Separating Favorable from Unfavorable Prognostic Markers in Breast Cancer: The Role of E-Cadherin

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ABSTRACT

Distant metastases are the major cause of morbidity and mortality in women with breast cancer. The ability to predict the metastatic proclivity is essential in choosing the optimal treatment. Tumor size and grade, which are frequently used markers in node-negative breast cancer patients, are inadequate markers for prognosis and individualized treatment design. The steps in metastatic progression include angiogenesis, invasion, and changes in adhesion characteristics. We developed a strategy for choosing biomarkers representing these steps in malignant progression to identify patients with occult metastases who will need chemotherapy and spare those women whose tumors have not developed the capacity to spread. To evaluate the added significance of E-cadherin to that of nm23-H1 and angiogenesis in determining metastatic proclivity, we used archival material from 168 node-negative breast cancer patients who were treated with mastectomy without any adjuvant chemotherapy or hormone therapy. Immunohistochemistry was used to detect E-cadherin and nm23-H1 expression, whereas angiogenesis was determined by microvessel count (MVC) after immunohistochemical staining. The median follow-up is 14 years. We found that E-cadherin is better in identifying the poor prognosis patients. The 14-year disease-free survival (DFS) is 84%, 80%, and 56% in patients with high, intermediate, and low E-cadherin. The worst prognosis group using nm23-H1 and MVC as biomarkers has a 14-year DFS of 62%. In this group, if E-cadherin is low, the 14-year DFS is further decreased to 44%. Nm23-H1 and MVC are better in identifying the good prognosis patients. The long-term DFS is >90% if MVC is low or if nm23-H1 is high. Multivariate analysis shows that E-cadherin, nm23-H1, and MVC are more significant prognostic biomarkers than tumor size or grade. Loss of E-cadherin appears to be a latter step in the metastatic progression compared to angiogenesis and the loss of nm23-H1 expression.

INTRODUCTION

Chemotherapy has been shown to decrease the risk of developing metastases and to result in a better average survival in all stages of patients (1), but it does not benefit all patients because in some, it is ineffective, and in others, it is unnecessary. Providing appropriate treatment recommendations to women with breast cancer requires an understanding of whether that particular patient will develop distant metastases. In present clinical practice, the risk of metastatic disease is estimated based on the number of positive axillary nodes, the tumor size, complemented with tumor grade, receptor status, ploidy, and proportion of cells in the S phase. But this prognostic information proves itself insufficient for clinical decision making. Following local therapy, women with node-negative breast cancer have an ~20–30% probability of developing metastatic disease (2, 3). The presently available markers of prognosis do not allow us to distinguish at diagnosis who the minority of the node-negative patients are who will actually develop distant metastases.

For metastases to occur, several progressive changes in the phenotype are needed. These include neovascularization, decreased adherence of the tumor cells to each other, increased motility, adhesion to the extracellular matrix, and degradation of the extracellular matrix (4–6). We have developed a strategy to identify prognostic markers based on biomarkers for these metastatic phenotypes.

Folkmn and colleagues (7–9) have shown that tumor growth, progression, and metastasis require angiogenesis. Growth of a tumor beyond 1–2 mm³ is dependent on angiogenesis (9). Tumor spread to distant sites is also dependent on access to the vasculature. The higher the count of microvessels and the larger the surface area of these vessels, the higher the probability that tumor cells will enter the circulation (10, 11). We, as well as others, have shown that when angiogenesis is measured by counting immunohistochemically stained microvessels, there is an excellent correlation between the MVC², the metastatic propensity, and the long-term DFS (12–15).

Nm23 is a tumor suppressor family of genes that inhibits metastases. Nm23-H1 has been shown to suppress metastatic potential in human carcinoma cell lines (16, 17). It has also been implicated in regulating basement protein deposition and restoring the normal phenotype to metastatic breast cells in culture. Transfection of the nm23-H1 gene suppresses the cytokine-stimulated motility of human breast carcinoma cells (18). A correlation between decreased levels of nm23-H1 expression and lymph node metastases (19–23), tumor grade (21, 24), or outcome (21, 25, 26) have been demonstrated. We have shown that, particularly in tumors that have high angiogenesis, if the expression of nm23-H1 is intact, the outcome is still excellent (27). None of these markers allowed us to distinguish a very poor prognosis group because even in our previously shown “bad” group, i.e., with high angiogenesis and low nm23-H1, two-thirds of the patients survived long-term. This indicated to us the need for additional markers.

E-cadherin is a calcium-regulated homophilic adhesion molecule. The gene has been cloned and is located on chromosome 16q22.1 (28). The extracellular domain is involved in cell-cell adhesion, whereas the intracellular domain connects to the actin cytoskeleton via catenins. It has a significant function in the epithelial intercellular junction complex, the establishment of epithelial polarization, glandular differentiation, and stratification (29). It is a component of the adherent junctions, and it concentrates the urokinase plasminogen activator and the epidermal growth factor receptor to cell contact sites (30, 31). In development, it is an important regulator of morphogenesis (32, 33). E-cadherin knockouts have been shown not to be viable and demonstrate abnormal epithelial morphogenesis (34).

Decreased E-cadherin-mediated adhesion is also one of the changes characterizing the invasive phenotype (29). In cell lines, an inverse relationship between levels of E-cadherin expression and invasion has been shown (35). In vitro data provide evidence that E-cadherin acts as an invasion suppressor molecule and that the level of expression of E-cadherin is related to invasive characteristics. Transfection of cDNA encoding E-cadherin into highly invasive mouse mammary tumor cell lines (36) resulted in decreased invasiveness in vitro and decreased metastasis in vivo (37).

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2 The abbreviations used are: MVC, microvessel count; DFS, disease-free survival; CI, confidence interval; ER, estrogen receptor.
Down-regulation of E-cadherin expression has been observed in many human carcinomas (29, 38). In general, tumors with lower expression of E-cadherin are more infiltrating, lower grade, and are more likely to have spread to lymph nodes. In breast cancer, a correlation between decreased levels of E-cadherin expression and lymph node metastases (39–41), tumor grade (40, 42, 43), or outcome (40, 42–44) have been demonstrated. The majority of these studies have either a short follow-up or include heterogeneous patient populations treated with varied adjuvant therapies that confound meaningful prognostic conclusions.

We hypothesized that E-cadherin, an epithelial-epithelial adhesion molecule, will add prognostic information to that of nm23-H1 and angiogenesis because it characterizes a different step in the malignant progression. We used archival material from our previously described database of patients treated with mastectomy between 1927 and 1987 (3, 45–50). None of the patients received adjuvant hormone therapy or chemotherapy, which may confound the natural history, and there is sufficient follow-up (median 14 years) so that the entire natural history of the disease has been expressed. This is particularly important in breast cancer because of its long natural history (48, 50).

**PATIENTS AND METHODS**

From our database of 2136 patients treated with mastectomy at the University of Chicago Hospitals between 1927 and 1987, we selected a group of node-negative patients who underwent mastectomy and did not receive any chemotherapy or hormonal therapy. Seven hundred and ninety-two patients satisfied those criteria. Sufficient archival material was available in 168 patients to assess E-cadherin expression. The median follow-up is 14 years (range, 3–36 years), and only 7% have a follow-up of <5 years. Thirty patients were dead of breast cancer, 84 were alive and free of disease, and 51 were dead of other diseases. Clinical data and follow-up information were obtained from the medical records and were further complemented by using telephone contacts with patients, family members, and physicians and by using the tumor registry. Details of the overall patient population in this database and clinical outcome have been previously published (3, 45–50). Characteristics of the patient population are detailed in Table 1. The median age of the patients is 57 years (range, 29–82 years). The treatment consisted of surgery only: radical or extended radical mastectomy in 73%, modified radical mastectomy in 23%, and other type of surgery in 4% of the patients. The median number of lymph nodes removed is 23 (range, 1–68). Tumor size was determined by gross measurements of the excised lesion or by the largest tumor diameter as measured on a histological section. The tumor size distribution is shown in Table 1. The majority of the tumors (88%) are ≤3 cm. Tumor nuclear grading was done using the pathological criteria described by Fisher et al. (51). In six patients, the tissue preservation was not satisfactory for nuclear grading.

### Immunohistochemistry.

**E-cadherin.** Standard immunohistochemical detection with minor modification was performed on sections from archival paraffin-embedded tissue to detect the expression of E-cadherin and nm23-H1 and to assess angiogenesis (anti-CD34 monoclonal antibody; Refs. 14, 27, 52). To detect E-cadherin, five-micrometer sections mounted onto pretreated slides were deparaffinized and rehydrated in graded alcohols and distilled water. The slides were rinsed in PBS and microwaved at high power for two cycles of 5 min each with a 10-min break between cycles (41) in citrate buffer (pH 6.0). The samples were allowed to cool to room temperature, rinsed with PBS, and incubated in 10% normal horse serum in PBS containing 1% BSA. After the PBS rinse, the tumor sections were incubated at 4°C overnight with primary antibody (mouse monoclonal antihuman E-cadherin antibody, clone HEC-1, Zymed Laboratories, San Francisco, CA) diluted 1:100 in PBS (40, 53). After rinsing in PBS, the slides were incubated with biotinylated antimmune immunoglobulin G (VectorBA-2000; Vector Laboratories, Burlingame, CA) for 1 h at room temperature. After rinsing with PBS, endogenous peroxidase activity was quenched with 0.3% H2O2 in PBS for 10 min at room temperature. The slides were again rinsed with PBS and then incubated for 1 h at room temperature with the avidin-biotin-peroxidase complex (Vectastain Elite ABC, Vector Laboratories). The slides were then again rinsed with PBS, developed with 3,3′-diaminobenzidine (Sigma Chemical, St. Louis, MO) chromogen solution (0.05% 3,3′-diaminobenzidine in PBS and 0.006% H2O2) dipped in 0.125% osmium tetroxide (Sigma Chemical) to enhance positivity, counterstained with 1% methyl green (Treven, Gaithersburg, MD), dehydrated in graded alcohol, air dried, and mounted using Pro-Tex (American Scientific Products, McGraw Park, IL) mounting medium under coverslips. Some variability in staining intensity was present. Previously described semiquantiative criteria for scoring, with minor modifications, was used (39, 40, 54). The tissue was scanned for areas of well-preserved tumor. Necrotic areas and areas where the tissue had deteriorated morphology were excluded. In areas of well-preserved tissue, the fraction of the positive staining cell was scored. The staining of normal duct epithelium was used as the internal control for each section. Normal skin was also included as the control. Staining of >75% of the cells and comparable to normal glands was scored as high = 3, clearly recognizable but weaker than normal, or heterogeneous in >25%, but <75% of the cells was scored as intermediate = 2, just identifiable staining in <25%, or none was scored as 1. Five consecutive high power fields images were captured and evaluated using Image Pro (Media Cybernetics, Silver Spring, MD) image analysis software.

**Nm23-H1.** To detect nm23-H1 protein, 5-μm sections mounted onto pretreated slides were deparaffinized and rehydrated in graded alcohols and distilled water. Tumor sections were incubated at 4°C overnight with primary antibody (mouse monoclonal anti-nm23-H1, Novocastra Laboratories, New-castle, United Kingdom) diluted 1:50. The immunoperoxidase detection system used (Vectastain Elite ABC) was used with 3,3′-diaminobenzidine and 0.006% H2O2 dipped in 0.125% osmium tetroxide (Sigma Chemical) to enhance positivity, counterstained with 1% methyl green (Treven, Gaithersburg, MD), dehydrated in graded alcohol, air dried, and mounted using Pro-Tex (American Scientific Products, McGraw Park, IL) mounting medium under coverslips. Some variability in staining intensity was present. Previously described semiquantiative criteria for scoring, with minor modifications, was used (39, 40, 54). The tissue was scanned for areas of well-preserved tumor. Necrotic areas and areas where the tissue had deteriorated morphology were excluded. In areas of well-preserved tissue, the fraction of the positive staining cell was scored. The staining of normal duct epithelium was used as the internal control for each section. Normal skin was also included as the control. Staining of >75% of the cells and comparable to normal glands was scored as high = 3, clearly recognizable but weaker than normal, or heterogeneous in >25%, but <75% of the cells was scored as intermediate = 2, just identifiable staining in <25%, or none was scored as 1. Five consecutive high power fields images were captured and evaluated using Image Pro (Media Cybernetics, Silver Spring, MD) image analysis software.

**Angiogenesis (MVC).** To assess angiogenesis, we used our previously described immunohistochemical method (33). In short, from the archival tissue block in which tumor was confirmed and graded, 5-μm sections mounted onto pretreated slides were deparaffinized and incubated at 4°C overnight with the primary antibody (monoclonal anti-CD34/QB-END, Novocastra Laborato-ries), diluted 1:25 in PBS. The immunoperoxidase detection system used for E-cadherin and nm23-H1 was also used to detect anti-CD34 binding (Vec-

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Table 1 | Selected characteristics of patients and their breast cancers

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* N/A, not available.
tastain Elite ABC; 3,3-diaminobenzidine as chromogen). Vascularity was determined by the number of vessels per field counted in the area of highest vascular density ("hot spots") at 400× magnification (0.1452 mm; ref. 2). Previously recommended guidelines were followed (15, 55–57). Single endothelial cells, endothelial cell clusters, and microvessels in the tumor clearly separated from adjacent microvessels were counted. Peritumoral vascularity and vascularity in areas of necrosis were not scored. Branching structures were counted as a single vessel. The presence of lumen or erythrocytes in the lumen was not required to classify a structure as a vessel. If the vascularity was uniform, microvessels in three fields were counted and averaged. If the vascularity in different fields was not uniform, up to 10 fields were counted, and the three highest counts were averaged. Low angiogenesis as previously detailed was defined as <15 microvessels/endothelial cells at 400× magnification (14, 15). E-cadherin, nm23-H1, nuclear grading, and the MVC were performed without the knowledge of the patients’ outcome.

**Statistical Analyses.** Actuarial survival curves were calculated according to the Kaplan-Meier method (58), and comparisons were made with the log-rank test (59). Patients were censored at last follow-up if they were free of disease and were considered dead of disease if they were dead and were known to have recurrent disease at the last evaluation. DFS was defined as the elapsed time from mastectomy to disease recurrence or death. Death of disease or any recurrent disease local or distant was considered as an event in DFS calculation. Patients were censored for death due to intercurrent disease.

**RESULTS**

**E-cadherin, nm23-H1, Angiogenesis, and Outcome.** The long-term DFS of all of the patients is shown in Fig. 1A. The 14-year DFS of the entire group is 78% (95% CI, 69–84). We choose the 14-year point because the median follow-up is 14 years. The long-term DFS as a function of E-cadherin expression is shown in Fig. 1B. There is a significant difference in outcome as a function of E-cadherin expression ($P = 0.005$). If E-cadherin is low, the 14-year DFS is 56% (95% CI, 34–71), but if E-cadherin is intermediate or high, the 14-year DFS is 80% (95% CI, 64–89) and 84% (95% CI, 72–92), respectively.

MVC and nm23-H1 alone are excellent in identifying the good prognosis patients. In univariate analysis, the 14-year DFS is 90% (95% CI, 74–96) if the MVC is low, whereas if the MVC is high, it is 74% (95% CI, 64–82; $P = 0.05$). Similarly, in the patients in whom the tumor had high nm23-H1, the 14-year DFS is 93% (95% CI, 81–97) compared to 69% (95% CI, 56–78) if nm23-H1 is decreased ($P = 0.008$). Thus, these two markers indicate a group of patients with an excellent prognosis. But even the high-risk group (high MVC and low nm23-H1) still contains patients with excellent prognosis in

![Fig. 1. Long-term DFS in node-negative breast cancer patients who did not receive adjuvant therapy. A, all patients. B, all patients as a function of E-cadherin expression (dotted line, high; solid line, intermediate; broken line, low E-cadherin). There is a significant difference in DFS ($P = 0.005$), particularly in the patients with low E-cadherin. C, patients who have tumors with high MVC and low nm23-H1 (i.e., “bad” prognostic biomarkers) as a function of high (dotted line), intermediate (solid line), and low (broken line) E-cadherin expression ($P = 0.01$). D, patients with high/intermediate E-cadherin and low MVC or high nm23-H1 (dotted line) compared to high/intermediate E-cadherin, high MVC, and low nm23-H1, (solid line; $P = 0.017$).](cancerres.aacrjournals.org)
whom the 14-year DFS is 62% (95% CI, 48–74) without any adjuvant therapy. Because E-cadherin is a marker of adhesion, different in function from nm23-H1 or angiogenesis, we expected that it would add prognostic information to MVC and nm23-H1. The DFS as a function of E-cadherin expression in patients with low nm23-H1 and high MVC (i.e., the “bad” prognostic group) is shown in Fig. 1C. There is a significant difference in outcome as a function of E-cadherin expression. The 14-year DFS is 76% (95% CI, 48–91), 61% (95% CI, 34–79), and 44% (95% CI, 22–65), respectively if E-cadherin is high, intermediate, or low (P = 0.01).

To determine which are the most significant prognostic biomarkers, we performed multivariate analyses, which are shown in Table 2. As a first step in a stepwise multivariate analysis, we included E-cadherin, nm23-H1, MVC, tumor size, tumor grade, age, and ER status. ER was the least significant; thus, as a first step, we excluded it from the analysis. Next, we excluded grade because of the high P (0.6) and age because of the noninformative hazard rate. This analysis demonstrates that the biomarkers E-cadherin, nm23-H1, and MVC appear to be more important variables than tumor size, grade, age, or ER.

Because E-cadherin is such a strong prognostic factor, it is important to determine whether it is sufficient by itself and whether nm23-H1 and MVC contribute any additional prognostic information. E-cadherin is not as good as nm23-H1 or MVC in identifying the “good” risk patients. The 14-year DFS of patients with high or intermediate E-cadherin is 84% and 80%, respectively. Because these values are close to each other, in our subsequent analyses, we combine the high and intermediate E-cadherin groups together. To further ascertain the contributory value of nm23-H1 and MVC to that of E-cadherin, we analyzed the DFS as a function of nm23-H1 or MVC in the combined group of high and intermediate E-cadherin patients. The 14-year DFS is 94% (95% CI, 81–98) if nm23-H1 is high or 91% if MVC is low. This confirms that nm23-H1 and MVC are better in identifying the good prognosis patients, whereas E-cadherin appears to be better in identifying the poor prognosis patients. Because there is no correlation between nm23-H1 and MVC (27), these two markers identify different patients and both contribute prognostic information. Fig. 1D shows the long-term DFS in patients with high/intermediate E-cadherin, comparing patients with either low MVC or high nm23 to those with both high MVC and low nm23. The 14-year DFS is 92% (95% CI, 82–97) versus 69% (95% CI, 50–82), respectively (P = 0.017). Thus, nm23 and MVC add prognostic information to that obtained from E-cadherin.

To further understand the relative importance of E-cadherin, nm23-H1 and angiogenesis, and breast cancer progression, we analyzed the outcome as a function of the combinations of the three biomarkers. Table 3 shows the 14-year DFS and number of patients in the eight possible combinations of the three biomarkers. This is also further detailed in Fig. 2. This figure allows us to identify events that are unlikely to occur and possibly assign some hierarchy to the events. The highest 14-year DFS is seen in patients in whom E-cadherin and nm23-H1 are high, and this is irrespective of the MVC count (93% and 94%). This group includes a substantial number of patients (54 patients). The group with slightly lower 14-year DFS is the one with high E-cadherin, low MVC, and low nm23-H1 (88%). There is a more substantial decrease in long-term DFS if MVC is high, nm23-H1 is low, but E-cadherin is high (69%). Interestingly, there are very few patients in three groups: low MVC, high nm23-H1, but low E-cadherin; low MVC, low nm23-H1, and low E-cadherin; and high MVC, high nm23-H1, and low E-cadherin, indicating that these combination of events are much less likely to occur. The worst 14-year DFS is in the group with low E-cadherin, low nm23-H1, and high MVC (44%).

E-cadherin and Other Tumor and Patient Characteristics. The relationship between E-cadherin score, tumor, and patient characteristics are shown in Table 4. In this table, we combined high and intermediate E-cadherin into one group and referred to it as high E-cadherin. The expression of E-cadherin was low in 19% of the tumors tested. There is a trend (P = 0.07) for decreased expression of E-cadherin with an increase in tumor size. A larger proportion of 2-cm tumors (25%) have low E-cadherin compared to ≤2-cm tumors (14%). A trend is also noted as a function of age. Older women have a smaller percent of low E-cadherin tumors. There is a strong correlation between nuclear grade and E-cadherin. Forty-nine percent of grade 3 tumors compared to 11% of grade 1 tumors have low E-cadherin expression (P < 0.001). The ER-positive tumors are more likely to also express E-cadherin (P = 0.04). Angiogenesis appears to be inversely correlated with E-cadherin expression (P = 0.05), whereas a trend for a direct correlation with nm23-H1 is noted.

DISCUSSION

Understanding the metastatic progression of breast cancer in humans offers the opportunity to tailor therapy based on individual tumor characteristics rather than that of the group. Approximately one-third of the node-negative breast cancer patients develop metastatic disease, whereas the other two-thirds never develop metastases despite the fact that they do not receive any chemotherapy. The search for prognostic biomarkers is important both to identify those patients

Table 2 Multivariate analysis of prognostic factors for DFS

<table>
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<th>Variables</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>HR (95% CI)</th>
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<td>E-cadherin (1–3)</td>
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Table 3 DFS in patient groups combining E-cadherin, nm23-H1, and MVC

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</tbody>
</table>

* The full model including all the variables is shown in column one. In the second column, ER is excluded first because it has the highest P. In column three, grade and age are also excluded: grade because of the high P and age because of the noncontributory hazard rate.

* Hazard rate and 95% CI.

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with occult metastases and also to spare chemotherapy treatments in those patients whose tumors have not developed the capacity for distant spread. Such tumor biomarkers have the potential to result in a significant reduction in unnecessary morbidity in those patients not needing the treatment and perhaps allow an increased intensity of therapy in those with occult disease that is destined to recur.

Several studies have demonstrated a correlation between E-cadherin expression, tumor pathological features, and outcome in breast cancer. Lipponen et al. (44) show a trend toward better relapse-free survival in node-negative patients who have high E-cadherin expression. The follow-up was 10 years, and some patients received systemic adjuvant therapy. Their entire patient population includes 208 patients (some have node-positive disease or metastatic disease), and in 13% of the cases, the pathological lymph node status was unknown. Thus, it is difficult to determine the number of node-negative patients. Guriec et al. (42) observed an association between mRNA expression and survival in 42 node-negative patients followed for a median of 90 months. Siitonen et al. (40) also show a significant

Table 4 E-cadherin expression compared with other tumor and patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Low E-cadherin n (%)</th>
<th>High E-cadherin n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>13 (14)</td>
<td>79 (86)</td>
<td>0.07</td>
</tr>
<tr>
<td>&gt;2</td>
<td>19 (25)</td>
<td>57 (75)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>14 (26)</td>
<td>39 (74)</td>
<td>0.10</td>
</tr>
<tr>
<td>&gt;50</td>
<td>18 (16)</td>
<td>97 (84)</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3 (11)</td>
<td>25 (89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>12 (12)</td>
<td>87 (88)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17 (49)</td>
<td>18 (51)</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13 (13)</td>
<td>86 (87)</td>
<td>0.04</td>
</tr>
<tr>
<td>Negative</td>
<td>15 (26)</td>
<td>43 (74)</td>
<td></td>
</tr>
<tr>
<td>MVC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4 (9)</td>
<td>40 (91)</td>
<td>0.05</td>
</tr>
<tr>
<td>High</td>
<td>28 (23)</td>
<td>96 (77)</td>
<td></td>
</tr>
<tr>
<td>nm23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>8 (13)</td>
<td>54 (87)</td>
<td>0.1</td>
</tr>
<tr>
<td>Low</td>
<td>20 (24)</td>
<td>65 (76)</td>
<td></td>
</tr>
</tbody>
</table>

n, patient number.
cantly higher DFS with invasive breast carcinoma expressing high E-cadherin. The prognostic value of E-cadherin was maintained in multivariate analysis. But the patient population in whom clinical follow-up was available is quite heterogeneous and includes 109 node-positive and node-negative patients, some of whom received various adjuvant therapies. Charpin et al. (43) limit their analysis to patients who did not receive adjuvant systemic therapy. At 10 years in the 82 node-negative patients, E-cadherin is a significant prognostic factor for overall survival. These studies indicate that E-cadherin has the potential to be a valuable prognostic marker, but most are heterogeneous, having many confounding variables.

Our patient population consists of node-negative patients who underwent mastectomy and received no other adjuvant treatment; thus, the natural history is not perturbed (48, 50). The long-term DFS of the patients in this group is 78%. We find that E-cadherin is the strongest prognostic factor for long-term outcome. Alone, it identifies a group of patients with a long-term DFS of 56%, and in combination with nm23-H1 and MVC, it identifies a group with an even lower long-term DFS of 44%. Hence, loss of E-cadherin expression appears to be a major determinative step in the metastatic progression. The combination of loss of E-cadherin, loss of nm23-H1, and high angiogenesis results in a worse long-term DFS. E-cadherin by itself is less valuable in identifying the good prognosis group. The long-term DFS in the patients with high or intermediate E-cadherin is 82%. In this group, nm23-H1 and angiogenesis identify the subgroups with higher 14-year DFS, 94% if nm23-H1 is high, or 91% if angiogenesis is low.

In Table 3 and Fig. 2, we attempt to combine the information on the three biomarkers. Multivariate analysis is excellent in indicating which is the most powerful prognostic factor, but it is also important to relate this acquired phenotype to each other. In Fig. 2, we assume that the tumors start out as having low angiogenesis and full expression of nm23-H1 and E-cadherin. The arrows are hypothetical paths that are proposed based on outcome, number of patients, and correlation between the markers. A bin with few patients may indicate that path to be an unlikely sequence of events. For example, there is only one patient with low angiogenesis and high nm23-H1 who has loss of E-cadherin; similarly, there are only a few patients (seven) who have high angiogenesis and high nm23-H1 who have loss of E-cadherin. Thus, irrespective of the extent of angiogenesis, there are few patients in whom E-cadherin expression is lost if nm23-H1 is fully expressed. A possible explanation may be that loss of E-cadherin occurs subsequent to the loss of nm23-H1.

It appears that among the three biomarkers, E-cadherin, nm23-H1, and MVC, MVC contributes the least prognostic information. If E-cadherin and nm23-H1 are high, even if MVC is high, the long-term survival is excellent. But if nm23-H1 expression is lost, the survival is lower, and if there is also a subsequent loss E-cadherin, there is an even further decrease in the long-term DFS to 44%. Angiogenesis appears to be the least significant of these biomarkers. If both nm23-H1 and E-cadherin expression are normal, an increase in angiogenesis does not result in further metastases. But if nm23-H1 expression is lost, then increased angiogenesis appears to be a facilitator of metastases because the long-term DFS is 88% if MVC is low and only 69% if MVC is high. There are very few patients with low MVC who have loss of E-cadherin, also indicating that loss of E-cadherin is a latter event in the metastatic progression. Both nm23-H1 and MVC are excellent in predicting the good prognosis patients. Although MVC may appear less significant, it still has prognostic value because there is no correlation between MVC and nm23-H1 and thus they identify different patients. However, the worst outcome is when all three biomarkers are “bad.” Hence, all three markers contribute prognostic information superior to the traditional prognostic markers, tumor size, and grade.

But even using the combined information from E-cadherin, nm23, and MVC, the worst outcome group still has a 44% long-term survival. Therefore, in this group, 44% of the patients would be receiving systemic therapy unnecessarily. Further biomarkers have to be identified in these patients. The most clinically relevant prognostic marker combination is that which identifies patients with very low or very high long-term DFS because those with very high long-term DFS will not need chemotherapy, whereas those with very low DFS need aggressive systemic treatments.

Promising biomarkers for predicting risk of metastases are likely to come from understanding the metastatic progression in human breast cancer. The markers analyzed in this study represent angiogenesis (MVC), epithelial-epithelial adhesion (E-cadherin), and motility/signal transduction (nm23-H1). Integration of biomarkers representing different steps in the metastatic progression will likely offer the best hope in prognosticating the outcome at diagnosis. Further, useful markers may come from invasion markers, such as proteolytic enzymes, and molecules characterizing the adhesion to the extracellular matrix. As in this study, the most valuable information both for the understanding of metastatic progression and prognosis will be obtained from studies done on patients followed for long periods of time and who received no adjuvant systemic therapy and therefore in whom the natural history is not perturbed.

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REFERENCES

PROGNOSIS MARKERS IN NODE-NEGATIVE BREAST CANCER


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