissue microarray study that the expression of these CKs was a marker of poor prognosis (7). Recent microarray studies have also suggested that tumors with a basal-like phenotype have a tendency towards visceral metastasis, especially in the lung (8). Moreover, they have identified a set of genes, which includes fascin, which mediates breast cancer metastasis to lung (8). Other recent cDNA and CGH-array and tissue microarray studies have shown that tumors with a basal-like phenotype, defined by the expression of CK5/6, had a highly proliferative immunophenotype and were frequently associated with p53 expression (9–14). In addition, molecular cytogenetic studies have shown

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Received 10/18/05; revised 12/2/05; accepted 12/21/05.

Grant support: Spanish Ministry of Education and Science grant SAF2004-08258-C02-01 and Instituto de Salud Carlos III grant PI051890; Fondo de Investigación Sanitaria, Spain (S.M. Rodríguez-Pinilla and D. Sarrió); and Asociación Española Contra el Cáncer (E. Honrado).

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doi:10.1158/1078-0432.CCR-05-2281

that basal-like tumors have specific chromosome aberrations (15–18), and cDNA and immunohistochemical studies have revealed that high proportions of *BRCA1* carcinomas, but only a small percentage of *BRCA2* carcinomas, express basal markers (19–22).

Despite the aggressive biological behavior of both sporadic basal-like phenotype and *BRCA1* germ-line mutated tumors, they seem to respond to chemotherapy. Some articles have reported the sensitivity of *BRCA1*-related breast cancer to neoadjuvant chemotherapy (23–25). Recently, Sakuma et al. (26) reported a case of locally advanced and metastatic sporadic breast cancer with cartilaginous and osseous metaplasia that was estrogen receptor, progesterone receptor, and HER2 negative, and had an excellent response to chemotherapy. Moreover, recent microarray studies revealed that sporadic basal-like phenotype tumors responded to chemotherapy and were associated with a high rate of complete pathologic response (27).

Fascin is an actin cross-linking protein that localizes to the core actin bundles of spikes and filopodia at the leading edge of migratory cells and that has been implicated in cell motility in several cell types. Fascin is not usually expressed by normal epithelial cells, but its overexpression has been reported in many different types of carcinomas, including ovary, colon, pancreas, esophagus, stomach, lung, and urinary bladder, as well as in other tumors, such as lymphomas, sarcomas, melanomas, and astrocytomas (28–43). Overall, the expression of fascin has been correlated with an aggressive clinical course and shorter survival. Two preliminary studies showed that fascin expression correlated inversely with estrogen receptor and progesterone receptor expression in breast cancer (44, 45).

In spite of these findings, basal-like breast cancers are a poorly characterized subgroup of tumors and the precise sets of basal markers that define this type of carcinoma remain to be established. Recently, Nielsen et al. (46) suggested that the use of four antibodies, estrogen receptor, CK5/6, HER2, and epidermal growth factor receptor (EGFR), can accurately identify basal-like tumors using standard available pathologic tools. By this definition, basal-like breast carcinomas had a worse prognosis than carcinomas with a luminal phenotype in several series.

In this study, we have examined whether a basal-like phenotype influences the prognosis and metastatic behavior of node-negative breast carcinomas. Taking into account that fascin is expressed by myoepithelial breast cells and in estrogen receptor–negative breast carcinomas (45), we have also evaluated whether fascin expression is associated with the basal-like phenotype and breast cancer outcome. Finally, because a basal-like phenotype is known to be more frequent in *BRCA1*-associated breast carcinomas, we have analyzed fascin expression in a group of hereditary breast cancers in *BRCA1* and *BRCA2* mutation carriers.

Materials and Methods

Tissue samples. This study comprises both familial and sporadic (nonfamilial) node-negative breast carcinomas. The familial breast cancer group was made up of 18 tumors from *BRCA1* mutation carriers and 10 tumors from *BRCA2* mutation carriers. The mutational study of the patients and some immunohistochemical and molecular characteristics of this group of tumors have been previously reported (12, 47).

We analyzed a group of 230 sporadic invasive breast carcinomas diagnosed in Hospital Universitario La Paz, Madrid between 1988 and 2002. None of them had criteria of familial cancer (48). Mean patient age at diagnosis was 53 years (range, 27-87 years); 46.6% and 53.4% of the patients were premenopausal and postmenopausal, respectively.

Breast surgery was done in all cases and consisted of segmental mastectomy with axillary node dissection when tumor size permitted or modified radical mastectomy according to the judgment of the multidisciplinary care team and patient preference.

Size information was available for 190 tumors: 119 (62.6%) tumors were pT_1 and 71 (37.4%) were pT_2. The histologic type of 226 cases was known: there were 167 (86.1%) invasive ductal carcinomas, 5 (5.2%) colloid carcinomas, 9 (4.6%) atypical medullary carcinomas, 5 (2.6%) micropapillary carcinomas, and 8 (4.1%) invasive lobular carcinomas. Information on histologic grade (according to the criteria of Elston and Ellis) was obtained for 198 tumors: 54 (27.3%) were grade 1, 59 (29.8%) were grade 2, and 85 (42.9%) were grade 3.

Regarding adjuvant therapy, 97 (44.5%) patients received tamoxifen, 43 (19.7%) patients were treated with tamoxifen and chemotherapy, and 71 (32.6%) patients received only chemotherapy. Adjuvant postoperative chemotherapy regimen consisted of cyclophosphamide-methotrexate-5-fluorouracil (5-FU). No other chemotherapeutic drugs were administered in any patient of this series. Radiotherapy was administered at the completion of chemotherapy for 92 (41.1%) patients because they underwent breast conservation surgery. All variables, including tumor size, grade, and immunohistochemical features, were similarly distributed between chemotherapy-receiving and non-chemotherapy-receiving groups, as shown by the absence of any statistically significant difference with the χ^2 test (data not shown).

We obtained follow-up information from 205 patients. The mean follow-up period was 101 months (range, 4-185 months). During the follow-up period, 14 patients suffered local recurrences and 25 experienced metastasis. The first metastatic location was bone in 15 patients and visceral in 10 patients. Visceral metastases were found only in lung parenchyma in five cases, in lung and pleura simultaneously in two cases, and in lung and liver parenchyma in three cases. It is not known whether the metastases of patients with liver and lung metastases appeared at different times or simultaneously.

Tissue microarray. Representative areas from formalin-fixed, paraffin-embedded infiltrating carcinomas and 30 samples from nonneoplastic breast tissue were carefully selected on H&E-stained sections and two tissue cores (1 mm in diameter) were obtained from each specimen. The tissue cores were precisely arrayed into a new paraffin block using a tissue microarray workstation (Beecher Instruments, Silver Spring, MD).

Immunohistochemistry. Immunohistochemical staining on tissue microarrays sections was done by the EnVision method with a heat-induced antigen retrieval step. Sections were immersed in boiling 10 mmol/L sodium citrate at pH 6.5 for 2 minutes in a pressure cooker. Mouse anti-human estrogen receptor (SP1, Master Diagnostica, Granada, Spain), progesterone receptor (1A6, Novocastra, Newcastle, United Kingdom), p53 (DO-7, Novocastra), CK5/6 (D5/16 B4, DakoCytomation, Glostrup, Denmark), EGFR (EGFR.113, Novocastra), Ki67 (MIB-1, Dako, Glostrup, Denmark), and fascin (55K-2, Dako) monoclonal antibodies were applied at dilutions of 1:50, 1:10, 1:25, 1:10, 1:10, 1:50, and 1:50, respectively. HER2 expression was evaluated using Herceptest (DakoCytomation). Cases were considered positive for hormonal receptors when any tumor cell was stained whereas the cutoff for p53 and Ki67 positivity was 30% and 15%, respectively (46). For HER2, only cases with a membranous staining score of 3+ were considered as positive. Unequivocal membrane and

Table 1. Results of immunohistochemical staining of this series

Molecular marker	Interpretable core	Positive cases
Estrogen receptor	227	167 (73.6%)
Progesterone receptor	228	152 (66.7%)
Ki67	230	48 (20.9%)
p53	223	60 (26.5%)
HER2/neu	230	37 (16.1%)
CK5/6	227	35 (15.4%)
EGFR	214	21 (9.8%)
Fascin	224	57 (25.4%)

cytoplasmic staining for EGFR and CK5/6, respectively, were considered as positive (19, 46, 49). Cases were considered to be positive for fascin if at least 10% of invasive tumor cells had unequivocal cytoplasmic staining (45). Normal breast tissue was also evaluated as an internal control. In negative controls, the primary antibodies were omitted.

In accordance with Nielsen et al. (46), we considered estrogen receptor/HER2 negative and CK5/6 and/or EGFR positive to be the basal-like phenotype.

Statistical analysis. To assess associations between categorical variables, we used the χ^2 contingency test with Yates' correction or Fisher's exact test as appropriate. Kaplan-Meier survival analyses were carried out for overall and breast cancer disease–specific survival using the log-rank test to examine differences between groups. A multivariate Cox regression model was also derived. Estimates were considered statistically significant for two-tailed values of $P < 0.05$. All analyses were carried out with the SPSS 12.0 statistical program (SPSS, Inc., Chicago, IL).

Results

Sporadic cases. Table 1 shows the immunohistochemical characteristics of this series with respect to estrogen receptor, progesterone receptor, Ki67, p53, HER2, CK5/6, EGFR, and fascin expression (Fig. 1). A basal-like phenotype (ER/ERBB2 negative, CK5/6 positive, and/or EGFR positive) was found in 27 of 227 tumors (11.9%). In addition, there were 10 estrogen receptor–positive and 10 progesterone receptor–positive cases

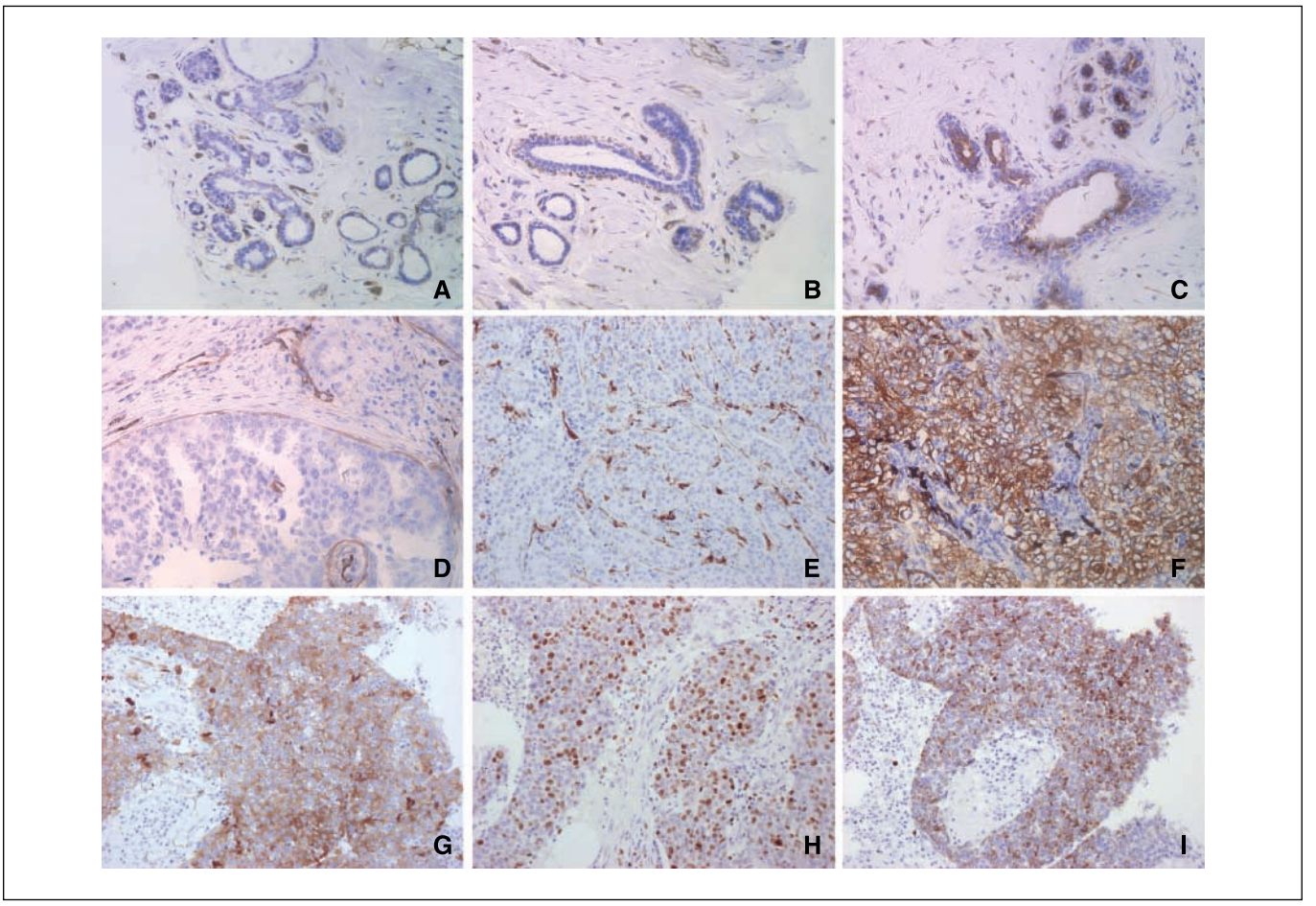

Fig. 1. Expression of proteins studied by immunohistochemistry in tissue microarray. *A,* fascin-positive myoepithelial cells of normal mammary ducts. *B,* fascin-positive myoepithelial cells of normal mammary terminal ductal-lobular unit. *C,* fascin immunoreactivity in normal mammary epithelial cells. *D,* fascin immunoreactivity of myoepithelial cells in the intraductal component of an invasive ductal carcinoma. *E,* fascin immunoreactivity of fibroblastic stromal cells and endothelial cells in a fascin-negative breast carcinoma. Note the difference with normal mammary stroma (*A* and *B*). *F,* fascin immunoreactivity of a *BRCA1*-related tumor. *G,* fascin immunoreactivity of a sporadic breast carcinoma. *H,* Ki67 immunoreactivity in the same tumor depicted in (*G*). *I,* CK5/6 immunoreactivity in the same tumor depicted in (*G* and *H*). [Original magnification, ×10 (*A* to *C*), ×20 (*D* to *I*)].

Table 2. Fascin immunoreactivity in relation to pathologic and clinical variables

Variables studied	Fascin positive	*P*
Age		0.23
Premenopausal	18 of 100 (18.0%)	
Postmenopausal	36 of 118 (30.5%)	
Size		0.125
pT_1	25 of 117 (21.4%)	
pT_2	21 of 70 (30.0%)	
Grade		0.002
1	7 of 54 (13.0%)	
2	11 of 57 (19.3%)	
3	32 of 84 (38.1%)	
Estrogen receptor		<0.001
Positive	26 of 165 (15.8%)	
Negative	31 of 59 (52.5%)	
Progesterone receptor		<0.001
Positive	27 of 150 (18.0%)	
Negative	30 of 75 (40.0%)	
Ki67		0.031
<15%	39 of 180 (21.7%)	
≥15%	18 of 47 (38.3%)	
p53		0.737
<30%	39 of 180 (21.7%)	
≥30%	17 of 61 (27.9%)	
HER2		0.743
Negative	49 of 190 (25.8%)	
Positive	8 of 37 (21.6%)	
CK5 of 6		0.003
Negative	40 of 190 (21.1%)	
Positive	16 of 34 (47.1%)	
EGFR		0.449
Negative	48 of 192 (25%)	
Positive	6 of 21 (28.6%)	
Basal-like phenotype		0.001
Negative	43 of 198 (21.7%)	
Positive	14 of 26 (53.8%)	

that were also positive for CK5 (data not shown). The basal phenotype was associated with larger tumor size because it was found in 16 of 70 (22.9%) pT_2 tumors but in only 7 of 117 (6%) pT_1 tumors ($P = 0.002$). A basal-like phenotype was more common among grade 3 tumors (21 of 85 cases, 24.7%) than in grade 1 (1 of 53 cases, 1.9%) and grade 2 (2 of 59 cases, 3.4%) tumors ($P < 0.0001$). In addition, sporadic carcinomas with a basal-like phenotype showed a high level of Ki67 staining in 12 out 48 (25%) cases, but in only 15 of 179 (8.4%) cases that were not of the basal-like phenotype. No association was found between this phenotype and age or p53 expression.

Fascin expression was observed in myoepithelial cells, but not in all ducts or acini. In addition, a few acini also expressed fascin in luminal cells. Fibroblasts and endothelial cells in intralobular stroma weakly expressed fascin. Fifty-seven of 224 (25.4%) sporadic invasive breast carcinomas exhibited fascin expression. There was a significant positive association between fascin expression and grade, Ki67 and CK5/6 expression, and the presence of the basal/myoepithelial phenotype. Conversely, there was a significant inverse association between fascin expression and estrogen receptor and progesterone receptor status. There was no correlation between fascin expression and HER2 or EGFR status (Table 2).

Fascin was highly expressed in myofibroblasts and endothelial cells of the stroma surrounding neoplastic epithelial cells. The intensity of the staining was higher than that observed in normal myofibroblasts and endothelial cells of normal breast parenchyma. Moreover, it was also expressed by histiocytes and some lymphoid cells.

Basal-like phenotype and fascin expression and prognosis in sporadic node-negative breast carcinomas. In this series, tumor size (pT_1 versus pT_2) and the basal-like phenotype were significantly associated with disease-specific survival in univariate analyses ($P = 0.01$ and $P = 0.04$, respectively); histologic grade (grade 1 versus grade 2/3) showed a nearly significant association with disease-specific survival ($P = 0.076$). Taking tumor size and the basal-like phenotype status together, a Cox model to predict disease-specific overall survival was developed from data from all patients. Only tumor size was prognostically significant with an odds ratio of 2.690 (95% confidence interval, 1.130-6.400; $P = 0.0025$). Fascin expression was not associated with prognosis (Fig. 2).

A basal-like phenotype was observed in 13 of 98 (13.3%) patients not receiving cyclophosphamide-methotrexate-5-FU and in 12 of 107 (11.2%) with adjuvant cyclophosphamide-methotrexate-5-FU. Five patients in the first group and one patient in the second group died from breast cancer. When cases were analyzed with respect to adjuvant chemotherapy, the basal-like phenotype was the only factor associated with prognosis ($P = 0.001$) in untreated patients (Fig. 3). In treated patients, tumor size, grade, and fascin expression showed a trend towards association with disease-specific survival ($P = 0.097$, $P = 0.067$, and $P = 0.063$, respectively).

There was no significant association between fascin expression and recurrence or metastasis location. In contrast, an association between recurrence/metastasis location and a basal-like phenotype was observed because these tumors showed local recurrence or visceral metastasis but not bone metastasis (Table 3).

Familial cases. Twelve of 28 familial cases had basal phenotype according to Nielsen et al.; 83.3% (10 of 12) and 16.7% (2 of 12) were *BRCA1*- and *BRCA2*-mutated tumors, respectively ($P = 0.061$). Fascin was expressed in all cases with basal phenotype and in three without it ($P = 0.004$). Fascin-expressing tumors were *BRCA1*-mutated carriers in 83.3% (10 of 12) of the cases and *BRCA2*-mutated carriers in 16.7% (2 of 12; $P = 0.048$) of the cases.

Discussion

Currently, there is no consensus about the set of markers that best define the basal-like phenotype although it has been suggested that the use of estrogen receptor, HER2, CK5/6, and EGFR can recognize breast carcinomas with a basal-like phenotype with high specificity. By this definition, we observed

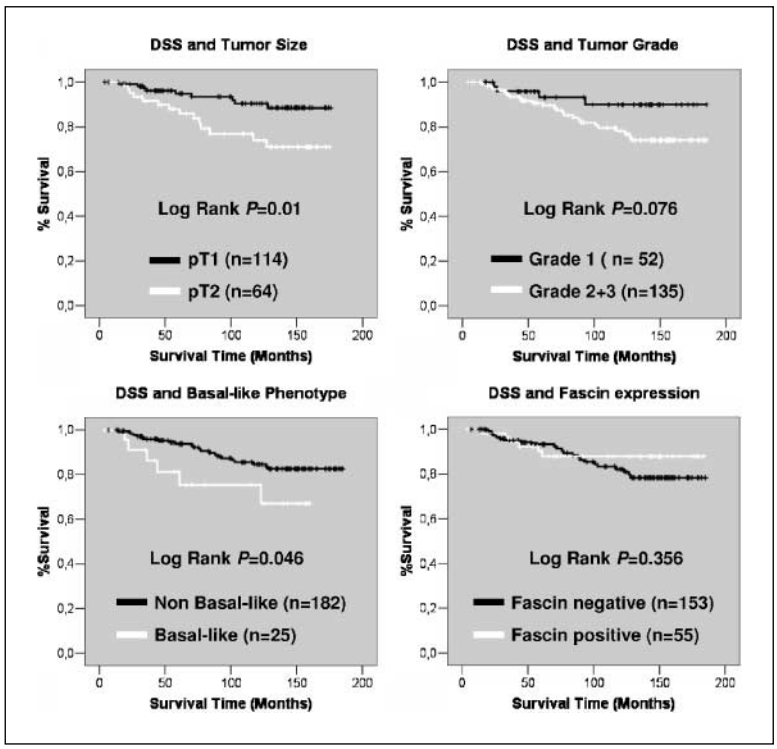

Fig. 2. Kaplan-Meier disease-specific survival (*DSS*) curves for breast cancer patients according to clinicopathologic and immunohistochemical features.

that 64.3% (10 of 18) of *BRCA1*-associated, 16.7% (2 of 12) of *BRCA2*-associated, and 11.9% (27 of 227) sporadic node-negative breast carcinomas had a basal-like phenotype. These figures are consistent with the results of other published studies (7, 9–14, 18–22, 49–52).

The distinction of basal-like carcinomas as a specific subgroup of breast cancer is of clinical relevance because differences in prognosis, chemotherapy response, and metastatic behavior have recently been reported in this set of tumors (5–9, 20, 26, 27). Several studies have suggested that breast cancers that express basal/myoepithelial markers have poor prognosis although results vary among series when node-negative and node-positive cases are considered. For example, van de Rijn et al. (49) reported that the expression of CK17 and/or CK5/6 was associated with significantly shorter survival in node-negative patients but had no predictive value in node-positive patients. In contrast, Nielsen et al. (46) found that the presence of either basal cytokeratin was associated with a significantly poorer outcome only in the lymph node-positive group. In our study, the univariate study suggested that node-negative breast carcinomas with a basal-like phenotype had shorter survival but this variable is highly correlated with tumor size. Our results suggest that differences in prognosis among series could be partially explained by differences in chemotherapy treatment because most studied series, including the current one, were retrospective and the criteria of the chemotherapy treatment in node-negative patients were not identical in all countries when patients were diagnosed.

Therefore, one of the most important findings in our study is that poor prognosis in basal-like carcinomas is associated with the absence of postoperative chemotherapy, suggesting an increased sensitivity of basal-like carcinomas to cyclophosphamide-methotrexate-5-FU. In contrast, chemotherapy did not improve prognosis in nonbasal-like grade 3 or HER2-positive breast carcinomas. These data suggest that the major molecular classes of breast cancer may vary in their sensitivity to cyclophosphamide-methotrexate-5-FU. Very recently, Rouzier et al. (27) have reported that basal-like or HER2-positive breast carcinomas showed a 45% complete pathologic response after preoperative treatment with paclitaxel followed by 5-FU, doxorubicin, and cyclophosphamide. The use of paclitaxel and doxorubicin could explain the high response rate of HER2-positive tumors in this later series.

Minn et al. (8) reported a higher incidence of visceral (lung) than bone metastasis in sporadic breast cancer with a basal phenotype. Consistent with these findings, in our series, basal-like sporadic carcinomas metastasized more frequently to lung and other visceral organs; in fact, no bone metastases were detected in this subgroup of tumors. Minn et al. also identified fascin as being one of the most important genes involved in the capacity of breast cancer to metastasize to lung. Because fascin plays an important role in the assembly of cell motility structures, which have been shown to be critical in cancer invasion and metastasis, we may speculate that fascin modulates metastatic behavior in basal-like tumors. To test this hypothesis, we analyzed fascin expression in this series of breast carcinomas and described for the first time an association between fascin expression and a basal-like phenotype. However, we did not find any association between fascin expression and metastasis location. Differences between our study and results reported by Minn et al. might have arisen from differences in the detection method used. We analyzed fascin expression by immunohistochemistry in neoplastic cells whereas

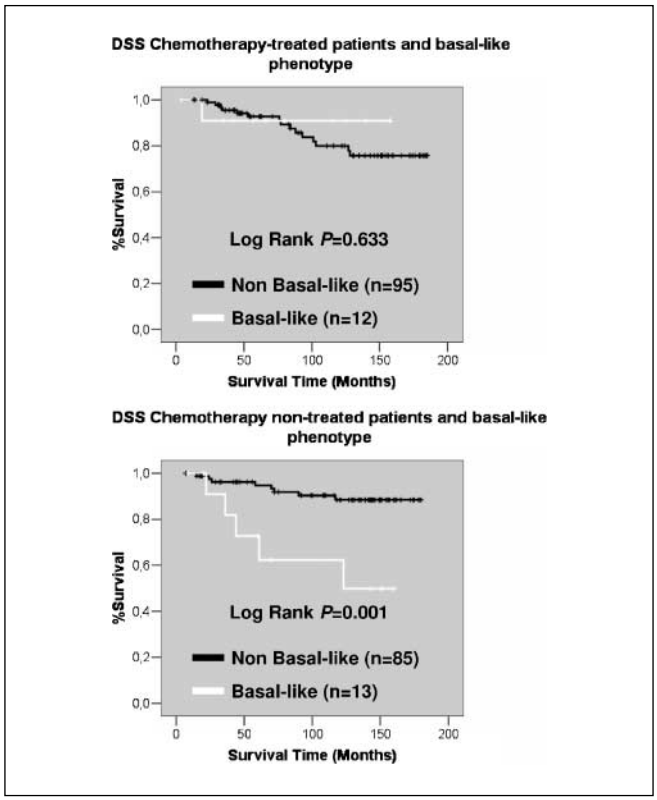

Fig. 3. Associations between basal-like phenotype and disease-specific survival in patients treated with and without chemotherapy.

Minn et al. studied it at the mRNA level by oligonucleotide array in tissue extracts. Because fascin expression is found in myofibroblasts, endothelial cells, and inflammatory cells, fascin expression data in the study of Minn et al. came from a mixed population of cells. It is interesting to note, however, that these findings could be biologically relevant because the level of expression of fascin that we observed in myofibroblasts and endothelial cells in the tumor stroma was higher than that observed in stromal fibroblasts and endothelial cells in normal mammary tissue (Fig. 1). This implies a possible role for tumor stroma in invasion and metastasis processes. There are only two previous studies analyzing fascin immunohistochemistry in breast cancer. Yoder et al. showed that fascin-expressing tumors were associated with an aggressive clinical course and poor disease-free and overall survival. Nevertheless, the multivariate analysis of these data showed that fascin expression was not independent of estrogen receptor or grade. Moreover, the study included both node-positive and node-negative cases (45). On the other hand, in keeping with our results, Grothey et al. (44) found no association between fascin expression and tumor stage, histology, grading, number of lymph nodes involved, or presence of metastasis at the time of surgery.

Fascin expression in hereditary breast cancer has not been analyzed before. In the current study, fascin was expressed mainly in hereditary cancers with a basal-like phenotype and, for this reason, was common among *BRCA1*-associated carcinomas. Fascin was expressed in nearly 84% of *BRCA1*-associated breast carcinomas, a frequency similar to those of other basal markers such as CK5/6. Future studies should evaluate the usefulness of this marker for more accurately predicting the probability of carrying a *BRCA1* mutation, as has been suggested for CK5/6.

Interestingly, previous studies have shown a characteristic pattern of metastatic spread in familial *BRCA1* tumors consisting of a low incidence of lymphatic spread to the axillary nodes, but a high incidence of hematogenous spread, mainly to the lung and brain (53, 54). This pattern of spread is similar to that observed in the work of Minn et al. (8) and in the present series, and suggests that it is related with a basal phenotype.

In summary, we found that the prognosis of basal-like, node-negative breast carcinomas depends on the use of postoperative chemotherapy and that these tumors have a greater tendency towards visceral metastasis than to bone metastasis in absence of treatment. Although fascin expression is associated with the basal-like phenotype, it is not associated with the metastatic behavior of these tumors. In addition, the current study reports for the first time fascin expression in hereditary breast cancer, which is characteristic of *BRCA1*-associated tumors.

Acknowledgments

We thank the Centro Nacional de Investigaciones Oncológicas Immunohistochemical Unit for expert technical assistance.

Table 3. Relationship between the basal-like phenotype and fascin expression and recurrence localization

	Recurrence				*P*
	Nonrecurrence	Local	Visceral	Bone	
Fascin					
Negative	123 of 152 (80.9%)	10 of 152 (6.6%)	7 of 152 (4.6%)	12 of 152 (7.9%)	0.727
Positive	45 of 54 (83.3%)	4 of 54 (7.4%)	3 of 54 (5.6%)	2 of 54 (3.7%)	
Basal-like phenotype					
Negative	150 of 182 (82.4%)	10 of 182 (5.5%)	7 of 182 (3.8%)	15 of 182 (8.2%)	0.001
Positive	16 of 23 (69.6%)	4 of 23 (17.4%)	3 of 23 (13.0%)	0 of 23 (0.0%)	

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Clin Cancer Res 2006;12:1533-1539.