Pharmacological and Biological Evidence for Differing Mechanisms of Doxorubicin Resistance in Two Human Tumor Cell Lines

Marilyn L. Slovak, Gerald A. Hoeltge, William S. Dalton, and Jeffrey M. Trent

Departments of Radiation Oncology [M. L. S., J. M. T.] and Medicine [W. S. D.], Arizona Cancer Center, University of Arizona, Tucson, Arizona 85724; and Department of Blood Banking, Cleveland Clinic Foundation, Cleveland, Ohio 44106 [G. A. H.]

ABSTRACT

The cellular pharmacology of doxorubicin resistance (DOXx) has most commonly been associated with decreased drug uptake, enhanced drug efflux, cross-resistance to multiple anticancer agents, and the overproduction of a P-glycoprotein mediated and may be related to an alternative mechanism capable of altering drug efflux or differential drug binding.

INTRODUCTION

Resistance to anticancer agents is a major limiting factor in curative cancer therapy. Unfortunately, clinically acquired resistance to a single anticancer agent such as DOX (Adriamycin) is frequently accompanied by the development of cross-resistance to multiple drugs (1-9). This phenomenon, termed MDR, appears to be due to a reduced net intracellular drug accumulation resulting from overexpression of a cell surface glycoprotein termed P-glycoprotein (10-14). This paper describes the stepwise selection and pharmacological characterization of two newly derived MDR DOXR tumor cell lines, HT1080 fibrosarcoma and LoVo colon adenocarcinoma. These two cell line models were chosen due to their strikingly different inherent sensitivities to DOX, with the HT1080 being very sensitive to DOX and LoVo being relatively resistant. The resulting pharmacological and biochemical characteristics of HT1080/DR and LoVo/DR cells suggest that DOX resistance in specific (and MDR in general) may result from more than one mechanism.

MATERIALS AND METHODS

Cell Lines, Culture Conditions, and Selection of DOXx Sublines. HT1080 fibrosarcoma cells were obtained from the American Type Culture Collection. The colon adenocarcinoma cell line, LoVo, was a gift from Dr. Benjamin Drewinko, M. D. Anderson Hospital and Tumor Institute, Houston, TX. The establishment and cell line characteristics of the parental cell lines have been described (15, 16). The parental and DOXx sublines were maintained as monolayer cultures as previously described (17). DOXx cells were selected by stepwise selection over a 2-year time period without mutagenic pretreatment.

Population Doubling Times. Growth rates were determined by counting replicate flasks at 24-h intervals by routine trypsinization procedures for monolayer cultures. The cell number and viability were determined by the trypan blue dye exclusion procedure. Doubling times were calculated by linear regression analysis.

In Vitro Drug Sensitivity Assays. Drug sensitivity testing was performed either by the dye exclusion assay after a 3-h pulse exposure to drug as previously described (6) or by the modified MTT assay (18). Briefly, HT1080 cells were plated at 3,000 (parental) or 6,000 (HT1080/DR4) cells/well in 96-well plates. LoVo cells were plated at 9,000 (parental) and 11,000 (LoVo/DR4) cells/well. Linearity curves demonstrated that cultures initiated at these cell densities continued to grow exponentially for at least 5 days for HT1080 cells and 7-10 days for LoVo cells. These incubation periods also allowed for at least three population doublings per cell line when grown in the absence of drugs. MTT assays were performed by continuous exposure to drug with 0.1 mg MTT (50 µl) added to each well 4 h before processing. Dimethyl sulfoxide (Sigma Chemical Co.) was used to solubilize the formazan crystals, plates were shaken for 5 min, and the absorbance was read at 570 nm, using a Dynatech MR600 microplate reader. All MTT assay results represent the average of a minimum of 8 wells with each drug tested in at least two separate experiments. Controls included medium with the highest drug concentration (blank) and cells with medium only. The percentage of survival was calculated by dividing the absorbance of drug-treated cells by the absorbance of control cells. The relative resistance was calculated by dividing the ID50 of the resistant sublines by the ID50 of the parental control.

Cross-resistance studies were performed by the methods described above with ARA-C, ACT-D, C219 antibody specific to P-glycoprotein revealed the overexpression of the M, 170,000 cell surface glycoprotein in LoVo DOXx cells but not in HT1080 DOXx cells. Second, LoVo DOXx cells are cross-resistant to vincristine, actinomycin D, colchicine, etoposide, and gramicidin D, but not to 1-β-D-arabinofuranosylcytosine. In contrast, HT1080 DOXx cells display cross-resistance to vincristine, actinomycin D, vinblastine, and etoposide; however, they are not cross-resistant to gramicidin D, and show an increased (~18-fold) cross-resistance to 1-β-D-arabinofuranosylcytosine. Third, intracellular DOX accumulation (as measured by 14CDOX at 1-h and high-performance liquid chromatography analysis) was decreased ~2.7-fold in LoVo DOXx cells and ~2.0-fold in HT1080/DR4 cells. However, while net accumulation studies in the presence of 5 mg/ml verapamil reversed DOXR to parental values in LoVo colon adenocarcinoma cells, it only minimally decreased DOX resistance (12.6%) in HT1080/DR4 cells. Efflux patterns of 14CDOX were similar for the MDR sublines with an ~50% decrease in DOX retention after 1 h when compared to their respective parental cell lines. Our results suggest that DOXx in LoVo/DR5 cells may result from overexpression of P-glycoprotein. In contrast, DOXx in HT1080/DR4 appears to be non-P-glycoprotein mediated and may be related to an alternative mechanism capable of altering drug efflux or differential drug binding.

Received 10/7/87; revised 2/11/88; accepted 2/18/88.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: DOX, doxorubicin; DOXx, doxorubicin resistant; MDR, multiple drug resistance; HT1080/DR, HT1080 fibrosarcoma doxorubicin-resistant cells; LoVo/DR, LoVo colon adenocarcinoma doxorubicin-resistant cells; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ID50, 50% inhibiting dose; ARA-C, 1-β-D-arabinofuranosylcytosine; ACT-D, actinomycin D; C219, C219 antibody specific to P-glycoprotein; HPLC, high-performance liquid chromatography; VER, verapamil. DE, dye exclusion assay.

2 Recipient of a Cancer Research Fellowship on behalf of the Ladies Auxiliary of the Veterans of Foreign Wars of the United States. To whom requests for reprints should be addressed at University of Arizona, Cancer Center, 1515 North Campbell Ave., 3rd floor, Tucson, AZ 85724.

3 Scholar of the Leukemia Society of America.

4 This study was supported in part by PHHS Grants CA-41183 (J. M. T.) and CA-43043 (W. S. D.).
Park, CA) were performed according to the method of Vistica et al. (19), using 0.5 μM DOX. Purity of 14C]DOX was determined to be >95% by HPLC.

HPLC Analyses. Intracellular DOX accumulation and metabolism comparison between the parental and DOX* sublines were performed by using reversed-phase HPLC. After a 60-min exposure of 0.5 μM DOX at 37°C, 2 × 10^6 cells were rinsed 3 times with ice-cold phosphate-buffered saline, pelleted, resuspended in 1 ml of double-distilled H_2O, and sonicated (10 s). Lysed cells were prepared for HPLC analysis according to the method described by Peng et al. (20), and fluorescent measurements of DOX were made with a Perkin-Elmer LS-1 fluorescence detector (excitation, 480 nm; emission, 550 nm).

VER Studies. Parental and DOX* cells were seeded at 2 × 10^6. Following a 15-min preincubation with 5 μg/ml VER, 0.5 μM [14C]DOX was added to parental and DOX* cells. After 1 h, the cells were quenched with ice-cold phosphate-buffered saline, centrifuged, processed by the silicone oil method, and read by liquid scintillation.

The possibility of VER reversal was also determined by continuous exposure by using the MTT assay described above with a 15-min VER preincubation before varying concentrations of DOX were added. Cells treated with VER (5 μg/ml) only served as a control to determine if VER alone had a cytotoxic effect on the cell cultures.

Immunoblot Analysis of Plasma Membrane Components. Plasma membrane preparations from HT1080, HT1080/DR4, LoVo, and LoVo/DR5 cells were purified according to the method of Riordan and Ling (21). Membrane protein determinations were assayed by modifications (24). The proteins were transferred onto polyacrylamide gel electrophoresis was performed according to the method of Fairbanks et al. (23) with modifications (24). The proteins were transferred onto nitrocellulose (25) and probed with [3H]-labeled C219 monoclonal antibody (a gift from Dr. Victor Ling, Toronto, Canada), as described by Kartner et al. (26). Membrane preparations from the multidrug-resistant CEM/VELB cell line were used as a positive control for P-glycoprotein overexpression (12). Approximately 50 μg of membrane protein were loaded in each lane.

RESULTS

Isolation of DOX* Subpopulations. DOX* cells were isolated without mutagenic pretreatment by the stepwise selection protocols outlined in Fig. 1. Initial dosages were based on ID_50 values established for the parental lines. The drug concentration was increased 2- to 4-fold after at least 8 weeks of continuous DOX exposure or when the subline demonstrated an adaptive growth pattern. The population doubling times for the DOX* sublines were increased when compared to their respective parental cell lines; HT1080 parent, 19 h; HT1080/DR4, 30 h; LoVo parent, 29 h; LoVo/DR4, 45 h; LoVo/DR5, 72 h.

Relative levels of DOX* compared to their parental cell lines were shown to be 129-fold for LoVo/DR4 cells (Table 1) and 222-fold in HT1080/DR4 cells, using the MTT dye assay (Table 2). The level of DOX* was less when performed by the dye exclusion test after a 3 h pulse exposure to drug (56-fold for LoVo/DR4 cells and 156-fold for HT1080/DR4). The level of resistance in the highest DOX* subline (LoVo/DR5) was ~285-fold over the parental control by the MTT assay (90-fold by dye exclusion) (Table 1). For subsequent pharmacological studies, the LoVo/DR4 and HT1080/DR4 sublines were utilized in all experiments.

Cross-Resistance Studies. To determine if the LoVo/DR4 and HT1080/DR4 sublines displayed cross-resistance to multiple pharmacological agents, cross-resistance studies with the use of a panel of drugs that differed in their structure and function were performed. As can be observed (Table 1), the LoVo/DR4 cells demonstrate a "typical" MDR phenotype which has been correlated with P-glycoprotein-mediated drug resistance and its associated cross-resistance to VCR, ACT-D, CLC, VP-16, and gramicidin D. Ara-C and dexamethasone were equally sensitive (Table 1).

In contrast, although HT1080/DR4 cells did demonstrate cross-resistance to VCR, ACT-D, and VP-16, VBL cross-resistance was minimal at the ID_50 value. However, the drug survival curve for VBL was shallow with ~20% survival even

Table 1 Cross-resistant patterns for LoVo parent and LoVo/DR4 cells with various pharmacological agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Assay</th>
<th>ID_50 (μg/ml)</th>
<th>Relative resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>MTT</td>
<td>0.093</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>DE</td>
<td>0.10</td>
<td>5.6</td>
</tr>
<tr>
<td>Doxorubicin + verapamil</td>
<td>MTT</td>
<td>0.060</td>
<td>1.25</td>
</tr>
<tr>
<td>Vincristine</td>
<td>DE</td>
<td>0.50</td>
<td>100.0</td>
</tr>
<tr>
<td>VP-16</td>
<td>MTT</td>
<td>0.68</td>
<td>43.0</td>
</tr>
<tr>
<td>Colchicine</td>
<td>DE</td>
<td>0.57</td>
<td>6.1</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>DE</td>
<td>0.53</td>
<td>4.9</td>
</tr>
<tr>
<td>Gramicidin D</td>
<td>MTT</td>
<td>34.0</td>
<td>1000.0</td>
</tr>
<tr>
<td>1β-D-Arabinofuranosylcytosine</td>
<td>DE</td>
<td>76.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>MTT</td>
<td>285.0</td>
<td>305.0</td>
</tr>
</tbody>
</table>

* Relative resistance: LoVo/DR4 ID_50.
* DE, dye exclusion study, performed after a 12-h exposure to VCR.
* Analysis could not be continued due to solubility problems and/or ethanol (vehicle) toxicity.

Table 2 Cross-resistance patterns of HT1080 parent and HT1080/DR4 cells with various pharmacological agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Assay</th>
<th>ID_50 (μg/ml)</th>
<th>Relative resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>MTT</td>
<td>0.0063</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>DE</td>
<td>0.0064</td>
<td>1.0</td>
</tr>
<tr>
<td>Doxorubicin + verapamil</td>
<td>MTT</td>
<td>0.0028</td>
<td>0.185</td>
</tr>
<tr>
<td>Vincristine</td>
<td>MTT</td>
<td>0.092</td>
<td>77.0</td>
</tr>
<tr>
<td>VP-16</td>
<td>MTT</td>
<td>0.00065</td>
<td>0.016</td>
</tr>
<tr>
<td>Colchicine</td>
<td>MTT</td>
<td>0.00055</td>
<td>0.0012</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>MTT</td>
<td>0.00048</td>
<td>0.0082</td>
</tr>
<tr>
<td>1β-D-Arabinofuranosylcytosine</td>
<td>MTT</td>
<td>0.014</td>
<td>0.25</td>
</tr>
<tr>
<td>Gramicidin D</td>
<td>MTT</td>
<td>0.31</td>
<td>0.33</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>MTT</td>
<td>48.00</td>
<td>53.00</td>
</tr>
</tbody>
</table>

* Relative resistance: HT1080/DR4 ID_50.

Fig. 1. Multistep selection protocols for isolating DOX* sublines. A, selection scheme for LoVo colon adenocarcinoma cells; B, selection scheme for HT1080 fibroblasts cells. DOX* cells were isolated and propagated at a fixed DOX concentration (left side of arrow) until the cells demonstrated an adaptive growth pattern. The time period maintained at a selected DOX concentration is to right of arrow. The subpopulations designated in bold type are referred to in this study.
at a concentration of 0.2 µg/ml VBL. Of interest, a 17-fold level of resistance was observed to ARA-C, while no cross-resistance was observed to gramicidin D (Table 2). Finally, HT1080 parental and HT1080/DR4 cells were shown to be equally sensitive to dexamethasone (Table 2).

Intracellular Accumulation and Efflux Studies. In order to characterize the nature of the observed DOXR, net intracellular DOX accumulation and efflux patterns were measured in the parental and LoVo/DR5 and HT1080/DR4 sublines. Steady-state concentrations of [14C]DOX after a 1-h drug exposure were found to be 2-fold less (50.3% less drug) in HT1080/DR4 relative to the parent line. A 2.7-fold decrease (63.4% less drug) in net accumulation was observed in LoVo/DR5 cells when compared to its parental cell line. HPLC analysis confirmed the results by using radiolabeled DOX, implying no difference in intracellular metabolism between the DOXR subpopulations and their parental counterparts. No additional HPLC peaks indicative of metabolites were observed in the chromatograms for LoVo/DR5 and HT1080/DR4. Although the presence of nonfluorescent metabolites cannot be entirely ruled out, the absence of any other metabolite suggests that this is an unlikely possibility.

To determine if drug retention differences could account for the differences observed on drug accumulation, efflux of [14C]DOX was determined in these cell lines after a 60-min incubation with 0.5 µM [14C]DOX to achieve steady-state levels. Fig. 2 compares [14C]DOX retention at various time points for LoVo (Fig. 2A) and HT1080 (Fig. 2B) cells. The amount of intracellular DOX in these cell populations is expressed as the percentage of [14C]DOX remaining at the selected time interval after 60-min pretreatment. After 1 h, the HT1080 parental cells retained 84% of the [14C]DOX, whereas only 40% remained in HT1080/DR4 cells. The efflux patterns for LoVo parental and resistant cells were quite similar with 80% DOX retention in the parent line and 41% retention in LoVo/DR5 cells. Of interest, LoVo/DR5 cells demonstrated a dramatic efflux of drug within the first 30 min in drug-free medium, whereas the parental cells appeared to retain most of the drug for that time period, followed by a notable loss of DOX over the next 90 min. Similar results were obtained with the LoVo/DR4 subline.

Verapamil Reversal of Drug Resistance. With a 15-min preincubation of 5 µg/ml of VER, [14C]DOX net accumulation was reversed to parental values in LoVo colon adenocarcinoma cells (Fig. 3). However, DOX resistance in HT1080/DR4 cells was augmented by only 12.6% in the presence of VER by this methodology. VER alone revealed 5% cytotoxicity in LoVo and 20% cytotoxicity in HT1080 cells. In both parental populations, the cytotoxic effects of DOX were potentiated by the presence of VER as reflected in the lower parental ID50 values (Tables 1 and 2). LoVo/DR4 cells showed a partial reversal of DOXR with an ID50 decrease from 12.0 to 1.25 µg/ml of DOX and the level of resistance was reduced from 222- to 20-fold.

By the MTT assay, VER also partially reversed DOXR in the HT1080/DR4 cells. HT1080/DR4 cells demonstrated a decrease in the ID50 value from 1.4 to 0.185 µg/ml with the level of resistance being reduced from 222- to 66-fold.

Western Blot Analysis. Immunoblot analysis was performed to determine if the HT1080 or LoVo DOXR sublines overexpressed P-glycoprotein. Using the C219 monoclonal antibody to P-glycoprotein, the M, 170,000 glycoprotein was shown to be significantly overexpressed in LoVo/DR5 cells when compared to the parental cell line (Fig. 4). In contrast, the HT1080/DR4 cells did not demonstrate overexpression of the P-glycoprotein compared to the parental cell line.

DISCUSSION

In this report, doxorubicin resistance has been characterized in two different human tumor cell lines in respect to cross-resistance to multiple drugs, enhanced drug efflux, and overexpression of P-glycoprotein. The results of this study suggest that despite identical in vitro selection strategies, the mecha-
DIVERSE MDR PHENOTYPES IN TWO DOX® CELL LINES

Fig. 4. Western blot analysis of plasma membrane components. Nitrocellulose blots were probed with the C219 monoclonal antibody to P-glycoprotein (P-gly). CEM/VLB® cells were used as a positive control. Ordinate, molecular weight in thousands, kDa.

The development of P-glycoprotein-mediated MDR (10-12) has been associated with some MDR tumors. Levels of ARA-C (antimetabolite) resistance may be associated with multiple drug resistance. Low levels of VP-16 resistance in HT1080 cells (28) and HT1080/DR4 cells do not exhibit resistance to gramicidin D. Marked cross-resistance to the Vinca alkaloids (5, 7, 14, 29) and gramicidin D, with no resistance to the antimitabolite, ARA-C (12, 27).

In contrast, HT1080/DR4 cells display a MDR cross-resistant pattern that is not usually associated with P-glycoprotein-mediated MDR. Even though these cells are clearly cross-resistant to the Vinca alkaloids, ACT-D, and VP-16, HT1080/DR4 cells do not exhibit resistance to gramicidin D. Marked cross-resistance to this channel-forming ionophore has been demonstrated in the 180-fold MDR CH²⁵C5 cell line (>5000-fold) (28), and was also evident in our LoVo/DR4 cells, presumably due to the membrane changes associated with P-glycoprotein overexpression (14). Moreover, reports of MDR-resistant subpopulations in general indicate that drug-resistant cells appear to overexpress the drug-resistant glycoprotein. CEM/VLB® cells were used as a positive control. Ordinate, molecular weight in thousands, kDa.

Using the MTT drug sensitivity assay, the level of DOXR in LoVo/DR4 cells is ∼130-fold, whereas HT1080/DR4 cells exhibit a ∼220-fold level of resistance. The cross-resistant pattern for LoVo/DR4 cells is similar to other MDR cell lines in that they show varying degrees of resistance to VCR, VP-16, ACT-D, CLC, and gramicidin D, with no resistance to the antimitabolite, ARA-C (12, 27).

A decrease in the steady-state drug level has been associated with the development of P-glycoprotein-mediated MDR (10-14, 28, 29) but not with the “atypical” MDR cell line selected with teniposide (34). In this study, differences in net drug accumulation after a 1-h exposure to drug were observed in the P-glycoprotein-mediated LoVo/DR5 cells as well as the non-P-glycoprotein-mediated HT1080/DR4 subline. The efflux of DOX was also considerably greater in the DOX® sublines relative to their respective parent cell lines. Thus, the differences observed in DOX accumulation at the steady-state level for the parental and DOX® sublines can be partially explained by an enhanced efflux of drug or by differential drug binding in the resistant sublines. Accordingly, the MDR phenotype observed in HT1080/DR4 is dissimilar to the atypical MDR coined by Danks et al. (34) in two important aspects, cross-resistance to the Vinca alkaloids and a decreased net intracellular accumulation of drug.

Immunoblot analyses using the C219 species-independent monoclonal antibody to P-glycoprotein recognized overexpression in DOX® LoVo colon carcinoma cells but failed to demonstrate overexpression in the 222-fold HT1080/DR4 cells. The apparent lack of P-glycoprotein recognized by Western blotting could be due to several possibilities. First, the level of expression of P-glycoprotein in the HT1080/DR4 subline may be below the detectable level for this assay. However, this does not seem likely since recognition of P-glycoprotein by this antibody has been shown to be overexpressed even in some normal tissues (27, 35). Further, in the results to be presented elsewhere, a study of RNA analysis of HT1080/DR4 cells using a complementary DNA to P-glycoprotein failed to demonstrate differential expression of P-glycoprotein in HT1080 parental versus resistant cells. Second, it could be argued that HT1080/DR4 cells may express a member of the P-glycoprotein gene family which does not share the epitope recognized by the C219 antibody. However, immunoblots that use a second monoclonal antibody. However, immunoblots that use a second monoclonal antibody to P-glycoprotein, (C494), again failed to detect P-glycoprotein overexpression. Finally, it appears equally plausible to suggest that these results are indeed consistent with the notion that the HT1080/DR4 subline does not differentially express P-glycoprotein in association with the development of multiple drug resistance.

In vitro multiple drug resistance has been shown to be reversed by calcium channel blockers such as verapamil (37). Although the precise biochemical mechanism for VER reversal of drug resistance is not known, it has been proposed that VER interferes with the binding of drugs to the P-glycoprotein efflux molecule (38, 39). Thus, it was anticipated that VER effects on P-glycoprotein versus non-P-glycoprotein-mediated MDR would differ in these two model systems. In the human colon adenocarcinoma LoVo/DR, VER appeared to completely reverse the decrease in net DOX accumulation after a 1-h exposure and displayed a 10-fold enhancement to DOX cytotoxicity by using the MTT continuous exposure assay. Although the cells remained ∼20-fold resistant to DOX in the presence of 5 µg/ml VER, a slightly higher dose of VER might be likely to completely reverse the acquired DOX® in these cells. Alternatively, differences in drug distribution and/or the intracellular target may account for the residual drug resistance phenotype.

Steady-state levels of DOX accumulation was only increased 12.6% by concomitant VER exposure in HT1080/DR4 cells. In addition, an ∼66-fold level of DOX® remained after 4 days of continuous VER plus DOX exposure. It should be noted that ∼20% of HT1080/DR4 cells were killed by the presence of VER.

of VER alone, precluding an increase in VER dose. These data suggest that the decreased drug accumulation and enhanced efflux seen in the HT1080/DR4 cells is associated with a non-P-glycoprotein efflux pump which is at best partially reversed by verapamil.

In summary, the observed differences in the apparent mechanisms of DOX\(^a\) in these two multiple drug-resistant cell lines provide strong evidence that different mechanisms of multiple drug resistance can be induced despite the use of similar selection strategies. One possibility which might explain these results is the presence of tissue-specific response to drug selection. For example, normal colonic tissue appears to be an intermediate to a high expressor of P-glycoprotein, while fibroblasts expressed P-glycoprotein at nondetectable or at extremely low levels (27, 35). Based upon the aforementioned data, we would speculate that acquired multiple drug resistance can occur by multiple mechanisms, including mechanisms independent of P-glycoprotein.

ACKNOWLEDGMENTS

We especially thank Dr. Victor Ling for providing us with the C219 monoclonal antibody to P-glycoprotein and to Dr. James H. Gerlach for suggesting and providing additional information on the C494 monoclonal antibody to P-glycoprotein and to Dr. James H. Gerlach for technical assistance, Lou Anne Alderman and Ann Monette for secretarial help, and Randy Summers for photographic assistance.

REFERENCES

Pharmacological and Biological Evidence for Differing Mechanisms of Doxorubicin Resistance in Two Human Tumor Cell Lines


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/10/2793

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.