Relationship between Paclitaxel Activity and Pathobiology of Human Solid Tumors

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ABSTRACT

We previously reported the pharmacodynamics of antiproliferative and apoptotic effects of paclitaxel in histocultures of bladder, breast, head and neck, ovarian, and prostate tumors obtained from patients. This study examined the relationship between paclitaxel pharmacodynamics and tumor pathobiological parameters [i.e., stage, grade, proliferation status, expression of P-glycoprotein (Pgp), p53, and Bcl-2]. Pgp, p53, and Bcl-2 proteins were detected by immunohistochemical methods. The drug sensitivity rank order of the five tumor types is as follows: prostate > head and neck > breast > ovarian for the antiproliferative effect and breast = ovarian = head and neck > prostate = bladder for the apoptotic effect. When the pathobiological parameters were considered as single parameters, the antiproliferative effect was inversely correlated with tumor stage, grade, labeling index (LI), and expression of Pgp, p53, and Bcl-2 (P < 0.05 in all cases). The apoptotic effect was positively correlated with Pgp expression, LI, and tumor grade (P < 0.01) but was not related to tumor stage and expression of p53 and Bcl-2 (P > 0.2). Results of multivariate analysis indicated that the maximum antiproliferative effect was best predicted by the combination of tumor stage and expression of Pgp and p53 (inverse correlation) and that the maximum apoptotic effect was best predicted by the combination of tumor LI and Pgp expression (positive correlation). In summary, these results indicate that the two major effects of paclitaxel in human solid tumors, i.e., antiproliferation and apoptosis, correlate with different tumor properties. The second finding that drug-induced apoptosis was equal or higher in tumors that expressed Pgp, p53, and Bcl-2 compared to tumors that did not express these proteins supports the use of paclitaxel in treating Pgp-, p53- and Bcl-2-positive tumors.

INTRODUCTION

Paclitaxel is active against ovarian, breast, non-small cell lung, head and neck, and bladder cancers, with an overall response rate of between 20 and 60% (1). In cell lines, paclitaxel promotes microtubule assembly and stabilizes microtubule dynamics, resulting in inhibition of cell proliferation and apoptosis (2–4). In human bladder, breast, head and neck, ovarian, and prostate tumors, paclitaxel inhibits tumor cell proliferation and induces apoptosis (5–9). Studies in cell lines have shown that resistance to paclitaxel appears to be related to the classical multidrug resistance phenotype (10), overproduction of tubulin, a slower rate of microtubule assembly (11), and overexpression of Bcl-2 (12). The biological factors that determine the antiproliferative and apoptotic effects of paclitaxel in patient tumors have not been identified.

We have studied the pharmacodynamics of paclitaxel in histocultures of tumors obtained from patients. The major advantages of the histoculture system are the maintenance of tissue architecture, cell-cell interaction, and inter- and intratumoral heterogeneity. The clinical relevance of the human tumor histoculture system has been demonstrated in retrospective and semiprospective preclinical and clinical studies, which show that drug response in histocultures correlates with the sensitivity and resistance of cancer patients to chemotherapy and patient survival (13–15). In the five tumor types that were studied, i.e., bladder, breast, head and neck, ovarian, and prostate, we observed several common properties of paclitaxel activity: (a) nearly all tumors showed incomplete response to the antiproliferative and apoptotic effects, (b) the maximum sensitivity of individual tumors to the two effects was inversely related, and (c) >95% of the apoptotic cells were labeled by DNA precursors (5–9). On the other hand, there was significant variability in the response of individual tumors to paclitaxel, which raises the question of whether there is a biological basis of the variability. In these previous studies, the pharmacodynamics in each tumor type were examined separately; the sample size was inadequate for evaluating the determinants of drug effect. In this study, we used the results on all of the five tumor types (n = 96) to analyze the relationship between paclitaxel pharmacodynamics and tumor pathobiology. The pharmacodynamic data were obtained from the previous studies (5–9). In addition, we compared the relative drug sensitivity of the five tumor types. For tumor pathobiology, we studied tumor stage, grade, and proliferation status because these parameters are readily available and/or routinely determined as a part of patient management.
Pgp expression was studied because of its role in paclitaxel resistance in cancer cell lines (10) and because we have observed that, in head and neck tumors, Pgp expression correlates with a higher sensitivity to the apoptotic effect but a lower sensitivity to the antiproliferative effect (5). p53 and Bcl-2 were studied because of their involvement in drug-induced apoptosis (16). The role of p53 in paclitaxel-induced apoptosis is unclear and may depend on the cell type. For example, paclitaxel induces apoptosis via both p53-dependent and -independent pathways in mouse embryonic fibroblasts (17). Disruption of wild-type p53 by SV40 T antigen or human papilloma virus E6 protein increases apoptosis in human fibroblasts (18), whereas deletion of wild-type p53 by E6 protein decreases apoptosis in a human ovarian cancer cell line (19). On the other hand, in several other human ovarian cancer cell lines, paclitaxel-induced apoptosis has no relationship with p53 status (20, 21). Transfection of leukemic cells with bcl-2 leads to a delayed onset of paclitaxel-induced apoptosis (12), whereas phosphorylation of Bcl-2 in leukemic and prostate cancer cells by Raf-1 activated by paclitaxel-induced microtubule damage is associated with a higher apoptosis (22, 23). The role of p53 and Bcl-2 expression in paclitaxel-induced apoptosis in patient tumors is unknown and was also studied.

**MATERIALS AND METHODS**

**Chemicals and Supplies.** The chemicals used to study the pharmacodynamics of paclitaxel were as described previously (5). Monoclonal antibodies against p53 (DO7) and Pgp (JSB-1) were obtained from BioGenex (San Ramon, CA); monoclonal antibodies against Pgp (4E3 and C494) and Bcl-2 and the Labeled Streptavidin-Biotin detection kit were from Dako (Carpinteria, CA); and Pgp polyclonal antibody (ab-1) was from Oncogene (Cambridge, MA). All chemicals and reagents were used as received.

**Procurement of Tumor Specimens.** Specimens of human bladder, breast, head and neck, ovarian, and prostate tumors were obtained via the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Tumor pathology was determined by the university pathologists. Of the 96 tumors studied, 95 were from chemotherapy-naive patients. The remaining tumor was from a head and neck cancer patient that had received paclitaxel treatment.

**Pharmacological Effects of Paclitaxel.** The pharmacodynamic data of the antiproliferative and apoptotic effects of paclitaxel were obtained from previous studies (5–9). Briefly, the tumor histocultures were treated with paclitaxel for 2 h (bladder tumors) or 24 h (all other tumors). The 2-h treatment is the duration of intravesical therapy of superficial bladder cancer, whereas 24-h exposure is one of the commonly used treatment schedules in patients. The antiproliferative effect was measured as the reduction in the fraction of tumor cells labeled by DNA precursor. Apoptosis was identified by morphological changes, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling, and/or DNA fragmentation.

Detection of Pgp, p53, and Bcl-2 Proteins. The expression of Pgp, p53, and Bcl-2 proteins, before and after paclitaxel treatment, was measured by immunohistochemical methods as described previously (5). Briefly, six continuous sections were mounted in a configuration of two rows and three columns on each slide. After dewaxing and sequential rehydration in xylene, ethanol, and water, tissue sections were boiled for 5 min in a 0.1 M citrate buffer (pH 6.0) in a microwave oven and then cooled and washed in PBS. After washing off the excess PBS, lines were drawn on the glass slide between the tissue sections, using a paraffin marker. The paraffin lines created a barrier that confined the different antibody solutions applied to different tissue sections. The tissue sections were incubated with Dako blocking solution for 10 min and subsequently with one of the following antibody solutions for 2 h: mouse antihuman Pgp antibody (JSB-1, 1:100 dilution; or C494, 1:300 dilution), a rabbit antihuman Pgp polyclonal antibody (ab-1, 1:200 dilution), a mouse antihuman p53 antibody (DO7, 1:100 dilution), and a mouse antihuman Bcl-2 antibody (1:50 dilution). Frozen sections of tumor tissues before culture were also stained for Pgp with the 4E3 antibody. The incubation was carried out in a humidified chamber at room temperature. The antibodies were diluted in PBS containing 5 mg/ml BSA. The negative controls used mouse IgG as the primary antibody. For positive controls, we used human adrenal gland for Pgp; human head and neck FaDu cancer cells, which are known to overexpress mutant p53, for p53; and human breast cancer MCF-7 cells or lymphocytes in the tissues for Bcl-2. After washing with PBS, the tissue sections were covered with the linker solution and then with peroxidase-conjugated streptavidin solution. After two washes with PBS, tissue sections were incubated for 5–7 min with diamobenzidine and counterstained with hematoxylin.

For each tumor, between 40 and 80 histocultures were processed for immunostaining of Pgp, p53, and Bcl-2. Only tumors that showed these proteins in at least two-thirds of the histocultures were considered positively stained. We used four Pgp antibodies to detect Pgp because of the variable staining of Pgp by different antibodies and to rule out antibody cross-reaction with non-Pgp proteins. Only tumors that showed staining by at least three antibodies were scored as Pgp positive. The intensity of staining was not reported because of the variability among the antibodies. Tumors that showed nuclear p53 staining in >10% of tumor cells were considered p53 positive. It is generally accepted that tumors with <10% stained cells are considered p53 negative (24, 25).

**Statistical Analysis.** Differences in mean values between groups were analyzed by the paired or unpaired Student's t test when the SDs were of similar magnitude; otherwise, the Wilcoxon nonparametric test was used. Software for statistical analysis (NPAR1WAY and TTEST procedures) was obtained from SAS (Cary, NC). Multiple comparisons used one way ANOVA testing with post hoc t test comparison (GLM procedure). Frequencies were compared by χ² test or Fisher’s exact test (FREQ procedure). Predictive relationships between tumor pathological parameters and tumor chemosensitivity were evaluated by linear regression analysis using the maximal r² selection method and the REG software routine of SAS. This model determines which model has the highest coefficient of determination for combinations of predictors. An accepted principle of

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1 The abbreviations used are: Pgp, P-glycoprotein; AIC, Akaike Information Criterion; LI, labeling index.
Table 1  Tumor pathobiological properties

Distribution of tumors by stage, grade, LI, and expression of Pgp, p53, and Bcl-2. For tumor stage, early, middle, and late refer to stages 0/I, II, and III/IV, respectively, for bladder, breast, head and neck, and ovarian tumors, or stage A, B, and C/D, respectively, for prostate tumors. For tumor grade, low, moderate, and high refer to grades I, II, and III/IV, respectively, for bladder, breast, head and neck, and ovarian tumors, and Gleason grades 1–3, 4–6, and 7–9, respectively, for prostate tumors. LI refers to untreated controls. The tumors that showed staining by at least three Pgp antibodies were scored as Pgp positive. Tumors that showed nuclear p53 staining in >10% of tumor cells were considered p53 positive.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>LI, % (mean ± SD)</th>
<th>No. of tumors (% frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage</td>
<td>Grade</td>
</tr>
<tr>
<td></td>
<td>Early</td>
<td>Middle</td>
</tr>
<tr>
<td>Bladder</td>
<td>43 ± 20</td>
<td>11 (69)</td>
</tr>
<tr>
<td>Breast</td>
<td>43 ± 17</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>53 ± 16</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>56 ± 19</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Prostate</td>
<td>42 ± 12</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Fig. 1  Immunohistochemical detection of Pgp, p53, and Bcl-2 in human tumors. A, Pgp stained by JSB-1 in a prostate cancer. B, p53 in a bladder cancer. C, Bcl-2 in a head and neck cancer. Chromogen: diaminobenzidine (brown) counterstained with hematoxylin (blue).

development of a model is to select the simplest model that gives a good description of the data (26). Generally, an increase in model complexity or number of predictors increases the goodness of fit or $r^2$. We used the AIC to balance model simplicity and goodness of fit (26).

RESULTS

Tumor Pathobiological Properties. Table 1 summarizes the pathobiological properties of bladder, breast, head and neck, ovarian, and prostate tumors. A significantly higher fraction of the breast, head and neck, and ovarian tumors studied were at late stage (i.e., >67%), compared to bladder and prostate tumors (≤35%). The LI in ovarian and head and neck tumors are significantly higher than those in other tumors ($P < 0.05$).

Fig. 1 shows the immunostaining of Pgp, p53, and Bcl-2 proteins. The rank orders of frequency of detection were: prostate > ovarian = breast = head and neck > bladder for Pgp; ovarian > breast = head and neck > bladder = prostate for p53; and breast > ovarian = prostate > head and neck > bladder for Bcl-2. Table 2 summarizes the results of the statistical analysis of the relationship among the different tumor pathobiological properties. Pgp expression was significantly and positively correlated with tumor stage, grade and LI, with the high-grade, late-stage, and highly proliferating tumors expressing Pgp more frequently. p53 expression was significantly and positively correlated with tumor LI. In contrast, Bcl-2 expression was not related to tumor stage, grade, and LI. Among the three proteins, only Pgp expression correlated with Bcl-2 expression. Of the 96 tumors, enhanced Pgp expression after paclitaxel treatment was detected in one head and neck and one prostate tumors, whereas no changes in p53 and Bcl-2 expression were detected.

Comparison of Paclitaxel Effects in the Five Tumor Types. The results of the comparison of paclitaxel effects in the five tumor types are summarized in Table 3. Paclitaxel produced antiproliferation in 86% (83 of 96) of tumors, with no effect (i.e., not significantly different from 0% inhibition) on the remaining 13 tumors (10 ovarian and 3 bladder). Only one prostate tumor showed a 100% response. In the responding tumors, the $E_{max}$ was achieved at 1 μM drug concentration and was not enhanced when the drug concentration was increased to

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Table 2  Relationship among tumor pathobiological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stage</th>
<th>Grade</th>
<th>LI</th>
<th>Pgp</th>
<th>p53</th>
<th>Bcl-2</th>
<th>Emax (%)</th>
<th>EC50 (µM)</th>
<th>r² (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>0.08</td>
<td>0.08</td>
<td>0.04</td>
<td>0.19</td>
<td>0.03</td>
<td>0.02</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
</tr>
<tr>
<td>LI</td>
<td>0.08</td>
<td>0.08</td>
<td>0.04</td>
<td>0.19</td>
<td>0.03</td>
<td>0.02</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
</tr>
<tr>
<td>Pgp</td>
<td>0.10</td>
<td>0.02</td>
<td>0.04</td>
<td>(&lt;0.001)</td>
<td>0.07</td>
<td>0.07</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
</tr>
<tr>
<td>p53</td>
<td>0.03</td>
<td>0.09</td>
<td>0.04</td>
<td>(&lt;0.001)</td>
<td>0.07</td>
<td>0.07</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>0.003</td>
<td>0.003</td>
<td>0.10</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
</tr>
</tbody>
</table>

Total 96 39

Table 3  Comparison of paclitaxel activity in five types of human tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>n</th>
<th>Emax (%)</th>
<th>EC50 (µM)</th>
<th>Emax (%)</th>
<th>EC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>16</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
<td>1.0 (0.1–10)</td>
</tr>
<tr>
<td>Breast</td>
<td>15</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
<td>1.0 (0.1–10)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>22</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
<td>1.0 (0.1–10)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>17</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
<td>1.0 (0.1–10)</td>
</tr>
<tr>
<td>Prostate</td>
<td>26</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
<td>1.0 (0.1–10)</td>
</tr>
<tr>
<td>Total</td>
<td>96</td>
<td>46 ± 32</td>
<td>1.2 (0.007–10)</td>
<td>10 ± 7 (0–29)</td>
<td>1.0 (0.1–10)</td>
</tr>
</tbody>
</table>

a Emax of the 83 responding tumors was 46 ± 19%.
b Emax of the 92 responding tumors was 13 ± 7%.

10 µM. Prostate, head and neck, and bladder tumors were the most sensitive to the antiproliferative effect of paclitaxel, followed by breast tumors, whereas ovarian tumors were the least sensitive.

For apoptosis, 86% (83 of 96) of untreated tumors showed <1% apoptotic cells, and the remaining 14% (13 of 96) showed between 1 and 5.5% apoptotic cells. Paclitaxel induced apoptosis in 96% (92 of 96) of the tumors, with no effect in the remaining 4 tumors. The maximal increase in apoptotic index due to paclitaxel treatment varied from 2 to 29%, which occurred at 0.1 µM in 62% (58 of 96), at 1 µM in 31% tumors (30 of 96), and at 10 µM in 4% (4 of 96) of the tumors. Although the maximum apoptotic index was comparable among the five tumor types, the rank order of tumor sensitivity to the apoptotic effect, measured by the drug concentrations needed to induce maximum apoptosis, was breast = ovarian = head and neck > prostate = bladder tumors.

Relationship between Tumor Pathobiological Properties and Paclitaxel Activity. Tables 4 and 5 summarize the relationship between paclitaxel activity and tumor pathobiological properties. As single parameters, tumor grade, stage, LI, and expression of Pgp, p53, and Bcl-2 were inversely correlated with drug-induced antiproliferation. On the contrary, Pgp expression, tumor LI, and grade were positively correlated with drug-induced apoptosis, whereas tumor stage and expression of p53 and Bcl-2 did not show a relationship with the apoptotic effect.

Table 5 shows the ranking of the tumor pathobiological indicators of the maximum paclitaxel effects. As single parameters, the rank orders of factors that gave the highest coefficient of determination (r²) and the lowest P values were: tumor stage > Pgp expression > tumor LI for the antiproliferative effect and Pgp expression > LI > tumor grade for the apoptotic effect. In general, inclusion of more parameters improved the regression. The two-, three-, four-, five-, and six-parameter combinations that resulted in the best fit for each category are shown in Table 5. The best-fitting regression for each effect was achieved with two- and three-parameter combinations, i.e., tumor stage, expression of Pgp, and p53 expression for the antiproliferative effect, and Pgp expression, tumor LI, and grade for the apoptotic effect. Addition of further parameters (i.e., Bcl-2 for the antiproliferative effect and tumor grade for the apoptotic effect) did not improve the goodness of fit, as indicated by no improvement in the AIC or r² values.

DISCUSSION

Comparison of Paclitaxel Activity in Five Tumor Types. Our results demonstrate the differences in the sensitivity of the five tumor types to the antiproliferative and apoptotic effects of paclitaxel. It is not known which of the two effects is responsible for the activity of paclitaxel in vivo and in patients. A recent study in human xenograft-bearing immunodeficient mice shows that the antitumor activity of paclitaxel in 16 tumor types (seven adenocarcinomas, two squamous cell carcinomas, six sarcomas, and one lymphoma) is correlated with
drug-induced apoptosis but not with mitotic arrest, suggesting that apoptosis is responsible for the drug effect in vivo (27). Our finding that the rank order of clinical activity of paclitaxel in different tumors (i.e., ovarian = breast = head and neck > bladder = prostate) is identical to the rank order of tumor sensitivity to the apoptotic effect but not the rank order of tumor sensitivity to the antiproliferative effect (i.e., prostate ≥ head and neck ≥ bladder ≥ breast ≥ ovarian) would suggest that

Table 4  Relationship between paclitaxel effects and tumor pathobiology

Results of previous studies on five types of human tumors (5-9) are compiled here to analyze for the relationship between paclitaxel pharmacodynamics and tumor pathobiology. E_max is the maximum effect and is expressed as mean ± SD. EC_{max} and EC_{30} are the drug concentrations needed to produce the maximum apoptotic effect and 30% inhibition of DNA precursor incorporation, respectively. LI of untreated controls and E_max are expressed as mean ± SD. EC values are expressed as median (range) values, because some tumors showed ECs of >10 μM, the highest concentration used. The significance of differences between mean values was analyzed by the two-tailed Student’s unpaired t test or one-way ANOVA test, and the significance of differences between median values were analyzed by the Wilcoxon nonparametric test.

Table 5  Correlation between pathobiological parameters and maximum paclitaxel activity

The correlations between the maximum antiproliferative and apoptotic effects with tumor pathobiological parameters were analyzed. LI refers to untreated controls. A higher r^2 and a lower AIC indicate a better predictive value. Statistical analysis was by linear regression analysis using REG software. The type of correlation, i.e., negative or positive, is indicated.
apoptosis may be more important than antiproliferation in determining the clinical activity of paclitaxel. However, it should be cautioned that the results in tumor histoculture may not be reflective of the clinical situation. The tumor specimens used in this study were selected based on availability and were not selected from patients who would have been candidates to receive paclitaxel treatment. Hence, we were not able to compare our in vitro finding with patient response. Confirmation that apoptosis plays a bigger role in the clinical activity of paclitaxel compared to antiproliferation would require in vivo studies in which the two effects are correlated with the therapeutic outcome.

Relationship between Paclitaxel Activity and Tumor Pathobiology. Because drug effects are expected to be a function of intracellular drug concentration, the nonparallel sensitivity of individual tumors to the antiproliferative and apoptotic effects indicate that the two effects are determined by additional factors that are unrelated to drug concentration. This is confirmed by the finding that the two drug effects were correlated with different tumor properties, i.e., the opposite relationships between antiproliferation and apoptosis with several tumor pathobiological parameters (i.e., Pgp expression, LI, and tumor grade).

Our finding of the opposite relationships between Pgp expression and the two major effects of paclitaxel is in line with the literature data that indicate a complex relationship between Pgp expression and tumor sensitivity to paclitaxel. For example, studies using cell lines indicate that increased drug efflux mediated by Pgp overexpression results in paclitaxel resistance (10), whereas the significant activity of paclitaxel in doxorubicin-refractory breast cancer patients (28), which often exhibit Pgp overexpression (29), suggests that Pgp overexpression does not necessarily result in paclitaxel resistance. The negative correlation between Pgp expression and drug-induced antiproliferation, observed in this study, may be due to the enhanced drug efflux in Pgp-positive tumors. On the other hand, the positive correlation between Pgp expression and drug-induced apoptosis is unexpected and suggests biological changes that are unrelated to drug efflux, resulting in enhanced apoptosis in the Pgp-expressing tumors. The latter is being addressed by ongoing studies in our laboratory using cells with negligible Pgp expression and their mdr-1-transfected variants; preliminary results confirmed the observation in patient tumors (30).

The positive correlation between LI of untreated controls and maximum apoptosis is in agreement with the observation in human cancer cell lines that paclitaxel-induced apoptosis correlated with cell proliferation (31–33) and with our previous finding that >95% of apoptotic cells are labeled by DNA precursors (5–9). The reason for the negative correlation between LI and the maximum antiproliferative effect is unclear.

In the multivariate regression model, inclusion of p53 expression resulted in a slight improvement of the $r^2$ and AIC values in the correlation between the $E_{\text{max}}$ of the antiproliferative effect of paclitaxel and tumor pathobiology. This indicates that the inverse relationship between p53 expression and drug-induced proliferation is not entirely due to the coincidence of p53 expression with the other two most significant prognostic indicators (i.e., tumor stage and Pgp expression) and may be directly related to the biological consequences of p53 expression. The mechanisms for this relationship are not apparent, in part because the immunohistochemical method used to detect p53 expression does not distinguish between overexpression of the functional wild-type p53 and expression of the mutated p53. For the apoptotic effect, the following two findings suggest that paclitaxel induces apoptosis in human solid tumors via p53-independent pathway(s): (a) there is a lack of correlation between p53 expression and the maximum apoptotic index and (b) nearly all apoptotic cells were labeled with DNA precursor (5–9), which, under the assumption that apoptotic cells cannot undergo DNA synthesis, implies that paclitaxel-induced apoptosis occurs after cells have passed through the G1-S checkpoint that is critical for the p53-dependent apoptotic pathway.

Bcl-2 expression is inversely related to the $E_{\text{max}}$ of the antiproliferative effect. However, results of the multivariate analysis indicate no improvement of the $r^2$ and AIC values by inclusion of Bcl-2 in the regression model, suggesting that the relationship is likely a result of the coincidence of Bcl-2 expression with the other parameters that are significant predictors of this drug effect (i.e., tumor stage, grade, and LI and expression of Pgp and p53). The lack of a correlation between Bcl-2 expression and drug-induced apoptosis suggests that the apoptotic pathway involving Bcl-2 is not a major pathway in human solid tumors.

Conclusions. In human bladder, breast, head and neck, ovarian, and prostate tumor histocultures, the antiproliferative and apoptotic effects of paclitaxel are determined by different tumor pathological properties. Because overexpression of Pgp, p53, and Bcl-2 in tumor cells is often associated with chemoresistance (16), our finding that the antitumor activity of paclitaxel is not diminished in Pgp-, p53-, and Bcl-2-positive tumors suggests its unique clinical utility for treating these tumors. Further studies are needed to confirm these observations in patients.

ACKNOWLEDGMENTS

We thank Jie Lu for her technical assistance.

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