Human Breast Cancer Susceptibility to Paclitaxel Therapy Is Independent of Bcl-2 Expression

Susan M. Poelman, Moses O. Adeyanya, Maria-Antonia Robertson, Wendy M. Recant, Theodore Karrison, Gini F. Fleming, Olufunmilayo I. Olopade, and Suzanne D. Conzen


ABSTRACT

In laboratory studies, ectopic overexpression of the antiapoptotic protein Bcl-2 has been shown to result in resistance to the cytotoxic effects of many chemotherapeutic drugs. Furthermore, posttranslational modification of moderately expressed endogenous Bcl-2 has been correlated with susceptibility to paclitaxel treatment in vitro. To determine whether tumor expression of Bcl-2 protein correlates with response and ultimate outcome in vivo, we quantified Bcl-2 expression by immunohistochemical analysis of archived biopsy specimens from metastatic breast cancer patients treated with single-agent paclitaxel. The statistical association between the degree of Bcl-2 expression, objective tumor response, and clinical outcome was then determined. In patients (n = 39) whose tumors had low (≤10% cells positive) Bcl-2 levels by immunohistochemical analysis, the overall response (complete response + partial response) rate was 21% versus an overall response rate of 22% in patients (n = 36) with high (>10% cells positive) Bcl-2 expression (P = 0.92). In patients with low Bcl-2 expression, the median time to progression was 126 days [95% confidence interval (CI), 63-160 days]. This was not significantly different than the 105 days for patients with high tumor Bcl-2 expression (95% CI, 84-214 days). The median survival time from initiation of paclitaxel therapy for patients with low Bcl-2 expression was 663 days (95% CI, 456-1119 days) and was not significantly different than the 450 days (95% CI, 239-1058 days) observed for patients with high Bcl-2 expression. In conclusion, we found that in metastatic breast cancer, there is no significant association between tumor Bcl-2 expression and response to paclitaxel, median time to progression, or survival, suggesting that the main mechanism of paclitaxel-induced cytotoxicity in breast tumors is independent of Bcl-2 expression.

INTRODUCTION

Paclitaxel (Taxol®) is an effective cytotoxic drug for the treatment of a wide range of cancers. In breast cancer therapy, the addition of paclitaxel to standard adjuvant chemotherapy has recently been shown to provide a significant survival benefit over therapy with doxorubicin and cyclophosphamide alone (1). In metastatic breast cancer, response rates to single-agent paclitaxel therapy have been reported to vary from 20–35% (2). Because susceptibility to chemotherapy is believed to reflect the ability of a tumor cell to undergo apoptosis, overexpression of antiapoptotic proteins such as Bcl-2 has been postulated to promote chemotherapy resistance in solid tumors. This hypothesis is supported by data from experimental systems, where ectopic overexpression of Bcl-2 can inhibit apoptosis in many different tumor cell lines derived from non-Hodgkin’s lymphoma, breast, lung, glioma, pancreatic, and ovarian cancers (3–8). In primary human breast cancer specimens, however, high Bcl-2 expression is associated with markers of good prognosis, including the expression of ER (9) and progesterone receptor and low tumor grade (9–11). Furthermore, within the subset of patients with ER-positive tumors, high Bcl-2 expression is associated with relatively indolent disease and predicts a good response to tamoxifen independent of the degree of ER positivity (12). In contrast, in patients with metastatic breast cancer treated with various combinations of non-taxane chemotherapy, high Bcl-2 expression has been associated with resistance to therapy (13) or has been shown to have no effect (14). The relationship between Bcl-2 expression and response to paclitaxel chemotherapy in patients with metastatic breast cancer has not been studied previously.

In vitro, paclitaxel treatment of tumor cell lines can result in the increased phosphorylation of Bcl-2, perhaps through a G2-M phase-dependent activation of an unidentified microtubule-associated kinase (15, 16). In a panel of tumor cell lines, Bcl-2 phosphorylation, in turn, has been shown to correlate with paclitaxel-induced apoptosis, prompting some investigators to speculate that both the level of Bcl-2 expression and its phos-
MATERIALS AND METHODS

Patients and Tumor Specimens. This retrospective chart review evaluated 87 patients with metastatic breast cancer treated with single-agent paclitaxel therapy at the University of Chicago from 1996–1999. Eligible patients included those who received paclitaxel as either a first-line \( (n = 57) \), second-line \( (n = 13) \), or third-line \( (n = 5) \) therapy for measurable or evaluable metastatic disease. Patients were also required to have a primary or metastatic tumor biopsy available for IHC analysis and clinical follow-up information that was accessible to the investigators. Twelve of 87 patients were not eligible (no measurable disease, lack of adequate archived material, or chemotherapy administered in addition to paclitaxel). Of the remaining 75 patients, 73 received paclitaxel every 21 days, and 2 received weekly paclitaxel. Tumor response was determined retrospectively based on radiological review of computed tomography scans. Evaluable disease response was determined for patients with local or bone disease based on reported physical exam and review of skeletal imaging (see below for response criteria). When possible, formalin-fixed paraffin-embedded blocks were collected from metastatic tumors resected before paclitaxel treatment \( (n = 32) \). If no biopsy for metastasis was performed \( (n = 43) \), tumor blocks from the primary or most recent recurrent breast cancer were analyzed. We allowed primary tumors to be used for analysis based on the results of a previous study \( (31) \) and unpublished data\(^4\) that suggest that levels of Bcl-2 expression remain constant between a primary tumor and a subsequent metastasis. In the study of Sjogren et al. \( (31) \), the authors analyzed Bcl-2 expression in asynchronous primary and metastatic tumors from 28 patients with breast cancer. The results revealed that only one patient developed a metastasis with a significant change in Bcl-2 expression compared with the primary breast cancer (a change from high Bcl-2 expression to low expression).

Response Criteria. Patients were evaluated for response by studies performed 4–6 weeks after the sixth cycle of paclitaxel therapy (or earlier if PD was evident). Computed tomography was used to evaluate tumor dimensions in patients with visceral disease \( (n = 55) \), physical examination was used to assess evaluable local disease \( (n = 17) \), and a combination of bone scan, plain films, and clinical assessment was used to evaluate patients with osseous disease only \( (n = 3) \). In patients with nonosseous disease, CR was defined as the disappearance of all evidence of disease. For patients with disease involving the bone, CR was defined as the disappearance of all nonosseous cancer, bone scans or skeletal radiographs without evidence of progression or new lesions, and the disappearance of bone pain. PR in patients without osseous disease was defined as a reduction of more than 50% in the sum of cross-sectional areas of all measured lesions in nonosseous sites. In patients with bony disease, bone scans or skeletal radiographs were required to show no progression or new lesions, and patients had to exhibit an improvement of bone pain. Stable disease was defined as a steady state (\( \pm 25\% \) increase in tumor dimension) or a response of \( < 50\% \). PD was defined as any new site of disease or a \( > 25\% \) increase in any measurable or evaluable disease. Time to progression was defined as the time from initial treatment with paclitaxel to the first occurrence of PD, discontinuation of treatment, or death.

IHC Analysis. Each paraffin block submitted was cut into 5-mm sections, the first of which was stained with H&E to ensure adequate quality of fixation and ensure that the number of invasive tumor cells was sufficient for IHC analysis. The remaining 5-mm sections were mounted positively charged slides and then heated to 60°C for 1 h, cooled, deparaffinized, and hydrated through three changes of xylene and graded alcohols. Slides were then washed with PBS three times, steamed with citric buffer in a rice cooker for 20 min, cooled, rinsed with PBS, quenched in 0.3% \( \text{H}_2\text{O}_2 \) in PBS for 30 min and blocked with 10% horse normal serum for 30 min. Specimens were incubated overnight in Bcl-2 monoclonal antibody 122 (Dako, Carpinteria, CA) using a stock concentration of 2.25 mg/ml at a 1:80 dilution. After rinsing with PBS, secondary biotinylated antimouse IgG antibody at a final concentration of 2.25 mg/ml was performed for each sample using an isotype-specific mouse antibody instead of the anti-Bcl-2 antibody. Human tonsil tissue was used as a positive control for Bcl-2 expression, as were tumor-infiltrating lymphocytes that were present in most samples.

\(^4\) S. M. Poelman and S. D. Conzen, unpublished data.
RESULTS

Seventy-five female patients were evaluable in this study with the following patient and tumor characteristics (median age, 49 years; age range, 25–77 years). Overall, 36 of 75 (48%) patients showed high levels of Bcl-2 expression (Fig. 1), as defined by >10% of cells with detectable IHC staining for Bcl-2 (a score of 3 or more; Table 1). The remaining 39 patients (52%) exhibited minimal staining for Bcl-2 (<10% of cells, scores of 2 or less). Tissue analyzed was from the following anatomical sites: (a) breast, n = 54; (b) lung, n = 2; (c) chest wall, n = 4; (d) lymph node, n = 8; (e) ovary, n = 4; (f) liver, n = 2; and (g) brain, n = 1. Bcl-2 expression was determined by staining performed either on the primary tumors (43 patients; 57%) or at the site of relapse (32 patients; 43%). The distribution of Bcl-2 expression in primary versus relapsed tumor samples was similar, with 23 of 43 (53%) primary tumors and 13 of 32 (41%) metastatic tumors exhibiting high Bcl-2 expression (P = 0.38; Table 2).

To determine whether the Bcl-2-positive and -negative patient groups were similar with respect to established prognostic characteristics, the two groups were compared. There was no significant association between Bcl-2 expression and age, grade, number of prior regimens, or location of metastases (Table 2). There was, however, evidence of an association between Bcl-2 expression (>10% of cells) and positive ER status (P = 0.084), although it did not reach statistical significance. It should be noted, however, that this association approached statistical significance when a cutoff of 75% positively stained cells was used (P = 0.052).

Because the low Bcl-2- and high Bcl-2-expressing groups were similar in terms of tumor characteristics, we next examined overall response. The overall response rate to chemotherapy was 21% (95% CI, 12–32%). In Bcl-2-positive patients, 8 of 36 (22%) responded (0 CRs and 8 PRs). In Bcl-2-negative patients, 8 of 39 patients responded (1 CR and 7 PRs (21%); see Table 3). Thus, there was no significant difference between response to paclitaxel in patients with either Bcl-2-positive or -negative tumors (P = 0.92). If a cutoff of 40% was used for Bcl-2 positivity [as was used in the report of Bonetti et al. (13), which found a correlation between CMF or CAF response to chemotherapy and Bcl-2 positivity], there was still no significant difference in response rate (21% for Bcl-2-negative patients versus 22% for Bcl-2-positive patients, P = 0.85). Moreover, we analyzed the relationship between those tumors that expressed very high levels of Bcl-2 (using a cutoff of 75% positively stained cells) and response rates and still found no significant association with response (P = 0.64).

Time to progression in the two Bcl-2 groups (using our original cutoff of 10% of cells to determine positivity) is shown in Fig. 2A. There was no significant difference between the two curves as measured by the log-rank test (P = 0.98). Median progression-free survival times were 126 days (95% CI, 63–160 days) in the Bcl-2-negative group and 105 days (95% CI, 88–214 days) in the Bcl-2-positive group. Similarly, overall

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**Table 1** Summary of Bcl-2 immunostaining

<table>
<thead>
<tr>
<th>IHC scoring</th>
<th>Low Bcl-2 expression (≤10% of cells stained positive)</th>
<th>High Bcl-2 expression (&gt;10% of cells stained positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>31</td>
<td>2</td>
</tr>
</tbody>
</table>

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**IHC Scoring.** Slides were scored for Bcl-2 expression with all patient identifiers removed, according to the percentage of tumor cells that stained positively. Staining intensity revealed that the vast majority of samples (>80%) had moderate to strong Bcl-2 intensity when compared with lymphocyte Bcl-2 expression. Thus, the percentage of positive cells rather than staining intensity was used for analysis of data. For every sample, at least 200 tumor cells were analyzed. To determine the distribution of Bcl-2 staining in patients’ tumors, we divided the results into six IHC scoring categories depending on the percentage of Bcl-2-positive cells: (a) 0, none; (b) 1, <1%; (c) 2, 1–10%; (d) 3, 11–30%; (e) 4, 31–75%; and (f) 5, >75%. Based on prior studies of breast cancer (12, 14, 32), lung (19), and head and neck cancers (28) examining Bcl-2 expression and patient tumor response, >10% positively stained cells was used to represent positive Bcl-2 expression for this report. Because an association between Bcl-2 expression in >40% of breast tumor cells and resistance to chemotherapy had been demonstrated previously (13), we also analyzed our data using a cutoff point of 40%. To ensure that there was no difference between tumors that were strongly positive and those that were less positive, we also analyzed our data using a cutoff of 75% positivity.

**Statistical Analysis.** χ² analysis was used to compare Bcl-2 IHC results with an objective tumor response (CR or PR) to paclitaxel. Estimation of progression-free and overall survival curves for patients with Bcl-2-positive or -negative tumors was determined by the Kaplan-Meier method (33). The log-rank test was used to test the null hypothesis that the underlying survivor curves were identical, with 23 of 43 (53%) primary tumors and 13 of 32 (41%) metastatic tumors exhibiting high Bcl-2 expression (P = 0.38; Table 2).

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**Fig. 1** Microscopic appearance of invasive breast cancers (×400). Anti-Bcl-2 IHC staining (A) and H&E staining (B) of a Bcl-2-positive tumor and IHC staining (C) and H&E staining (D) of a Bcl-2-negative tumor.

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survival rates (Fig. 2B) were not significantly different (log rank 
$P = 0.37$) with a median survival time of 663 days (95% CI,
456–1119 days) in the Bcl-2-negative group and 450 days (95% CI, 239–1058) in the Bcl-2-positive group. Thus, tumor Bcl-2 
expression did not correlate with either response to paclitaxel,
time to progression, or time to death.

**DISCUSSION**

Failure to undergo apoptosis is considered a major 
mechanism of chemoresistance. Bcl-2, a potent antiapoptotic 
protein, is highly expressed in up to 70% of low-grade breast 
cancers and in 50% of breast cancers overall (35). In vitro 
studies performed using cell lines with genetically engi-
nereed overexpression of Bcl-2 have consistently shown a 
strong positive correlation between Bcl-2 expression and 
resistance to cytotoxic chemotherapy. However, the degree of 
Bcl-2 overexpression in genetically modified cell lines is 
typically much greater than even strong endogenous expres-
sion (36). Indeed, most studies performed with a variety of 
clinical human tumor specimens have not found a significant 
association between Bcl-2 expression and chemotherapy re-
sistance (17–30). One exception to this finding is a study by 
Bonetti et al. (13) in women receiving CMF or CAF for 
metastatic breast cancer in which a correlation was found 
between resistance to CMF or CAF therapy and tumor Bcl-2 
expression. In contrast, Sjostrom et al. (14) did not find a 
correlation between Bcl-2 expression and response to 5-flu-
ourouracil, epirubicin, and cyclophosphamide in 103 patients 
with metastatic breast cancer. In the current study, we inves-
tigated whether an association exists between tumor Bcl-2 
expression and cytotoxic response to paclitaxel therapy in 
patients with metastatic breast cancer, and we found that 
response to paclitaxel therapy is independent of the degree of 
Bcl-2 expression in the patient’s tumor.

Because paclitaxel treatment can result either directly or 
indirectly in Bcl-2 hyperphosphorylation and consequent func-
tional inactivation, one might alternatively hypothesize that 
tumors with moderate Bcl-2 expression (in contrast to tumors 
having little or no expression) might be more susceptible to 
paclitaxel-induced apoptosis. However, we did not find a cor-
relation with moderate Bcl-2 expression (11–75% of cells) and 
response in this group of patients (data not shown). This indi-
cates that Bcl-2 is unlikely to directly mediate paclitaxel-
induced cytotoxicity in metastatic breast cancer. The relation-
ship between tumor Bcl-2 phosphorylation status and response 
to paclitaxel must await the development of a phospho-specific 
anti-Bcl-2 antibody. Low levels of Bax, a proapoptotic protein, 
have been associated with resistance to chemotherapy and short-
In summary, to our knowledge, this is the first study to examine the relationship between tumor Bcl-2 expression and the corresponding clinical response to paclitaxel. Contrary to what might be expected based on results obtained in defined tissue culture systems, there is no evidence to support a correlation between tumor Bcl-2 expression levels and resistance to paclitaxel treatment for metastatic breast cancer. It remains possible, however, that earlier stage or lower grade tumors (presumably with fewer accumulated genetic changes) might show a relationship between Bcl-2 expression and susceptibility to paclitaxel treatment. The results from this study, however, suggest that Bcl-2 expression does not influence breast cancer susceptibility to treatment with paclitaxel.

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REFERENCES


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