Molecular Markers in Male Breast Carcinoma

Daniel Rayson, M.D.
Charles Erlichman, M.D.
Vera J. Suman, Ph.D.
Patrick C. Roche, Ph.D.
Lester E. Wold, M.D.
James N. Ingle, M.D.
John H. Donohue, M.D.

Mayo Clinic Cancer Center, Mayo Clinic, Rochester, Minnesota.


Supported in part by the Mayo Clinic Cancer Center Support Grant (CA-15083-24).

The authors would like to thank Steven C. Zeismer for assistance with the immunohistochemistry.

Address for reprints: Charles Erlichman, M.D., Mayo Clinic, 200 First Street, SW, Rochester, MN 55905.

Received October 28, 1997; revision received March 18, 1998; accepted April 6, 1998.

© 1998 American Cancer Society

BACKGROUND. In contrast to female breast carcinoma, information regarding the prevalence and prognostic information of new molecular markers is limited in male breast carcinoma. The objective of this study was to assess the degree of expression and prognostic value of estrogen receptors (ER), progesterone receptors (PR), androgen receptors (AR), bcl-2, p53, HER-2/neu, cyclin D1, and MIB-1 in a cohort of male breast carcinoma patients.

METHODS. A computerized search of the medical index, tumor registry, and tissue registry was used to identify 111 male patients with a diagnosis of primary adenocarcinoma of the breast seen between 1950–1992 at the Mayo Clinic. Of these, 77 patients had adequate tissue specimens available for the immunohistochemical analysis of the markers. Immunoperoxidase staining was performed by an automated avidin-biotin complex method. Progression free (PFS) and overall (OS) survival distributions were estimated using the Kaplan–Meier method. The log rank test was used to determine whether any patient characteristic, tumor feature, or molecular marker was associated significantly with PFS or OS.

RESULTS. The majority of tumor specimens were positive for ER (91%), PR (96%), AR (95%), and bcl-2 (94%). Fewer positive specimens were found for cyclin D1 (58%), MIB-1 (38%), HER-2/neu (29%), and p53 (21%). The 5-year PFS and 10-year OS for the entire patient cohort was estimated to be 66% (95% confidence interval [CI], 57–77%) and 38% (95% CI, 29–50%), respectively. PFS was decreased significantly for those patients with tumors staining positively for MIB-1 (P = 0.012) or negatively for cyclin D1 (P = 0.009). OS was not found to differ significantly with respect to these markers.

CONCLUSIONS. The nearly universal expression of hormone receptors in these tumors suggests a central role for endogenous hormones in male breast carcinoma. The high degree of AR expression would suggest that antiandrogen therapy should be explored further. The high frequency of bcl-2 positivity may implicate antiapoptotic mechanisms in the carcinogenesis of male breast carcinoma. The finding of decreased PFS in MIB-1 positive tumors supports the role of proliferative activity as a negative prognostic factor in male breast carcinoma. Positive cyclin D1 expression is associated with increased PFS in male breast carcinoma patients, which suggests that interactions among cell cycle regulatory proteins may be important in this disease. Cancer 1998;83:1947–55.

© 1998 American Cancer Society.

KEYWORDS: male breast carcinoma, molecular markers, hormone receptors, immunohistochemistry.
Prognostic variables are well defined in the female breast carcinoma population and include tumor size, lymph node involvement, histologic type, nuclear grade, and hormonal receptor status.\textsuperscript{10,11} There has been a proliferation of new tumor markers in female patients and clinical practice guidelines on their use recently have been published.\textsuperscript{12} Although the clinical utility of many of these markers remains under investigation, the study of protooncogenes (e.g., p53 and HER-2/neu), cell cycle regulatory proteins (e.g., cyclin D1 and MIB-1), and markers of apoptosis (e.g., bcl-2 and bax) in female breast carcinoma has led to new insights into the biology of the disease and attempts at utilizing molecular markers as prognostic factors.

The natural history of male breast carcinoma has been described in a number of recent studies,\textsuperscript{2–9,13} but data regarding the presence of newer molecular markers and their prognostic significance in males with breast carcinoma are limited in comparison with that in females. Carcinomas of the male breast arise in a different hormonal milieu compared with female breast tumors but appear to behave similarly. The elucidation of new molecular markers in male breast carcinoma may provide insights into the biologic differences between male and female breast carcinoma patients and could aid in clinical management.

The objectives of this study were to describe the tumor characteristics, treatment, and outcome in a cohort of male breast carcinoma patients treated at our institution and to determine the degree of expression and prognostic value of the following molecular markers: estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), p53, bcl-2, HER-2/neu, MIB-1, and cyclin D1. The majority of these markers were chosen because of the availability of data from female patients and because they represent active areas of investigation in female breast carcinoma.

\textbf{PATIENTS AND METHODS}

\textbf{Patients}

Utilizing a computer search of the medical index, tumor registry, and tissue registry, all male patients with a diagnosis of adenocarcinoma of the breast seen at the Mayo Clinic between the years 1950–1992 were identified. All charts were reviewed and confirmation of the treatment of primary breast carcinoma was obtained for 111 patients. Thirty-four patients did not have pathologic specimens available primarily because they were seen in consultation only and the tumor specimens either were not reviewed or saved at Mayo Clinic. Seventy-seven patients had adequate tissue specimens available for immunohistochemical analysis of the 8 markers and form the basis of this report. In all cases, tissue specimens analyzed were from the initial surgical treatment. No patients received neoadjuvant therapy. Information for the entire cohort of 111 patients regarding risk factors, clinical presentation, primary surgical and adjuvant therapies, tumor recurrence, and subsequent treatment as well as date and cause of death were obtained from the medical record. All therapies not administered at Mayo Clinic were confirmed by review of the clinical notes in the patient’s oncology history when they returned for follow-up visits. No patients were contacted. This study was approved by the Institutional Review Board of Mayo Clinic.

\textbf{Immunohistochemistry}

Standard pathologic features of each case including tumor size, nuclear grade, and lymph node status were reviewed and classified according to the TNM system.\textsuperscript{14} Immunoperoxidase staining for ER, PR, AR, MIB-1, p53, c-erb B-2, bcl-2, and cyclin D1 were performed by an automated avidin-biotin complex (ABC) method on a Vantana Techmate 1000 (Vantana, Tucson, AZ). Six-micron paraffin sections were mounted on silanized glass slides, deparaffinized, and rehydrated through graded alcohols to water. Endogenous peroxidase activity was blocked by incubation with 0.6% hydrogen peroxide. Sections were immersed in 10-mM sodium citrate buffer (pH 6.0) subjected to heat antigen retrieval for 30 minutes in a steamer, and then cooled for 5 minutes. Sections then were treated with 10% normal goat serum for 10 minutes to block nonspecific protein binding. Antibody characterization, tissue localization, and positive controls are shown in Table 1. The negative control for each antibody was a nonimmunogenic immunoglobulin (Ig) G antibody.

The mouse monoclonal antibodies to human ER, human PR, MIB-1, p53, c-erb B-2, and bcl-2 were applied and tissue sections were incubated for 30 minutes at room temperature. The monoclonal antibodies to AR and cyclin D1 were incubated with sections overnight at 4 °C. After brief rinsing, sections were treated with biotinylated antimouse IgG for 30 minutes, rinsed, and then incubated with peroxidase-labeled ABC for 30 minutes. After brief washing, sections were incubated with dianinobenzidine or aminoethylcarbazole (AEC) and hydrogen peroxide for 5 minutes. Detection chemistries were all purchased from BioTek, and used according to the manufacturer’s instructions. Sections were lightly counterstained with hematoxylin and then mounted with a coverslip.

The immunostaining was assessed by three reviewers (D.R., L.W., and P.R.) who were blinded with regard to the tumor characteristics and outcomes of the patients. The degree of immunostaining for MIB-1,
-2, cyclin D1, and p53 was assessed jointly by the three reviewers and a consensus was reached in each case. For these markers, specimens with $\geq 20\%$ of tumor cells staining for the marker were deemed positive. Immunostains for ER, PR, AR, and HER-2/neu were assessed independently by each reviewer. Any tumor cell staining for ER, PR, AR, or HER-2/neu was scored positive. The concordance between reviewers ranged from 86% for AR to 97% for ER. In cases in which there was not unanimous agreement, the result recorded by two of the three reviewers was used.

### Statistical Analysis

The distributions of OS and progression free survival (PFS) were estimated using the Kaplan–Meier method. For the endpoint of PFS, patients who did not have progression documented in the medical record were censored at the last date their disease status was known. Patients who died of breast carcinoma without documented progression were declared to have progressed on the date of death. The log rank test was used to determine whether any tumor feature, patient characteristic, or molecular marker was significantly associated with PFS or OS. Fisher’s exact test was used to assess whether there was a significant pairwise association between positive staining for each marker. Concordance between two tumor markers was said to be observed if both stained positively or both stained negatively.

### RESULTS

**Patient and Tumor Characteristics**

The patient and tumor characteristics for the entire cohort as well as for those patients with adequate tissue are shown in Table 2. All but two of these characteristics, as well as PFS and OS, were not significantly different between the group with tissue for analysis ($n = 77$) and the group for whom tissue was unavailable ($n = 34$). The exceptions were stage and lymph node status documentation, in which 24% and 65%, respectively, of patients without tissue samples for analysis had no stage and lymph node status documentation compared with 5% and 32%, respectively, of those with tissue samples for analysis.

The most common surgical therapies were modified radical mastectomy, which was performed in 61 patients (55%), and a radical mastectomy, which was performed in 26 patients (23%). There was a trend toward less extensive surgery in more recent decades. Seventy-five percent of the 16 patients diagnosed between 1950–1959 underwent radical mastectomy, as did 48% of the 23 patients treated between 1960–1969, and 10% of the 31 patients treated between 1970–1979; none of the 41 patients treated between 1980–1992 underwent this procedure.

The median tumor size was 20 mm among the 86 patients for whom this information was available. For the endpoint of PFS, patients who did not have progression documented in the medical record were censored at the last date their disease status was known. Patients who died of breast carcinoma without documented progression were declared to have progressed on the date of death. The log rank test was used to determine whether any tumor feature, patient characteristic, or molecular marker was significantly associated with PFS or OS. Fisher’s exact test was used to assess whether there was a significant pairwise association between positive staining for each marker. Concordance between two tumor markers was said to be observed if both stained positively or both stained negatively.

### TABLE 1

<table>
<thead>
<tr>
<th>Antibody Characterization, Tissue Localization, and Positive Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antigen</strong></td>
</tr>
<tr>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>Progesterone receptor clone 1A6</td>
</tr>
<tr>
<td>Androgen receptor clone 2F1Z</td>
</tr>
<tr>
<td>MIB-1 clone MIB-1</td>
</tr>
<tr>
<td>bcl-2 clone 124</td>
</tr>
<tr>
<td>C-neu clone CB11</td>
</tr>
<tr>
<td>Cyclin D1 clone P2011F11</td>
</tr>
</tbody>
</table>

Ig: immunoglobulin; ER: estrogen receptor; PR: progesterone receptor; AR: androgen receptor.
TABLE 2
Patient and Tumor Characteristics

<table>
<thead>
<tr>
<th>All patients (n = 111)</th>
<th>Patients with available tissue (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range) (yrs)</td>
<td>64.0 (33–85)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18 (16%)</td>
</tr>
<tr>
<td>Negative</td>
<td>80 (72%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>13 (12%)</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>7 (6%)</td>
</tr>
<tr>
<td>Lumpectomy</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Simple mastectomy</td>
<td>11 (10%)</td>
</tr>
<tr>
<td>Modified radical mastectomy</td>
<td>61 (55%)</td>
</tr>
<tr>
<td>Radical mastectomy</td>
<td>26 (23%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Tumor size (median) (range) (mm)</td>
<td>20 (n = 86) (1–50)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>43 (39%)</td>
</tr>
<tr>
<td>IIA</td>
<td>27 (24%)</td>
</tr>
<tr>
<td>IIB</td>
<td>22 (20%)</td>
</tr>
<tr>
<td>IV</td>
<td>7 (6%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (11%)</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (6%)</td>
</tr>
<tr>
<td>No</td>
<td>82 (74%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>22 (20%)</td>
</tr>
<tr>
<td>Adjuvant hormonal therapy</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (14%)</td>
</tr>
<tr>
<td>No</td>
<td>89 (80%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Adjuvant radiation</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>33 (30%)</td>
</tr>
<tr>
<td>No</td>
<td>72 (65%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (5%)</td>
</tr>
</tbody>
</table>

TABLE 3
Immunohistochemistry Results

<table>
<thead>
<tr>
<th>Marker</th>
<th>No.</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>76</td>
<td>69 (91%)</td>
</tr>
<tr>
<td>PR</td>
<td>76</td>
<td>73 (96%)</td>
</tr>
<tr>
<td>AR</td>
<td>74</td>
<td>70 (95%)</td>
</tr>
<tr>
<td>HER-2/neu</td>
<td>76</td>
<td>22 (29%)</td>
</tr>
<tr>
<td>p53</td>
<td>77</td>
<td>16 (21%)</td>
</tr>
<tr>
<td>bcl-2</td>
<td>77</td>
<td>72 (94%)</td>
</tr>
<tr>
<td>MIB-1</td>
<td>77</td>
<td>29 (38%)</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>73</td>
<td>42 (58%)</td>
</tr>
</tbody>
</table>

ER: estrogen receptor; PR: progesterone receptor; AR: androgen receptor.

Progression Free Survival and Overall Survival

At the time of last follow-up, 5 patients were alive and had not progressed, 37 had progressed but were still alive, and 69 had died. Among the 69 patients who had died, 24 had died of breast carcinoma, 35 died of other causes, and 10 died of unknown causes. Among the 42 patients who were alive at last follow-up, the median length of follow-up was 7.8 years (range, 9 days–19.1 years).

The 3-year and 5-year PFS rate for the entire cohort was estimated to be 72% (95% confidence interval [CI], 64–82%) and 66% (95% CI, 57–77%) respectively Fig. 1. The 5-year and 10-year OS rate for the entire cohort was estimated to be 62% (95% CI, 53–72%) and 38% (95% CI, 29–50%) respectively Fig. 2.

Neither PFS or OS was found to be significantly worse for the 64 patients (58% of the entire cohort) for whom lymph node status was known (P = 0.081 and P = 0.792, respectively). PFS was found to be significantly decreased for those males with tumors staining positively for MIB-1 (P = 0.012) Fig. 3, as well as for those with tumors staining negatively for cyclin D1 (P = 0.009) Fig. 4. OS was not found to differ significantly with respect to either marker. Due to the high frequency of marker positivity, the association be-

Immunohistochemistry

The results of immunohistochemical analysis of the studied markers are summarized in Table 3. The majority of tumor specimens were positive for ER (91%), PR (96%), AR (95%), and bcl-2 (94%). A smaller proportion of tumors with positive staining was found for cyclin D1 (58%), MIB-1 (38%), HER-2/neu (29%), and p53 (21%).

Among the 67 tumor specimens analyzed for ER, PR, and bcl-2 expression, 57 cases (85%) were concordant for ER and bcl-2 and 61 cases (91%) were concordant for PR and bcl-2. A strong association was found between positive staining for p53 and cyclin D1 (P < 0.001). Other significant associations also were found between ER and MIB-1 (P = 0.036), ER and AR (P = 0.031), and cyclin D1 and AR (P = 0.028).
tween ER, PR, AR, and bcl-2 and PFS or OS could not be assessed. There was no evidence to suggest that p53 or HER-2/neu positivity was associated with PFS or OS.

**DISCUSSION**

The main results of our study can be summarized as follows: 1) male breast carcinoma demonstrates a high degree of hormone receptor positivity; 2) bcl-2 also is highly expressed in these cancers; 3) positive staining for MIB-1 was associated with a significantly decreased PFS \((P = 0.012)\); 4) negative staining for cyclin D1 was associated with a significantly decreased PFS \((P = 0.009)\); 5) there was a significant association between p53 and cyclin D1 expression \((P < 0.001)\); and 6) none of the studied markers were found to be significantly associated with OS.

The presence of ERs in male breast carcinoma tumor specimens was first described by Wittliff in 1974.\(^\text{17}\) Subsequent reports have found a higher proportion of ER positive cases in males compared with females. Approximately 64–85.7% of all cases of male breast carcinoma have been found to express ER.\(^\text{18–22}\) The prognostic significance of ER remains uncertain in male breast carcinoma. In what to our knowledge is the largest population-based study of hormone receptors in male breast carcinoma performed to date, Stalsberg et al.\(^\text{19}\) in a study of 282 men, found a marginally significant trend for patients with ER positive tumors to present with higher stage disease although the influence on disease free and OS was not stated. Data regarding PR are even more limited. Stalsberg et al.\(^\text{19}\) in a study of 282 men, found a marginally significant trend for patients with ER positive tumors to present with higher stage disease although the influence on disease free and OS was not stated. Data regarding PR are even more limited. Stalsberg et al.\(^\text{19}\) found PR to be positive in 76.3% of 139 cases and reported a significant correlation between ER and PR positivity. Our study found ER positivity in 91% of cases and PR positivity in 96% of cases. Although these figures are higher than generally reported, they are consistent with the high degree of expression of hor-
mone receptors that previously has been reported in male breast carcinoma. Stalsberg et al.19 used a biochemical assay to assess for ER and PR that may be less sensitive than the immunohistochemical analysis utilized in our study, especially for very small lesions and for paraffin embedded tissues.23 We found no evidence to suggest a significant association exists between age and ER positivity. Thirty-one of the 34 patients age >60 (91%) were ER positive and 38 of the 42 patients age ≥60 years (90%) were ER positive (P = 1.00). This is in agreement with the results from recent studies18,21 but differ from those of Weber-Chappuis et al.21, who found that patients age >60 years were more likely to be ER positive (87% ER positive vs. 64% for those patients age <60 years).

Our finding that nearly 95% of tumors in our patient cohort had immunohistochemically detectable ARs is consistent with a previous report in a small group of patients24 and provides a molecular basis for the response to androgen manipulation that have been reported in cases of metastatic disease. Our results, in conjunction with the reports of Lopez et al.25 and Doberauer et al.,26 would suggest that in addition to antiestrogens, antiandrogen therapy should be explored in male breast carcinoma. Due to the high degree of ER, PR, and AR positivity, we were unable to assess whether the presence of these of these receptors increased PFS or OS.

bcl-2 is part of a large family of protooncogenes whose expression serves to block apoptosis.27 bcl-2 expression in female breast carcinoma has been found to be associated with favorable prognostic features including the presence of ER.28,29 It also has been demonstrated that bcl-2 expression is enhanced in ER positive tumors after exposure to tamoxifen30 and may play a role in predicting the efficacy of adjuvant therapies.31 bcl-2 was expressed in 94% of the cases in our study. Due to the high degree of bcl-2 positivity we were unable to assess the prognostic value of this marker. bcl-2 expression in the female breast has been associated with hormonal and other growth factors that regulate hyperplastic and involutional changes.28 The high degree of bcl-2 expression in our male breast carcinoma cohort suggests that the male breast may be under similar growth factor control and that resistance to apoptotic cell death may be a factor in the carcinogenesis of this disease.

Mutations in the p53 tumor suppressor gene located on the short arm of chromosome 17 are among the most commonly observed oncogenic abnormalities in human malignancies.32,33 Immunohistochemical detection of p53 has been found in 0–54% of male breast tumors.21,22,34 In our series, 21% of the tumors were p53 positive. This result, although within the range previously reported for male breast carcinoma, may be an underestimation when compared with results obtained by polymerase chain reaction techniques.35,36 The prognostic significance of p53 mutations in male breast carcinoma remains unclear. Anelli et al.36 found a trend toward a longer disease free survival in a cohort of 34 patients with wild-type p53. Pich et al.37 found a survival advantage for those cases with p53 negative tumors (median survival of 99 months for p53 negative tumors vs. 33 months for p53 positive tumors). In our study, p53 was not found to be associated with PFS or OS.

The product of the HER-2/ neu protooncogene is a cell surface growth factor receptor of the tyrosine kinase family, which is related closely to the epidermal growth factor receptor.38,39 HER-2/ neu expression occurs in 20–30% of female breast tumors and has been correlated with a number of adverse prognostic factors.40,41 Immunohistochemically detected HER-2/ neu expression has been found in 0–95% of male breast carcinoma specimens.9,21,34,42–44 One study3 found that HER-2/ neu positivity was associated with a poorer outcome although this conclusion is limited by the fact that there were only 17 cases studied. Our study found 29% of cases to be HER-2/ neu positive, an incidence in high agreement with that of female breast carcinoma. In contrast to findings in female breast carcinoma, there was no evidence to suggest a significant association between HER-2/ neu expression and lymph node or ER status exists nor was HER-2/ neu expression found to be associated significantly with PFS or OS. This may reflect the small number of patients in our cohort with lymph node positive disease because in female breast carcinoma, HER-2/ neu provides more prognostic information for lymph node positive compared with lymph node negative patients.39 Alternatively, other growth factor receptors produced by different protooncogenes may play more important roles than that of by HER-2/ neu.

The MIB-1 antibody is directed against part of the Ki-67 antigen, a proliferation-associated antigen expressed in all cells that are not in the resting phase (G0) of the cell cycle, and has been associated with decreased survival in female breast carcinoma.45 Pich et al.,46 investigating the role of proliferative activity in male breast carcinoma, studied the immunohistochemical expression of silver-staining nucleolar organizer regions (AgNOR), proliferating cell nuclear antigen, and MIB-1 in 27 cases. Their results suggested that proliferative activity, as assessed by positive staining for AgNORs, may offer significant prognostic information. Our finding of a significantly decreased PFS in MIB-1 positive cases (P = 0.012) supports the con-
cept that proliferative activity is a significant adverse prognostic factor in male breast carcinoma.

Cyclin D1 complexes with cyclin-dependent kinases that bind and phosphorylate the retinoblastoma gene protein (pRB) and is rate limiting for cell entry into S-phase. It acts as a growth factor sensor and depends on mitogenic stimulation for synthesis. Cyclin D1 is overexpressed in a number of human malignancies as a result of gene amplification or translocations involving the D1 locus on chromosome 11q13.47,48 In female breast carcinoma, approximately 50% of tumors have been found to overexpress cyclin D149–51 and cyclin D1 expression has been found to be associated with more aggressive tumor behavior.52,53 Cyclin D1 overexpression has been assumed to confer a proliferative advantage to tumor cells and thus contribute to a worse prognosis. In a previous study, overexpression of cyclin D1 was found to occur in 41% (18 of 44) of male breast carcinoma specimens.49 We found that 58% of our cases stained positively for cyclin D1. Contrary to expectation, cyclin D1 negativity was associated with a significantly decreased PFS (P = 0.009). This finding is consistent with Gillett et al.51 who, in a recent study of cyclin D1 expression in 345 female breast carcinoma patients, also found that moderate or strong cyclin D1 staining was associated with a significant disease free survival and OS benefit. One possible explanation for this result may involve the interactions of cyclin D1 with the pRB. It has been demonstrated that mutations or inactivation of pRB leads to down-regulation of cyclin D1.47,48,54 Tumors with normal pRB may have increased expression of cyclin D1 compared with tumors with mutated pRB. This could account for the improved PFS provided by positive immunostaining for cyclin D1 observed in our study as well as that of Gillett et al.51 We currently are testing the hypothesis that cyclin D1 may serve as a ‘surrogate’ marker of pRB in male breast carcinoma and that low levels of expression may indicate the presence of mutated pRB.

The strength of the conclusions from our study is limited by a number of considerations. The small size of our patient cohort precluded a multivariate analysis of the data to assess the independent strength of the observed associations between tumor markers and outcome. Also, due to the small numbers the analysis had limited power to detect small differences among the studied variables. Finally, the use of multiple comparisons increases the possibility of detecting spurious associations between the individual markers themselves and in their relation to PFS and OS. Despite these limitations, a number of interesting avenues of further investigation are suggested by our data.

The nearly universal expression of hormone receptors as well as the significant associations found for ER and MIB-1, ER and AR, and cyclin D1 and AR suggests a central role for endogenous hormones in male breast carcinoma. Based on extrapolation from the female population, tamoxifen is the most commonly used adjuvant hormonal therapy in male patients, although there is no prospective data to confirm efficacy. Further research is needed to clarify the relation between hormone receptor expression and response to hormonal therapies. The role of medical androgen blockade in the treatment of metastatic disease and as adjuvant therapy also remains to be elucidated but is supported by our finding of a high degree of AR expression in male breast carcinoma.

The high frequency of bcl-2 expression implicates antiapoptotic mechanisms as playing a role in male breast carcinoma. bcl-2 is only one of a large and growing family of regulators of apoptosis that includes molecules with either agonistic or antagonistic activities. The most powerful bcl-2 antagonist is bax and there is evidence that the physiologic bcl-2/bax balance may play a critical role in apoptotic cell death in female breast carcinoma.55 We observed a high concordance rate between bcl-2 and the expression of ER and PR. It is possible that the action of endogenous hormones may influence the interactions of bcl-2 and bax by shifting the balance toward bcl-2, thereby causing inhibition of apoptosis and promotion of uncontrolled cellular proliferation.

Due to the rarity of male breast carcinoma and the lack of standardized treatment guidelines, large collaborative studies will need to be performed using strict clinical and laboratory criteria to advance our understanding of this disease as well as to identify the most effective treatment approaches. Although a rare disease, the proliferation of reports from large single institution series describing the natural history and outcomes of male breast carcinoma suggests that prospective clinical trials and collaborative laboratory research potentially are feasible.

REFERENCES


