Amplification and Overexpression of Topoisomerase IIα Predict Response to Anthracycline-based Therapy in Locally Advanced Breast Cancer

John S. Coon, Elizabeth Marcus, Shalina Gupta-Burt, Steven Seelig, Kris Jacobson, Shande Chen, Vivian Renta, Geraldo Fronda, Harvey D. Preisler


ABSTRACT

Purpose: The putative association between erbB-2 overexpression and favorable response to anthracycline-based therapy in breast cancer is controversial, and the mechanism unclear. We sought to determine whether coamplification and overexpression of the topoisomerase IIα gene, near erbB-2 on chromosome 17, and a known anthracycline target, may underlie the association.

Experimental Design: Thirty-five patients who had locally advanced breast cancer (LABC) and who had received neoadjuvant, anthracycline-based therapy were studied. Copy number of topoisomerase IIα and erbB-2 was determined by fluorescence in situ hybridization, and expression by immunohistochemistry.

Results: Of 8 patients with erbB-2 amplification, 5 had a complete response (CR) or minimal residual disease (MRD), 3 had a partial response (PR), and none had stable (StD) or progressive disease (PD) at the time of mastectomy, versus 3 CR or MRD, 16 PR, and 8 StD or PD for patients without amplification (P = 0.008). In contrast, erbB-2 overexpression was not significantly associated with response (P = 0.114). Of 6 patients with topoisomerase IIα amplification, 4 had CR or MRD, 2 PR, and none StD or PD, versus 4 CR or MRD, 17 PR, and 8 StD or PD for patients without amplification (P = 0.034). All of the tumors with topoisomerase IIα amplification also had erbB-2 amplification, but not vice versa. Overexpression of topoisomerase IIα (9 patients) was also associated with favorable response (P = 0.021).

Conclusions: Coamplification of erbB-2 and topoisomerase IIα is significantly associated with favorable local response to anthracycline-based therapy in LABC. The expression data favor a plausible mechanism based on topoisomerase IIα biology.

INTRODUCTION

The therapy of LABC continues to be a difficult problem. Historically, patients had a very poor rate of survival with single modality treatment. Recently, with the routine use of multimodality therapy, specifically chemotherapy, surgery, and radiation, survival has improved (1). Favorable response to neoadjuvant chemotherapy is associated with improved survival (1). Several tumor markers have been reported to be predictors of neoadjuvant chemotherapy response in LABC, including estrogen receptor (2), progesterone receptor (2), proliferation index (2, 3), ploidy (3), and apoptotic index (3). These studies have not provided a basis for selecting optimal chemotherapy for individual patients, however. This results in some patients receiving aggressive therapy without significant benefit. A possible approach to this problem is to customize therapy for individual patients based on demonstrating molecular targets in their tumor cells for specific drugs. Defining such targets may be a prerequisite for improving outcome in this disease.

The controversial and seemingly paradoxical observation that breast cancer patients with erbB-2 overexpression may benefit more than others from anthracycline-based therapy may provide a lead to defining a useful target for chemotherapy (4, 5). This observation seems paradoxical because erbB-2 is a well-known oncogene the amplification and overexpression of which have long been linked to adverse prognosis in breast carcinoma (6, 7). Furthermore, attempts to link erbB-2 up-regulation to increased sensitivity to anthracyclines via manipulating expression in cell lines have been unsuccessful (8). The report that the gene for topoisomerase IIα, a well-established molecular target for anthracyclines in experimental systems, is frequently coamplified with erbB-2 in breast cancer, because of their proximity on chromosome 17, now provides a plausible, although unproven, mechanism for the putative link between erbB-2 overexpression and anthracycline response (9). That a gene amplification event commonly encountered in clinical malignancies makes affected tumors more drug-sensitive, rather than drug-resistant, runs counter to a well-accepted paradigm in...
tumor biology (9). Another recent insight that may bear on the significance of erbB-2 in LABC is the demonstration that only high-level erbB-2 overexpression in breast cancer is associated with gene amplification and is more biologically significant than low-level overexpression (compared with normal breast epithelium; Ref. 10). This appears to underlie the observation that assessing erbB-2 overexpression by immunohistochemistry may be less informative clinically than measuring gene amplification by FISH (11). Diverse methodology for assessing erbB-2 probably contributes significantly to the controversy concerning the significance of erbB-2 overexpression in this and other important contexts. The recent report that approximately 12% of breast cancers that express erbB-2 also express an activated, phosphorylated form of erbB-2, by immunohistochemistry, may further complicate the assessment of expression (12). Because of these observations, we studied both overexpression and gene copy number of both erbB-2 and topoisomerase IIa in a group of 35 patients with LABC treated with anthracycline-based neoadjuvant chemotherapy.

MATERIALS AND METHODS

Patients. Patients with LABC who were treated with neoadjuvant, anthracycline-based chemotherapy at Cook County Hospital between 1996 and 1999 were included in this retrospective study, provided that there was sufficient paraffin-embedded tissue available from the diagnostic (pretherapy) biopsy. All of the patients had a mastectomy after completion of chemotherapy, permitting pathological assessment of the response of the breast tumor. CR was defined as no residual tumor identified in the mastectomy specimen, based on the study of H&E-stained sections. MRD was defined as no gross tumor and microscopic invasive carcinoma present in two or less high-power fields. PR was defined as >50% reduction in the sum of the products of the perpendicular diameters of all measurable lesions within the pathological specimen compared with the clinical measurements taken at diagnosis by physical examination. MR was defined as a 26–49% decrease in the size of measurable lesions at physical examination taken at diagnosis by physical examination compared with the H&E-stained sections. MRD was defined as no gross tumor and response of the breast tumor. CR was defined as no residual tumor chemotherapy, permitting pathological assessment of the re-

Lab Vision Corp. (Fremont, CA) at a dilution of 1:100. A single block from the pretherapy biopsy was selected for analysis for each patient on the basis of having the greatest area of well-preserved tumor. Immunostaining frequency of all tumor cells on each slide was scored subjectively on a scale of 0 to 4 (actual cell counting was not performed) without knowledge of clinical patient data, as described previously (13). The scoring was as follows: <1% positive tumor cells, 0; 1–10%, 1; 10–35%, 2; 36–70%, 3; and >71%, 4. Tumor cell staining intensity was also scored on a scale of 0 to 4 but was found to be so closely related to frequency that it was not further considered in this study. Only cell membrane-associated staining was considered for erbB-2. Only nuclear staining was considered for topoisomerase IIa.

FISH. Paraffin blocks were sectioned at 5 μm and mounted onto SuperFrost positively charged slides (Shandon, Inc., Pittsburgh, PA) and baked at 56°C overnight to fix the tissue onto the slides. After baking, the slides were treated with Pretreatment Solution (Vysis, Inc., Downers Grove, IL) and protease I (Vysis, Inc) according to a protocol similar to that published by Hopman et al. (14). Slides were dewaxed and then incubated in pretreatment solution for 10 min at 80°C. Slides were washed and then incubated in 4 mg/ml protease I in 0.2 N HCl at 37°C for 15 min, dehydrated in graded ethanol baths, and air dried. Sections were hybridized with a FISH probe combination containing a prototype SpectrumGreen LSI Topo II-α, SpectrumOrange LSI HER-2 (erbB-2), and SpectrumAqua CEP-17 (all probes from Vysis, Inc.) using a HYBrite instrument that performs codenaturation and hybridization (Vysis, Inc.). The topoisomerase IIa probe covers ~280 kb, including the entire topoisomerase IIa gene and the sequence tag site markers, WI-15402, SGC31792, and WI-17575 (a subsequently released commercial version of this Vysis multicolor probe set included an LSI Topo II-α probe targeting a larger, 400-kb region). The LSI HER-2 probe covers ~190 kb, including the entire erbB-2 gene. The CEP 17 probe contains sequence homologous to the D17Z1 satellite repeat sequence. Probe hybridization mixture was applied to each section, and glass coverslips were immediately applied and sealed with rubber cement. Slides were then codenatured at 73°C for 5 min and were hybridized at 37°C for 16–18 h. After removing the coverslips, the slides were incubated at 73°C in 2× SSC/0.3% NP40 for 2 min to remove nonspecifically bound FISH probe, and then were air dried in the dark. DAPI I counterstain (Vysis, Inc.) was applied to the specimen to allow visualization of the nuclei.

The FISH slides were evaluated using a Zeiss Axioskop epifluorescence microscope (Carl Zeiss, Inc, Thornwood, NY). Signals were visualized, and counting was performed using a DAPI single-band pass filter set to visualize nuclei, a green single-band pass filter to visualize SpectrumGreen TopoII-α, an orange single-band pass filter set to visualize SpectrumOrange HER-2, and an aqua single-band pass filter set to visualize SpectrumAqua CEP-17. Signals for each probe were counted in 25–30 tumor-cell nuclei. For nuclei with greater than 20 erbB-2 or topoisomerase II signals, an estimate of 21 signals was used for the purpose of calculating signal ratios. A ratio of 2.5 or greater for erbB-2/CEP-17 or for topoisomerase II/CEP-17 was considered to indicate amplification.
Response of the tumor in the breast at the time of mastectomy was divided into three categories: CR or MRD, PR, and StD or PD. Immunohistochemistry data were categorized as overexpressed, or not, and FISH data were categorized as amplified, or not. Fisher’s exact test (2-tailed) was used to examine associations between each of the markers and response of the breast tumors to chemotherapy. Results were considered statistically significant at the 0.05 level. The sample size was small in this study. A power calculation based on Fisher’s exact test for $2 \times 2$ table (from PASS), assuming 8 CR or MRD patients and 27 other patients, showed a power of 48% if the percentage of amplified is 50% among CR or MRD patients and is 10% among other patients. The same technique was used for other categorical data. To investigate associations between patient age and response and other markers, the two-sample Wilcoxon test or the Kruskal-Wallis test (for more than two groups) was used. Age was treated as a continuous variable and was grouped by the category for response or the markers.

### Table 1 Comparison of erbB-2 amplification and overexpression

<table>
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<tr>
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<th>erbB-2 overexpressed</th>
<th>erbB-2 not overexpressed</th>
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<tbody>
<tr>
<td>erbB-2 amplified</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>erbB-2 not amplified</td>
<td>9</td>
<td>18</td>
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<tr>
<td>Total patients</td>
<td>15</td>
<td>20</td>
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</table>

Statistical Methods. Response of the tumor in the breast at the time of mastectomy was divided into three categories: CR or MRD, PR, and StD or PD. Immunohistochemistry data were categorized as overexpressed, or not, and FISH data were categorized as amplified, or not. Fisher’s exact test (2-tailed) was used to examine associations between each of the markers and response of the breast tumors to chemotherapy. Results were considered statistically significant at the 0.05 level. The sample size was small in this study. A power calculation based on Fisher’s exact test for $2 \times 2$ table (from PASS), assuming 8 CR or MRD patients and 27 other patients, showed a power of 48% if the percentage of amplified is 50% among CR or MRD patients and is 10% among other patients. The same technique was used for other categorical data. To investigate associations between patient age and response and other markers, the two-sample Wilcoxon test or the Kruskal-Wallis test (for more than two groups) was used. Age was treated as a continuous variable and was grouped by the category for response or the markers.
Age was then compared among the groups. This was done to compare the conditional distributions of age-grouped categories. If there is no association between age and response (or the markers), then there should be no difference among groups. The sample size is important in this context. For example, if the mean age is 55.3 (SD, 4.5) in 6 topoisomerase IIα amplified patients and the mean age is 47.1 (SD, 8.8) in 29 nonamplified patients, the power (based on a 2-sided level of 0.05) is ~95%. However, if the mean age is 46.8 (SD, 7.1) in 9 patients with topoisomerase IIα overexpression, and the mean age is 49.2 (SD, 9.3) in 29 patients without overexpression, the power (based on a 2-sided level of 0.05) is only ~13%.

**RESULTS**

**Patients.** A total of 35 patients with LABC who were treated with anthracycline-based therapy before mastectomy at Cook County Hospital between 1996 and 1999 were included in the study. All of the patients had biopsy-proven invasive breast carcinoma: 19 ductal, 5 lobular, and 11 diagnosed as breast adenocarcinoma, not otherwise specified. The ages of the patients ranged from 35 to 69 (mean, 48.5) years. Seven patients had stage IIB tumors, 18 had IIA, and 10 IIB. Twenty-two patients received six 21-day cycles of 50 mg/m² doxorubicin (300 mg/m² total) and 75 mg/m² docetaxel (450 mg/m² total) as part of a clinical trial; the others received CAF. CAF therapy comprised six 21-day cycles of 500 mg/m² cyclophosphamide (3000 mg/m² total), 50 mg/m² doxorubicin (300 mg/m² total), and 500 mg/m² 5-fluorouracil (3000 mg/m² total). Five patients achieved a CR and 3 a MRD rating in the breast at the time of mastectomy, 19 had a PR, none had MR, 5 had StD, and 3 had PD while on chemotherapy. The fraction of patients with positive lymph nodes at mastectomy in each response group was as follows: CR, 0 of 5; MRD, 1 of 3 (microscopic); PR, 9 of 19; StD, 1 of 5; and PD, 3 of 3.

**FISH Analysis.** Eight patients’ tumors (23%) showed amplification of the erbB-2 gene as assessed by FISH, with erbB-2/CEP-17 ratios ranging from 2.5 to 4.3 (Fig. 1). None of the two patients with erbB-2 overexpression and StD or PD had erbB-2 amplification, and in the 7 patients with PR, only one overexpressing tumor had amplification. To further analyze the possible link between erbB-2 overexpression and chemotherapy response, we considered separately the 27 patients without erbB-2 amplification, including 9 with overexpression (Table 5). Among these patients, there was no evidence of an association between erbB-2 overexpression and tumor response (P = 0.843).

<table>
<thead>
<tr>
<th>Topoisomerase IIα amplified</th>
<th>Topoisomerase IIα not amplified</th>
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<tr>
<td>Total patients</td>
<td>9</td>
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| erbB-2 amplified | 5 | 3 | 0 |
| erbB-2 not amplified | 3 | 16 | 8 |

| Total patients | 8 | 19 | 8 |

**Table 3** Amplification of erbB-2 is related to chemotherapy response

**Immunohistochemistry.** Fifteen patients’ tumors were interpreted as overexpressing erbB-2 because they showed widespread (3+ or 4+) membrane staining that surrounded individual tumor cells (Fig. 2a). The association between erbB-2 overexpression and gene amplification had borderline statistical significance (P = 0.051). Nine tumors had erbB-2 overexpression without amplification, whereas two had amplification without apparent overexpression (Table 1). Topoisomerase IIα staining was largely confined to the nucleus of the tumor cells in which it was detectable, with rare tumors exhibiting faint cytoplasmic staining. Only nuclear staining was considered in scoring. Nine patients had topoisomerase IIα overexpression, defined as 3+ or 4+ staining (Fig. 2b). There was no evidence for an association between topoisomerase IIα overexpression and gene amplification (P = 0.635; Table 2). Most notably, only two of nine tumors with topoisomerase IIα overexpression had amplification. There was also no evidence of an association between expression of topoisomerase IIα and expression of erbB-2 (P = 0.700).

**Association of Molecular Markers with Response to Therapy.** The clinical end point in this study was the local tumor response in the breast, defined by comparing the breast tumor at presentation with the tumor, if any, at the time of mastectomy after chemotherapy. Amplification of erbB-2 was significantly associated with a favorable local tumor response to chemotherapy (Table 3). Of eight patients with erbB-2 amplification, five had a CR or MRD, three had a PR, and none had StD or PD at the time of mastectomy. In contrast, the 27 patients without amplification had 3 CR or MRD, 16 PR, and 8 StD or PD (P = 0.008). If CR or MRD versus all of the other responses are compared, P = 0.007.

Unlike erbB-2 gene amplification, erbB-2 overexpression was not significantly associated with breast tumor response to chemotherapy (P = 0.114) although if CR or MRD versus all of the other responses are compared, there was a borderline association (P = 0.051; Table 4). Neither of the two patients with erbB-2 overexpression and StD or PD had erbB-2 amplification, and in the 7 patients with PR, only one overexpressing tumor had amplification.
Amplification of topoisomerase IIα was significantly associated with a more favorable tumor response to chemotherapy (Table 6). Of six patients with amplification, 4 had CR or MRD, 2 PR, and none StD or PD, versus 4 with CR or MRD, 17 with PR, and 8 with StD or PD among patients without amplification (P = 0.034). If CR or MRD versus all of the other responses are compared, P = 0.016. Topoisomerase IIα overexpression was also significantly associated with favorable chemotherapy response (Table 7). Of 9 patients with overexpression, 5 had a CR or MRD, 2 had a PR, and 2 had StD or PD, versus 3 with CR or MRD, 17 with PR, and 6 with StD or PD among those without overexpression (P = 0.021). If patients with CR or MRD versus all of the other responses are compared, P = 0.015. None of the four patients with overexpression who failed to achieve a CR or MRD had amplification, which suggests that gene amplification is a more specific marker for favorable response than overexpression as detected by immunohistochemistry. Also, two of the three patients without overexpression who achieved CR or MRD had amplification, which suggests that immunohistochemistry alone should not be used to infer that a patient is unlikely to benefit from anthracycline-based therapy. Nevertheless, three of the five patients with overexpression who achieved CR or MRD were not amplified. This suggests that overexpression of topoisomerase IIα by immunohistochemistry, in the absence of amplification, may sometimes be biologically meaningful. Of the eight patients who achieved CR or MRD, only one had neither overexpression nor amplification.

Consideration of Other Tumor and Patient Factors.

Other available patient and tumor factors for which an association with response to therapy seemed reasonably likely were tested for such an association. There was no evidence for an association between response and patient age, the histological type of the tumor (ductal, lobular, or unspecified), tumor grade (II or III), tumor stage (IIB, IIIA, or IIIB), estrogen or progesterone receptor status (immunohistochemistry by a commercial reference laboratory), or type of chemotherapy (doxorubicin and docetaxel versus CAF). None of these factors was significantly associated with those which predicted response (erbB-2 amplification, topoisomerase IIα amplification or overexpression) except patient age, which was associated with topoisomerase IIα amplification (P = 0.015).

DISCUSSION

Some large studies have reported a positive association between erbB-2 overexpression and favorable response to anthracyclines in breast cancer (4, 5), but the existence of this association and its underlying mechanism remain controversial (15). This study sought to clarify several issues related to this putative association. The patients included were quite uniform with regard to stage and presentation, all falling into the relatively uncommon category of LABC. This group of patients permitted assessing a very well-defined measure of response to neoadjuvant chemotherapy, namely the effect on the tumor in the breast at the time of mastectomy. Clearly, systemic tumor response (development of clinically evident metastases) and survival are also extremely important in evaluating the efficacy of chemotherapy and will be evaluated in this group of patients when sufficient data become available. Nevertheless, these other end points appear to involve a multitude of tumor and host factors, in addition to drug sensitivity of the tumor cells. Other important features of this study are that we determined both overexpression and gene amplification for erbB-2, using a FISH strategy the performance characteristics of which have been well documented (11). Also included were expression and gene amplification studies on topoisomerase IIα, based on the recent documentation that this gene is often coamplified with erbB-2 in breast cancer and is a well-known target for topoisomerase IIα poisons, including the anthracyclines (9, 16). This suggests that coamplification of topoisomerase IIα with erbB-2 may be the principal mechanism underlying the putative relationship between erbB-2 overexpression and response to anthracyclines.

Our data showed a highly significant association between erbB-2 amplification and favorable response to anthracyclines. The association between overexpression and response nevertheless failed to reach statistical significance, and no evidence was found for a link between overexpression and response in patients without amplification. These observations suggest that favorable response is associated with erbB-2 amplification, rather than overexpression itself. Although erbB-2 amplification and high-level overexpression are strongly linked in breast cancer, overexpression (compared with normal breast epithelium) without amplification is also well documented in the literature (6, 10). A recent report using special methods for quantifying overexpression suggests that overexpression in the absence of amplification is at a lower level than that associated with amplification (10). It is also important to recognize that using immunohistochemistry as a quantitative measure of erbB-2 expression is sometimes quite difficult in the small needle biopsies that comprised the patient material in this study, because of tissue edge artifacts and uncertain discrimination between specific and nonspecific staining. Therefore, some of
Table 6  Amplification of topoisomerase IIa is related to chemotherapy response

Amplification of topoisomerase IIa, as determined by FISH, was significantly associated with more favorable tumor response to chemotherapy (P = 0.034). If CR or MRD versus all of the other responses are compared, P = 0.016. Response was determined by comparison of the breast tumor at presentation with the mastectomy specimen. CR = complete response, MRD = minimal residual disease, PR = partial response, SD = stable disease, PD = progressive disease.

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<tr>
<th>CR or MRD</th>
<th>PR</th>
<th>STD or PD</th>
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<tr>
<td>Topoisomerase amplified</td>
<td>4</td>
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</tr>
<tr>
<td>Topoisomerase not amplified</td>
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<td>17</td>
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<td>Total patients</td>
<td>8</td>
<td>19</td>
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Table 7  Overexpression of topoisomerase IIa is associated with chemotherapy response

Topoisomerase IIa overexpression was significantly associated with favorable chemotherapy response (P = 0.021). If patients with CR or MRD versus all of the other responses are compared, P = 0.015. Response was determined by comparison of the breast tumor at presentation with the mastectomy specimen.

<table>
<thead>
<tr>
<th>CR or MRD</th>
<th>PR</th>
<th>STD or PD</th>
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<tbody>
<tr>
<td>Topoisomerase overexpressed</td>
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</tr>
<tr>
<td>Topoisomerase not overexpressed</td>
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<td>17</td>
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<td>Total patients</td>
<td>8</td>
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Both the gene amplification and expression data for topoisomerase IIa are consistent with the notion that this protein is an important determinant of sensitivity of breast cancer to anthracycline-based therapy. This potentially important conclusion requires confirmatory evidence from additional studies because of the small size and retrospective nature of the study population. The disparity between amplification and overexpression may be partly explained by the fact that topoisomerase IIa is a cell cycle-regulated protein. In fact the frequency of topoisomerase IIa expression in a population of cells is tightly linked to the rate of cell proliferation, and topoisomerase IIa has been suggested as a proliferation marker (17). This probably tends to obscure the relationship between gene amplification and overexpression. Attempting to quantify protein expression by immunohistochemistry in small biopsy samples is also difficult, as discussed above. Our data suggest that measuring topoisomerase IIa amplification by FISH is more specific than measuring expression by immunohistochemistry for predicting anthracyline responsiveness, because four of six patients with amplification had CR or MRD, and the other two had PR. Previous studies of topoisomerase IIa expression versus response to drugs that target it have been inconsistent in finding a significant relationship (18, 19). It also appears likely, however, that FISH may miss some tumors with biologically significant topoisomerase IIa overexpression, because four of six patients with amplification had CR or MRD, and all but one of these had topoisomerase IIa overexpression. It may be that patients who have neither amplification nor overexpression should be offered an alternative to anthracyclines, because only one such patient had CR or MRD in our series. Clearly, however, more patients must be studied to arrive at conclusions sufficiently secure to provide the basis for planning treatment. It also appears likely that developing an optimal clinical assay for topoisomerase IIa may be at least as complex as the process for erbB-2. Measuring phosphorylation of topoisomerase IIa may also be useful because its activity may be more regulated (20).

This study does not completely settle the question of whether erbB-2 amplification plays a functional role in determining favorable response to certain anticancer drugs, or is only a marker for topoisomerase IIa coamplification. We found only two patients with isolated erbB-2 amplification; one had MRD, and the other PR. More patients of this kind are needed to test the hypothesis that LABC patients with erbB-2 amplification alone will not respond as well to anthracycline-based therapy as those who have coamplification of topoisomerase IIa.

Amplification of topoisomerase IIa in Breast Cancer

The tumors that were scored as high-level overexpressers and that did not have amplification may actually represent low-level overexpressers (false positives). Two of the patients with erbB-2 amplification were not scored as overexpressers and are presumed to be false negatives. This problem has been recognized in previous studies comparing FISH with immunohistochemistry for erbB-2, and has been attributed to protein degradation in the paraffin-embedded material, in addition to the interpretation problems mentioned above (11). This implies that the FISH assay may be technically more robust for evaluating the clinically important erbB-2 status of tumors, especially in needle biopsies such as were used in this study. This idea is supported by a recent report demonstrating greater reliability of amplification assays than immunohistochemistry for predicting progression based on erbB-2 status (11). The marginal association between overexpression and response is also consistent with an important role for topoisomerase IIa, because amplification of the two genes was tightly linked in our patients and in other studies (9).

Our data support the idea that topoisomerase IIa amplification in clinical breast cancer specimens occurs as a result of proximity to the oncogene, erbB-2, because all cases with topoisomerase IIa amplification had erbB-2 amplification, but not vice versa. In contrast to the findings of Jarvinen et al. (9), however, who described deletion of topoisomerase IIa with frequency equal to amplification in tumors with erbB-2 amplification, we did not detect topoisomerase IIa deletions. The probe set used in our study was designed primarily for detecting amplification of the topoisomerase IIa region in a three-probe multicolor assay. This provides the advantage of measuring the erbB-2, topoisomerase IIa, and chromosome 17 centromere copy numbers in each cell individually, allowing accurate identification of clonal variations. As a result, the topoisomerase IIa target region was significantly larger than that used by Jarvinen et al. (9). Therefore, their probe may have detected smaller deletions that may have been missed with our probe. Another possibility is that their smaller probe is associated with lower hybridization and/or detection efficiency, leading to the artifactual appearance of deletions in some cases. It is unclear at present whether discriminating between tumors that have deletion of one topoisomerase IIa allele, versus those that have two alleles (not amplified), would be biologically or clinically significant.
overexpression has been associated with a favorable response to taxanes, perhaps because erbB-2 overexpression activates mitogen-activated protein kinase, which mediates Taxol-induced cell killing (21). A mechanism based on facilitating response to taxanes does not appear to explain our data, however, because there was no apparent association between tumor response and whether or not a taxane (versus cyclophosphamide and 5-fluorouracil) was combined with doxorubicin. Prospective clinical studies in which patients receive a course of anthracycline alone and taxane alone may be needed to further resolve this important question.

REFERENCES


