Androgen-dependent and -independent Human Prostate Xenograft Tumors as Models for Drug Activity Evaluation

Chiung-Tong Chen, Yuebo Gan, Jessie L-S. Au, and M. Guillaume Wientjes

ABSTRACT

The present study evaluated three human prostate xenograft tumors (CWR22, CWR22R, and CWR91) as models for drug activity evaluation. The chemosensitivity and the expression of several proteins (i.e., p-glycoprotein or Pgp, prostate specific antigen or PSA, p53, and Bcl-2) in xenograft tumors were compared with those in patient tumors obtained through radical prostatectomy (n = 26). CWR22 is androgen-dependent, CWR22R is the androgen-independent subline of CWR22, and CWR91 is a separately derived androgen-independent tumor. The results of immunohistochemical and/or Western blot analysis indicate that the expression of PSA, Pgp, p53, and Bcl-2 in the three CWR xenograft tumors are representative of their expression in 100, 85, 90, and 60%, respectively, of patient tumors. The responses of histocultures of xenograft tumors to doxorubicin and paclitaxel, including inhibition of DNA precursor incorporation and cell death induction, were qualitatively similar to the responses of patient tumors. For example, in all three xenograft and patient tumors, doxorubicin produced complete antiproliferation and cytotoxicity (i.e., cell kill) whereas paclitaxel produced incomplete effects. A comparison of the concentration-effect relationships in xenograft and patient tumors (population median values) indicates that the chemosensitivity observed in patient tumors is representative of the chemosensitivity of one or more of the three xenograft tumors, as follows: (a) the three xenograft tumors and patient tumors responded equally to doxorubicin-induced antiproliferation; (b) CWR22R, CWR91 and patient tumors responded equally to doxorubicin-induced cytotoxicity, whereas CWR22 was 2-3-fold less sensitive; (c) CWR22 and CWR22R tumors were less sensitive to paclitaxel-induced antiproliferation compared with patient tumors, whereas CWR91 was several-fold more sensitive; and (d) CWR22, CWR22R and patient tumors responded equally to paclitaxel-induced cytotoxicity, whereas CWR91 was 2-3-fold more sensitive. The results of this study indicate that the three xenograft tumors, which show chemosensitivity comparable with the results of ≥50% patient tumors and encompass the majority of the heterogeneous patient prostate tumors in the expression of Pgp, PSA, p53 and Bcl-2 proteins, are useful models for drug activity evaluation.

INTRODUCTION

Prostate cancer is the most common malignancy in men. Hormone refractory prostate cancer has a poor prognosis with an overall median survival of 9–18 months (1, 2). Secondary hormonal manipulation may provide symptomatic relief in about 38% of patients and demonstrate a 40–90% subjective response in 30% of patients, with a limited response duration ranging from 3–16 months (3, 4). A similar response rate has been achieved with systemic chemotherapy (5). None of the treatments presently available produce survival advantage (1, 2). The low response rate and short-lived response in patients underscores the need of developing more effective treatments.

The development of new treatments for hormone refractory prostate cancer has been slow, in part because of the lack of appropriate experimental models. Several series of rat prostate tumor models are available (6, 7), but their clinical relevance is questionable because of the known differences between rat and human tumors (8).

Our laboratories have used histocultures of surgical specimens of prostate tumors from patients to evaluate drug activity. The major advantages of the histoculture system are the use of patient materials, the maintenance of three-dimensional tissue architecture with intact stroma, cell-cell interaction, and inter- and intratumoral heterogeneity (9). The interaction between tumor and stromal cells is thought to play a role in epithelial growth and response to androgen stimulation (10, 11). The clinical relevance of the histoculture model has been shown by Hoffman and colleagues in retrospective and semiprospective preclinical and clinical studies; the responses of human tumor histocultures to mitomycin C, doxorubicin, 5-fluorouracil, or cisplatin correlate with the tumor sensitivity, resistance, and survival of head and neck, colorectal, and gastric cancer patients treated with these drugs (12–14). The limitations of human prostate tumor specimens as models for evaluating drug activity are: (a) sources of tumors are nonrenewable; (b) the tumors cannot be readily grown in animals and, therefore, cannot be extended to in vivo studies; and (c) because they are mainly obtained through radical prostatectomy of locally confined, early stage tumors, they may not be representative of the advanced disease. Additional experimental models that retain the advantages and are devoid of the limitations of patient tumor histocultures may improve therapy development.

Presently, there are nine human prostate cancer cell lines and 21 xenograft tumors, derived from primary and metastatic human prostate tumors (15–17). Three of the xenograft tumors (CWR22, CWR22R, and CWR91) were evaluated in the present study. CWR22 was derived from a Gleason tumor grade 9, stage D, prostate carcinoma with osseous metastasis. It is androgen-dependent, does not grow in female mice, and regresses in male mice after orchietomy. CWR22R, a subline of CWR22 that recurred after regression after androgen withdrawal, is not dependent on androgen and is able to grow in female and castrated animals. CWR22 and CWR22R represent the first pair of human androgen-dependent and -independent xenograft tumors that are derived from the same patient tumor. CWR91 is derived from a Gleason tumor grade 7, stage C, androgen-independent prostate carcinoma (17–19).

The major goal of the present study was to evaluate whether CWR22, CWR22R, and CWR91 tumors are clinically relevant models for drug activity evaluation. Doxorubicin and paclitaxel were used as the test drugs. Doxorubicin produces one of the highest combined partial and complete response rates for single agents, of about 30% (5). Although paclitaxel as a single agent has limited clinical activity in prostate cancer (20), more recent data indicate encouraging clinical response for the combination of paclitaxel with estramustine (21). The second reason for selecting these two drugs is that we have shown that these drugs produce qualitatively different pharmacodynamics in tumors obtained from patients with locally confined and early stage disease (22, 23). Doxorubicin produces complete antiproliferation and cytotoxicity (i.e., cell kill) whereas paclitaxel produces only partial cytotoxicity. Similar qualitative differences in the response of xenograft and patient tumors to these two drugs will suggest that the

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chemosensitivity of xenograft tumors is representative of the chemosensitivity of patient tumors. In addition, comparison of the chemosensitivity of CWR22 and CWR22R tumors may suggest the effect of tumor progression to the androgen-independent state on tumor sensitivity to doxorubicin and paclitaxel. We also evaluated the expression of the multidrug resistance Pgp, p53, and Bcl-2 in xenograft and patient tumors, to determine whether the xenograft tumors show similar profiles of these proteins as in patient tumors. These proteins were studied because doxorubicin and paclitaxel are substrates for Pgp, and because p53 and Bcl-2 play a role in drug-induced apoptosis (27). As shown below, the expression of Pgp, PSA, p53, and Bcl-2 in the three xenograft tumors and their sensitivity to the antiproliferation and cytotoxicity induced by doxorubicin and paclitaxel are comparable with those observed in patient tumors.

MATERIALS AND METHODS

Chemicals and Reagents. Paclitaxel was a gift from Bristol-Myers Squibb Company (Wallingford, CT), and doxorubicin was provided by Pharmacia Company (Dublin, OH). The following were purchased from the indicated companies: testosterone tablets (12.5 mg each) from Innovative Research of America (Toledo, OH); Matrigel from Becton Dickinson Labware (Bedford, MA); sterile pigskin collagen (Spongostan standard) from Health Designs Industries (Rochester, NY); tissue culture supplies (i.e., L-glutamine, sodium pyruvate, fetal bovine serum, gentamicin, MEM, MEM, MEM nonessential amino acids solution, and MEM vitamin solution) from Life Technologies, Inc. (Grand Island, NY); ApopTag In Situ Apoptosis Detection Kit from Oncor Inc. (Gaithersburg, MD); BrdUrd, proteina K, and mouse normal IgG from Sigma Chemical Company (St. Louis, MO); mouse BrdUrd monoclonal antibody, Pgp monoclonal antibody JSB-1, and liquid 3,3′-diaminobenzidine substrate kit from BioGenex (San Ramon, CA); mouse Bcl-2 monoclonal antibody, mouse PSA monoclonal antibody, mouse Pgp monoclonal antibody 4E3, mouse p53 monoclonal antibody DO-7, and labeled streptavidin-biotin universal detection kit from DAKO Corporation (Carpinteria, CA); rabbit Pgp polyclonal antibody ab-1 from Oncogene (Cambridge, MA); PSA detection kit from Hybritech (San Diego, CA); and ECL Western blotting kit from Amer sham Corporation (Arlington Heights, IL). All chemicals and reagents were used as received.

Patient Tumors. Archived specimens of human primary prostate tumors were used for immunohistochemical analysis of the expression of Pgp, PSA, Bcl-2, and p53. We have reported the pharmacodynamic data of doxorubicin and paclitaxel on these tumors (22, 23). These tumors were obtained from the peripheral zone of the prostate gland in patients, who had organ-confined prostatic adenocarcinoma, during radical prostatectomy.

Transplantation of Xenograft Tumors. Male athymic Nu/Nu Balb/C retired breeder mice were purchased from the National Cancer Institute (Bethesda, MD). Three human prostate xenograft tumors (CWR22, CWR22R, and CWR91) were kindly provided by Dr. Thomas G. Pretlow at Case Western Reserve University, Cleveland, OH. The tumors were transplanted in nude mice according to the method described previously (17, 19). Briefly, the minced tumor fragments were mixed with an equal volume of Matrigel and about 0.3 ml of the mixture was injected s.c. into each flank of a mouse, through an 18-gauge needle. The host animals for the androgen-dependent CWR22 tumors were supplemented with testosterone. Three days before transplantation of the CWR22 tumor, the animals were castrated and sustained-release testosterone pellets were implanted s.c. to maintain testosterone levels in the range found in humans (i.e., 6 ng/ml; Ref. 28). The host animals for the androgen-independent CWR22R and CWR91 tumors did not require testosterone supplementation. When the tumor reached about 1 g in size, in about 1.5–2.5 months, they were resected and used for histoculture.

Histoculture. Patient and xenograft tumor specimens were cultured as described previously (22, 23). Tumors were cut into ≤1 mm³ pieces under sterile conditions. Five to six tissue pieces were placed on a 1 cm³ medium-soaked collagen gel and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The culture medium consisted of a 1:1 mixture of MEM and DMEM, 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 40 μg/ml gentamicin, 0.1 mM MEM nonessential amino acids, and concentrated MEM vitamin solution (100-fold concentrated, 10 ml/liter). The histocultures were fed every other day and used for pharmacodynamic studies on day 4. We have shown that testosterone supplementation had no effect on the LI of patient tumor histocultures, presumably because these were short-term cultures (29). Hence, the culture medium for the CWR22 tumor was not supplemented with testosterone.

Immunohistochemical Detection of Pgp, p53, Bcl-2, and PSA Proteins. The expression of Pgp, p53, Bcl-2, and PSA proteins was detected by immunohistochemical methods as described previously (23, 30). To minimize data variability due to intratumoral heterogeneity, adenocarcinoma cells were used to simultaneously detect these markers. Briefly, dewaxed and rehydrated tissue sections were boiled in a 0.1 M citrate buffer (pH 6.0) for 5 min, washed briefly in PBS, and incubated with DAKO blocking solution for 10 min and then with an antibody for 2 h. The following antibodies were used: Pgp antibody (JSB-1, 1:100 dilution in PBS containing 5 mg/ml BSA), PSA antibody (ab-1, 1:150 dilution), Pgp antibody (DO-7, 1:100 dilution), Bcl-2 antibody (1:50 dilution), and PSA antibody (1:20 and 1:100 dilution). After applying biotin-anti-IgG linker solution and peroxidase-conjugated streptavidin solution, tissue sections were incubated with 3′-3″-diaminobenzidine and hydrogen peroxide and then counterstained with hematoxylin. The negative controls used mouse IgG as primary antibody. For positive controls of these proteins, we used human prostatic cancer FaDu cells with mutant p53 and Pgp overexpression (30, 31), human breast MCF-7 cancer cells which overexpress Bcl-2 (32), and human prostate tumors which secreted PSA. All tumors were analyzed simultaneously to eliminate variability due to day-to-day variations.

Detection of Pgp, p53, Bcl-2, and PSA by Western Blot Analysis. The levels of Pgp, p53, Bcl-2, and PSA in CWR xenografts were analyzed by Western blotting, as described previously (30). Briefly, tumors were homogenized and DNA was sheared by passing through a 21-gauge needle. Cell debris were removed by centrifugation and the protein concentration in the supernatant was measured by the Bicinchoninic Acid Protein Determination Kit (Sigma Chemical Company). Protein (25 μg) was loaded on a 7.5 (for Pgp detection), 10 (for p53), or 12% (for Bcl-2 and PSA) polyacrylamide slab gel and then transferred onto a nitrocellulose filter by electro-blotting. After blocking the nonspecific binding sites with 5% skim milk in TBST, the filter was incubated with antibody in TBST containing 2% BSA. The antibodies for Pgp (JSB-1, 4E3, and ab-1), p53 (DO-7), and PSA were diluted 750-fold, and the Bcl-2 antibody was diluted 250-fold. The linker solution and the peroxidase-conjugated streptavidin (from the labeled streptavidin-biotin kit, 1:5 dilution in TBST containing 1% BSA) were applied sequentially. The signal was developed using the Amersham ECL chemiluminescence method.

Drug Treatment. Tumor histocultures were treated with paclitaxel (0.00012–12 μM) for 24 or 96 h and with doxorubicin (0.000017–17 μM) for 96 h. These treatment durations were used in our previous studies on patient tumors (22, 23). Paclitaxel was dissolved in ethanol and added to culture medium, at a final ethanol concentration of 0.1%. The same concentration of ethanol was added to the controls. Doxorubicin was dissolved in distilled water. After drug treatment, tumors were incubated with 40 μM BrdUrd for 48 h and then embedded in paraffin. Controls were processed similarly, with the exception of drug treatment. Histological tissue slides were processed for drug effect measurement, as described previously (22, 23). A typical experiment used a total of 8–15 tumor pieces for each drug concentration. For measurement of antiproliferation effect, a minimum of 200 cells/piece, or >1,600 cells, were counted/concentration. For cytotoxicity measurement, we evaluated >1,600 cells/piece in samples which had more than 200 cells/piece remaining after drug treatment or all surviving cells in samples which had less than 200 cells/piece remaining.

Drug-induced Antiproliferation. Drug-induced antiproliferation was measured by inhibition of BrdUrd labeling. BrdUrd-labeled cells were detected by immunohistochemical method using the LSAB kit. Tissue slides were dewaxed, rehydrated, washed, and incubated with the mouse BrdUrd monoclonal antibody (1:250 dilution in PBS containing 5 mg/ml BSA). The brown-colored BrdUrd-labeled cells were scored under microscope and the fraction of labeled cells, the LI, was determined as described previously (30).
Drug-induced Cytotoxicity. Drug-induced cell kill was measured by counting cells with damaged DNA as identified by the terminal TUNEL assay, as described previously (23, 30). Briefly, tissue slides were deparaffinized, rehydrated, and treated with proteinase K. Endogenous peroxidase was quenched using 2% hydrogen peroxide. Tissue slides were then incubated sequentially with terminal deoxynucleotidyl transferase, antidigoxigenin peroxidase, and chromogen 3,3′-diaminobenzidine plus the substrate hydrogen peroxide. Afterward, the tissue sections were counterstained with hematoxylin, dehydrated, and coverslipped, and the brown-colored TUNEL-positive cells were scored.

Quantitation of PSA Secretion. Reduction of PSA secretion is a clinically relevant and widely used objective criterion for monitoring patient response (2). Drug-induced reduction of PSA secretion in histoculture was monitored by the PSA concentrations in culture medium. PSA was measured by a sandwich-immunoassay using two antibodies against two different epitope sites on PSA. PSA measurement was performed by the Immunology Laboratory of the James Cancer Hospital (Columbus, OH).

Data Analysis. The drug concentration-effect relationships were analyzed using equations (A) and (B) for antiproliferation and cytotoxicity, respectively.

\[
E_{\text{antiproliferation}} = \frac{E_{\text{max}}}{1 + \left(\frac{C}{K_{\text{antiproliferation}}} + C\right)^n} + 100 - E_{\text{max}} \quad (A)
\]
\[
E_{\text{cytotoxicity}} = \frac{(E_{\text{max}} - E_{\text{baseline}}) \cdot C^n}{K_{\text{cytotoxicity}} + C^n} + E_{\text{baseline}} \quad (B)
\]

where \(E_{\text{antiproliferation}}\) is the LI of drug-treated specimens expressed as a percentage of the LI of untreated controls; \(E_{\text{cytotoxicity}}\) is the fraction of TUNEL-labeled cells; \(C\) is the drug concentration; \(E_{\text{max}}\) is the maximum drug effect; \(K_{\text{antiproliferation}}\) and \(K_{\text{cytotoxicity}}\) are the drug concentrations that produce 50% antiproliferation and cytotoxicity, respectively; \(n\) is a curve shape parameter; and \(E_{\text{baseline}}\) is the fraction of dead cells in untreated controls. Values for IC\(_{50}\) and LC\(_{50}\) were determined.

Statistical Analysis. Differences between groups were evaluated using the Mann-Whitney U test, t test, or ANOVAs. Software for statistical analysis (ANOVA, T-TEST and NPAR1WAY procedures) were obtained from SAS Institute, Inc. (Cary, NC).

RESULTS

Histocultures of Xenograft Tumors. Fig. 1 shows the micrographs of histocultures of the CWR22, CWR22R, and CWR91 tumors. The histocultures of all three tumors maintained the three-dimensional structure, and showed presence of epithelial tumor cells and normal stroma (fibroblasts and muscle cells). The CWR22 tumor also retained scattered acinus-like structures. The BrdUrd LI of the xenograft tumor histocultures was in the following rank order: CWR91 > CWR22R > CWR22 (Table 1). Our previously reported LI for patient tumor histocultures was higher than the LI of the androgen-dependent CWR22 tumor, but lower than the LI of the...
Expression of proteins in archived patient tumor slides and xenograft tumor slides was detected using immunohistochemical methods. Protein expression in xenograft tumors was also analyzed by Western blotting of tissue extracts. For PSA, Pgp, and Bcl-2 proteins, tumors were scored as either positively or negatively stained and the data was expressed as fraction (F) of tumors that was stained positive. For p53, because its immunostaining resided in the nucleus, individual cells were scored according to their different staining intensities.

### Expression of Pgp, PSA, p53, and Bcl-2 Proteins

<table>
<thead>
<tr>
<th>Tumors</th>
<th>LI % (mean±SD)</th>
<th>Immunostaining</th>
<th>Western</th>
<th>Immunostaining</th>
<th>Western</th>
<th>Immunostaining</th>
<th>Western</th>
<th>Immunostaining</th>
<th>Western</th>
<th>Immunostaining</th>
<th>Western</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (n = 26)</td>
<td>42 ± 12</td>
<td>++ + +</td>
<td>100</td>
<td>NA</td>
<td>-</td>
<td>15</td>
<td>NA</td>
<td>-</td>
<td>60</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>CWR22 (n = 36)</td>
<td>35 ± 5</td>
<td>++ + +</td>
<td>100</td>
<td>++ + + +</td>
<td>100</td>
<td>++</td>
<td>0</td>
<td>+</td>
<td>40</td>
<td>+</td>
<td>&lt;0</td>
</tr>
<tr>
<td>CWR91 (n = 32)</td>
<td>63 ± 8</td>
<td>++ + +</td>
<td>100</td>
<td>++ + + +</td>
<td>100</td>
<td>+</td>
<td>0</td>
<td>++</td>
<td>100</td>
<td>++ + + +</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 1 Expression of PSA, Pgp, Bcl-2, and p53 proteins in patient and xenograft tumors

<table>
<thead>
<tr>
<th>PSA</th>
<th>Pgp</th>
<th>Bcl-2</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunostaining</td>
<td>Western</td>
<td>Immunostaining</td>
<td>Western</td>
</tr>
<tr>
<td>F%</td>
<td>NA</td>
<td>F%</td>
<td>NA</td>
</tr>
<tr>
<td>100</td>
<td>+</td>
<td>100</td>
<td>++</td>
</tr>
<tr>
<td>60</td>
<td>+</td>
<td>100</td>
<td>++</td>
</tr>
<tr>
<td>40</td>
<td>++</td>
<td>100</td>
<td>++</td>
</tr>
<tr>
<td>0</td>
<td>+ + +</td>
<td>100</td>
<td>++</td>
</tr>
<tr>
<td>0</td>
<td>++ + + +</td>
<td>100</td>
<td>++</td>
</tr>
</tbody>
</table>

* Differences in the LI between patient tumors and the three xenograft tumors are significant (P < 0.05).

**androgen-independent CWR22R and CWR91 tumors. The LI in patient and xenograft tumor histocultures is higher than the proliferation index of between 1.6–16% found in other studies that used pulse labeling with DNA precursors or snapshot measurement of the expression of proliferation markers such as proliferation cell nuclear antigen or Ki-67 (33). This is probably because the LI of histocultures represents the cumulative LI measured over 48 h, or a 48 to 500-fold longer labeling period, compared with the short-term labeling studies (34, 35).

A comparison of the untreated controls before and after culture shows no changes in morphology nor expression of PSA (data not shown), indicating the preservation of tumor morphology and functional characteristics during histoculture.

**Expression of Pgp, PSA, p53, and Bcl-2 Proteins.** Twenty-six patient tumors were analyzed for expression of Pgp, PSA, p53, and Bcl-2 proteins by immunohistochemistry, whereas the three CWR xenografts were analyzed by both immunohistochemistry and Western blotting. The results are shown in Fig. 1 and 2, and Table 1. The immunostaining for Pgp, PSA, and Bcl-2 resided in cell membrane or cytoplasm which was continuous and prevented the scoring of individual cells with different staining intensity. The immunostaining for p53 resided in the nucleus, which was discrete and enabled the determination of the fraction of cells showing different staining intensity.

The three CWR tumors and 85% of patient tumors showed immunostaining for Pgp. Among the patient tumors, the less differentiated tumors showed a homogeneous staining throughout the cells, whereas the highly differentiated tumors showed a localized staining at the luminal side of apical cells.

Forty percent of patient tumors showed immunostaining for Bcl-2 protein, whereas 60% did not. In comparison, Bcl-2 was not detected immunohistochemically and was detected only by Western blot analysis in the three xenograft tumors with the highest expression in CWR22R tumor (Fig. 2). This indicates a lower Bcl-2 expression in the xenograft tumors as compared with 40% of patient tumors.

CWR22 and CWR22R tumors contained <10% scattered p53-positive cells. In contrast, the CWR91 tumor did not show p53-positive cells by immunohistochemical staining and showed only a faint band by Western blot analysis. The majority of patient tumors (>90%) contained ≤10% p53-positive cells, which is comparable with the results in the three xenograft tumors.

Immunohistochemical detection of PSA in the CWR22 tumor was achieved with a lower antibody concentration (dilution 1:100) compared with that in CWR22R and CWR91 tumors (dilution 1:20), indicating a higher PSA secretion in the CWR22 tumor. This is consistent with the rank order of the signals in the Western blot (Fig. 2), the rank order of the PSA concentrations in the culture media of untreated controls (i.e., 33 ± 14 ng/ml (n = 8) in the CWR22 tumor; 4.4 ± 1.3 ng/ml (n = 6) in the CWR22R tumor; below the detection limit of 0.03 ng/ml in the CWR91 tumor), and the rank order of the reported plasma PSA concentrations in mice bearing these xenograft tumors (18). The PSA concentrations in the culture medium of the three xenograft tumors are comparable with our reported PSA concentrations in the culture media of patient tumors (i.e., range, 0.03–48.8 ng/ml; average, 12.3 ng/ml; Refs. 22, 23).

A comparison of the immunostaining or Western blot results indicates that the CWR22R tumor showed a lower PSA expression and no changes in Pgp expression, but a higher expression in Bcl-2 and p53 compared with its parent CWR22 tumor.
Drug activity in xenograft tumors was established in the present study. Drug activity in patient tumors were obtained from our previous studies (22, 23). The data represent mean ± SD, with the exception that for the paclitaxel-induced antiproliferation which showed not normally distributed data, the data represent the median values with ranges in parentheses.

<table>
<thead>
<tr>
<th>Tumors and drug treatments</th>
<th>Antiproliferation</th>
<th>Cytotoxicity</th>
<th>PSA reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50} nM</td>
<td>p</td>
<td>E_{max} %</td>
</tr>
<tr>
<td>Doxorubicin (96 h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient (n = 17)</td>
<td>61 ± 73</td>
<td>NA</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>CWR22 (n = 5)</td>
<td>151 ± 40</td>
<td>0.01</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>CWR22R (n = 5)</td>
<td>62 ± 15</td>
<td>0.37</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>CWR91 (n = 5)</td>
<td>70 ± 24</td>
<td>0.20</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Paclitaxel (24 h)</td>
<td>&gt;10 μM (0.2 -&gt; 12,000)</td>
<td>NA</td>
<td>47 ± 19</td>
</tr>
<tr>
<td>Patient (n = 26)</td>
<td>899 (202→12,000)</td>
<td>0.57</td>
<td>49 ± 10</td>
</tr>
<tr>
<td>CWR22 (n = 5)</td>
<td>&gt;12 μM</td>
<td>0.05</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>CWR22R (n = 4)</td>
<td>376 (82→12,000)</td>
<td>0.97</td>
<td>59 ± 8</td>
</tr>
<tr>
<td>Paclitaxel (96 h)</td>
<td>73 (1.5→12,000)</td>
<td>NA</td>
<td>70 ± 12</td>
</tr>
<tr>
<td>Patient (n = 11)</td>
<td>185 (100→12,000)</td>
<td>0.04</td>
<td>58 ± 8</td>
</tr>
<tr>
<td>CWR22R (n = 5)</td>
<td>538 (23→12,000)</td>
<td>0.08</td>
<td>56 ± 12</td>
</tr>
<tr>
<td>CWR91 (n = 4)</td>
<td>26 ± 12</td>
<td>0.24</td>
<td>76 ± 4</td>
</tr>
</tbody>
</table>

a P, comparison between patient and xenograft tumors.

b E_{max}, maximum drug effect at the highest drug concentration (paclitaxel 12 μM or doxorubicin 17 μM).

c NA, not available.

**Drug Effects in Xenograft Tumors.** In all three CWR xenograft tumors, paclitaxel and doxorubicin produced antiproliferation, reduced PSA secretion, and produced cytotoxicity, in descending order with respect to both drug potency and E_{max} (Table 2). For example, the same drug concentration (e.g., 0.1 μM) produced a greater antiproliferative effect (~50% for doxorubicin and 30–70% for paclitaxel) compared with the cytotoxic effect (≤5% for doxorubicin and <30% for paclitaxel).

Fig. 3 shows the sigmoidal, concentration-dependent antiproliferation induced by doxorubicin and paclitaxel. The most notable difference between the two drugs is the different extent of E_{max}. In all three xenograft tumors, doxorubicin produced 100% antiproliferation, whereas paclitaxel treatment for 24 or 96 h produced a maximal inhibition of between 50 and 80% with no further increase even when drug concentrations were increased by 10–100-fold. The 24 h paclitaxel treatment was less effective compared with the 96 h treatment. A comparison of the IC_{50} values shows a higher molar potency for doxorubicin in CWR22 and CWR22R tumors compared with paclitaxel, whereas the reverse was observed in CWR91 tumors.

Fig. 4 shows the cytotoxicity induced by doxorubicin and paclitaxel in the three xenograft tumors. Untreated controls showed an average of 1% TUNEL-labeled cells. Doxorubicin produced concentration-dependent and nearly complete cytotoxicity, whereas both the 24 and 96 h paclitaxel treatments produced an incomplete effect. A comparison of the IC_{50} values shows a higher molar potency for doxorubicin in all three xenograft tumors compared with paclitaxel.

Drug-induced reduction of PSA concentrations was studied in CWR22 and CWR22R tumors but not in CWR91 tumors because the PSA concentration of the latter tumor could not be quantified by immunoaassay. The results are shown in Fig. 5 and Table 2. Doxorubicin produced concentration-dependent and nearly complete elimination of PSA concentrations in CWR22 and CWR22R tumors. In contrast, treatment with paclitaxel for 96 h produced incomplete inhibition of PSA secretion in the CWR22 tumor and highly variable PSA concentrations in the CWR22R tumor (data not shown). In general, the extent of drug-induced reduction in PSA levels is consistent with the extent of drug-induced antiproliferation and cytotoxicity.

**Comparison of Drug Effects in Xenograft and Patient Tumors.** Fig. 3 and 4 and Table 2 compare the chemosensitivity of the three xenograft tumors with our previously obtained data on the antiproliferative and cytotoxic effects of doxorubicin and paclitaxel in histocultures of patient tumors obtained via radical prostatectomy (22, 23). For doxorubicin, the antiproliferative effect in the three xenograft tumors and the cytotoxic effect in the two androgen-independent tumors are nearly identical with its effects in patient tumors (i.e., P >0.05), whereas the CWR22 tumor was 2–3-fold less sensitive than patient tumors to drug-induced antiproliferation and cytotoxicity (P ≤0.01). For paclitaxel, the CWR22 and CWR22R tumors were less sensitive to the antiproliferative effect but equally sensitive to the cytotoxic effect.

![Fig. 3. Concentration-dependent antiproliferation induced by paclitaxel and doxorubicin. CWR22 (●), CWR22R (△), and CWR91 (□) tumor histocultures were treated with paclitaxel for 24 or 96 h and doxorubicin for 96 h. Inhibition of cumulative BrdUrd LI was expressed as the percentage of untreated controls. Results in xenograft tumors were obtained in the present study. Results in patient tumors (○) were obtained from our published results (22, 23). The sigmoidal lines are computer-fitted lines according to Equation A. The data represent mean ± SD.](image-url)
CWR91 tumor was more sensitive than patient tumors to the antiproliferative and cytotoxic effects of paclitaxel, as indicated by a several-fold lower IC₅₀ and a 2-3-fold higher maximal cytotoxicity.

**DISCUSSION**

Comparison of Chemosensitivity in Patient and Xenograft Tumors. The heterogeneity in patient tumors limits the likelihood of finding experimental models that can precisely predict the chemosensitivity of all patient tumors. Our goal is to develop models that represent a majority of patient tumors. The present study was to determine whether the three human prostate xenograft tumors (CWR22, CWR22R, and CWR91) represent clinically relevant models for drug activity evaluation. Our results show that these tumors have a proliferation status and expression of PSA, Pgp, Bcl-2, and p53 proteins that are consistent with a majority of patient tumors. More importantly, the sensitivity of the three xenograft tumors to doxorubicin and paclitaxel are qualitatively similar to the median response of patient tumors. In all three xenograft tumors and patient tumors, doxorubicin produced complete antiproliferation and cytotoxicity whereas paclitaxel produced incomplete effects. Furthermore, a comparison of the concentration-effect relationships in xenograft and patient tumors indicates that the chemosensitivity observed in patient tumors is represented by the chemosensitivity of one or more of the three xenograft tumors: (a) the three xenograft tumors and patient tumors responded equally to doxorubicin-induced antiproliferation; (b) the CWR22R, CWR91, and patient tumors responded equally to doxorubicin-induced cytotoxicity, whereas the CWR91 tumor was 2-3-fold less sensitive; (c) the CWR22 and CWR22R tumors were less sensitive to paclitaxel-induced antiproliferation compared with patient tumors, whereas the CWR91 tumor was several-fold more sensitive; and (d) the CWR22, CWR22R, and patient tumors responded equally to paclitaxel-induced cytotoxicity, whereas the maximal cytotoxicity in the CWR91 tumor was two to three times that in patient tumors. The similar chemosensitivity between the xenograft tumors and patient tumors to paclitaxel is noteworthy because other more commonly used experimental models, such as monolayer cultures of human prostate cancer PC3 and DU145 cell lines, typically show 50% effective drug concentration (i.e., 1–3 nM in monolayers; Ref. 36) that are 20- to >4,000-fold lower than the IC₅₀ and LC₅₀ values in patient tumors.

Bcl-2 and p53 play a role in chemosensitivity; overexpression of Bcl-2 is linked to reduced apoptosis and chemoresistance, whereas p53 is linked to apoptosis induced by chemotherapeutic drugs and ionizing radiation (37). However, a comparison of the p53 and Bcl-2 levels among the three xenograft tumors indicates no consistent relationship between the expression of these proteins and chemosensitivity. For example, CWR91 had the lowest levels of p53 and Bcl-2 among the three tumors, yet it is equally or more sensitive than the CWR22 tumor to the antiproliferative and cytotoxic effects of doxorubicin and paclitaxel. A separate study in our laboratory, which examined the relationship between the expression of p53 and Bcl-2 and the paclitaxel sensitivity in 96 bladder, breast, head and neck, ovarian, and prostate tumors from patients, indicates no relationship between these parameters (30).

**Progression to Androgen-independent State Is Accompanied by an Enhanced Drug-induced Cytotoxicity.** The second goal of the present study was to examine the effects of tumor progression to the androgen-independent state on chemosensitivity. Such comparison in patient tumors has not been possible because advanced prostate tumor samples are not readily available. Our results show that progression of the androgen-dependent CWR22 tumor to the androgen independent CWR22R tumor resulted in no change in tumor sensitivity to the antiproliferation effect of doxorubicin and paclitaxel, but did result in a higher sensitivity to the cytotoxic effect of these drugs. Additionally, the androgen-independent CWR91 tumor also was more sensitive than the CWR22 tumor to...
the antiproliferative and/or cytotoxic effects of these two drugs. The higher drug-induced cytotoxicity in the CWR22R and CWR91 tumors compared with the androgen-independent tumors was further confirmed in a separate study using two new agents, cytochalasin E and geldanamycin. The enhanced drug-induced antiproliferation and/or cytotoxicity in the two androgen-independent tumors compared with the CWR22 tumor was further confirmed in a study where the potential of the histoculture system to contribute to cancer patient survival. Clinical Cancer Res., 1: 1537–1543, 1995.


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