Human NADPH-Cytochrome P450 Reductase Overexpression Does Not Enhance the Aerobic Cytotoxicity of Doxorubicin in Human Breast Cancer Cell Lines

Shairoz Ramji, Chunja Lee, Tadanobu Inaba, Adam V. Patterson, and David S. Riddick

Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario, M5S 1A8 Canada [S. R., C. L., T. I., D. S. R.], and Auckland Cancer Society Research Centre, University of Auckland, Auckland 1000, New Zealand [A. V. P.]

ABSTRACT

Doxorubicin is a useful antineoplastic drug with multiple mechanisms of cytotoxicity. One such mechanism involves the reductive bioactivation of the quinone ring to a semiquinone radical, which can exert direct toxic effects and/or undergo redox cycling. We hypothesized that human NADPH-cytochrome P450 reductase (CYPRED) catalyzes doxorubicin reduction and that overexpression of this enzyme sensitizes human breast cancer cell lines to the aerobic cytotoxicity of doxorubicin. cDNA-expressed human CYPRED catalyzed doxorubicin reduction, measured as the rate of doxorubicin-stimulated NADPH consumption. Using a bank of 17 human liver microsomal samples, the rate of doxorubicin reduction correlated with CYPRED catalytic activity and CYPRED protein immuno-reactivity. Diphenyliodonium chloride, a mechanism-based inactivator of CYPRED, inhibited CYPRED activity and doxorubicin reduction in human liver microsomes with similar concentration dependence. Stably transfected clones of MDA231 human breast cancer cells overexpressing human CYPRED immuno-reactive protein and catalytic activity showed enhanced sensitivity to the aerobic cytotoxicity of tirapazamine, a bioreductive drug known to be activated by CYPRED; however, no sensitization to the cytotoxic effects of doxorubicin was observed. Although human CYPRED is an important catalyst of doxorubicin reduction, overexpression of this enzyme does not confer enhanced sensitivity of human breast cancer cells to the aerobic cytotoxicity of doxorubicin.

INTRODUCTION

Doxorubicin is an antineoplastic drug with a wide range of clinical activity against several solid and hematological malignancies (1). Several mechanisms appear to contribute to the cytotoxic effect of doxorubicin and related anthracyclines. These include DNA intercalation, DNA alkylation and cross-linking, direct membrane effects, initiation of DNA damage via inhibition of topoisomerase II, and free radical production with consequent DNA damage and lipid peroxidation (2).

Free radical formation from anthracyclines such as doxorubicin generally results from processes of drug biotransformation, and these compounds display complex metabolic patterns. The major metabolite in humans is doxorubicinol, produced via cytosolic carbonyl reductase-catalyzed reduction of the ketone at C-13 of doxorubicin (3). Although this is generally viewed as a detoxication step, there is some evidence that doxorubicinol contributes to the dose-limiting cardiotoxicity that characterizes the clinical use of doxorubicin (4). Reductive deglycosylation of doxorubicin to the 7-deoxyaglycone and two-electron reduction of the quinone moiety to the hydroquinone catalyzed by cytosolic NAD(P)H:quinone-oxido-reductase are generally viewed as detoxication pathways (5). Free radical production by doxorubicin involves the one-electron reductive bioactivation of the quinone ring to a semiquinone radical (6), which can exert direct toxic effects and/or undergo redox cycling. Under aerobic conditions, the semiquinone radical reacts rapidly with oxygen to generate superoxide anion, which in turn may produce ROS3 such as hydrogen peroxide and hydroxyl radical (7). This may result in oxidative stress leading to cell death, events that seem to be particularly important in doxorubicin cardiotoxicity (8, 9); however, the role of free radical formation and oxidative stress in tumor cell killing remains controversial (2). Under hypoxic conditions, the unstable and reactive semiquinone radical and/or subsequent breakdown products may contribute to cytotoxic outcomes via covalent binding to cellular macromolecules (10–12). In addition, the iron-chelating ability of doxorubicin contributes to the production of formaldehyde during oxidative stress, and doxorubicin-formaldehyde conjugates contribute to DNA covalent binding (13, 14).

Our main interest is enzyme-catalyzed reductive bioactivation of doxorubicin to free radicals and subsequent redox cycling under aerobic conditions and whether these events can be manipulated to make human cancer cells more sensitive to the cytotoxic effects of anthracyclines. Although several enzymes can potentially catalyze the one-electron reductive bioactivation of doxorubicin to the semiquinone radical, our focus is on CYPRED (EC 1.6.2.4). Rat and rabbit CYPRED catalyze the one-electron reduction of doxorubicin to the semiquinone radical (15–17). Furthermore, purified rat CYPRED enhances doxorubicin toxicity toward human breast cancer cells via a mechanism that involves covalent binding to macromolecules (18, 19). Transfection of human CYPRED cDNA into Chinese hamster ovary cells enhances doxorubicin cytotoxicity by 1.8–3.3-fold (20), whereas a similar manipulation in V79 Chinese hamster fibroblasts had no effect on doxorubicin toxicity (21). In addition to CYPRED, the major phenobarbital-inducible CYP in rat liver, CYP2B1, can also catalyze the one-electron reduction of doxorubicin to the semiquinone radical (22). Phenobarbital-inducible CYPs play a role in the cytotoxicity of doxorubicin in rat hepatocytes (23, 24), but not in V79 Chinese hamster fibroblasts genetically engineered to overexpress CYP2B1 (24). When considering the effects of CYPRED on doxorubicin cytotoxicity, it is important to keep in mind that CYPRED also plays an important role in the detoxication of this anthracycline via the reductive deglycosylation pathway. Consistent with this, it was shown that decreased CYPRED expression (achieved via both pharmacological and antisense approaches) in human lung cancer and lymphoma cell lines resulted in enhanced doxorubicin toxicity (25).

Although it is clear that rat and rabbit CYPRED catalyze the reductive bioactivation of doxorubicin to the semiquinone radical, the role that CYPRED plays in modulating the cytotoxicity of doxorubicin remains controversial. We are particularly interested in the role that human CYPRED plays in the reductive bioactivation of doxorubicin and whether enhanced expression of this enzyme can sensitize human breast cancer cells to the cytotoxic effects of this anthracycline. Because many human tumors and cancer cell lines display low levels of CYPRED and associated CYP enzymes (26, 27), these studies may suggest a future treatment strategy combining conventional chemotherapy with a gene therapy approach designed at selec-
tively enhancing drug bioactivation within tumor cells. In this regard, it is particularly desirable to characterize human enzymes to minimize clinical complications that could result from immune responses directed toward enzymes of foreign origin (28).

In the present investigation, we have demonstrated that human CYPRED is an important catalyst of doxorubicin reduction. To examine the potential chemotherapeutic significance of this finding, we analyzed stable transfectants of the estrogen receptor-negative human breast cancer cell line MDA231 overexpressing human CYPRED protein. Overexpression of human CYPRED in the MDA231 cell line confers enhanced sensitivity to the bioreductive drug tirapazamine (SR4233; Ref. 29), a positive control in the present study. Although human CYPRED is an important catalyst of doxorubicin reduction, our results suggest that overexpression of this enzyme does not confer enhanced sensitivity of human breast cancer cells to the aerobic cytotoxicity of doxorubicin.

MATERIALS AND METHODS

Source of Chemicals. Doxorubicin hydrochloride, diethylenetriaminepentaacetic acid, cytochrome c, MTT, puromycin, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). DPIC was obtained from Aldrich Chemical Co. (Milwaukee, WI). Tirapazamine was a kind gift from Dr. A. M. Rauth (Ontario Cancer Institute, Toronto, Ontario, Canada). Fetal bovine serum was purchased from Invitrogen Life Technologies, Inc. (Carlsbad, CA). Gentest (Woburn, MA). Electrophoresis and blotting equipment and reagents were obtained from Bio-Rad Laboratories Inc. (Hercules, CA).

Source of Microsomes. Microsomes from human lymphoblastoid cells transfected with human CYPRED cDNA or vector were purchased from BD Gentest (Woburn, MA). ECL detection reagents were obtained from Amer sham Biosciences (Piscataway, NJ). Electrophoresis and blotting equipment and reagents were obtained from Bio-Rad Laboratories Inc. (Hercules, CA).

Catalytic Activity Assays. CYPRED activity was assayed under aerobic conditions at 37°C in 1-ml incubation mixtures containing 300 mM potassium phosphate (pH 7.7), 70 μM cytochrome c, and microsomal protein (100 μg for human liver, 25 μg for human lymphoblastoid cell lines, and 100 μg for human breast cancer cell lines). Reactions were initiated by the addition of 1 mM NADPH, and the rate of cytochrome c reduction was determined spectrophotometrically at 550 nm based on ε = 21 mmol⁻¹ cm⁻¹ (35). The rate of the enzyme-catalyzed reaction was determined by subtracting the rate of the reaction occurring in the absence of microsomes. Product formation was linear with respect to protein concentration and incubation time.

Doxorubicin reduction was measured indirectly as the doxorubicin-stimulated microsomal oxidation of NADPH, based on the method of Goeptar et al. (22). NADPH oxidation was assayed under aerobic conditions at 37°C in 1-ml incubation mixtures containing 50 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 0.5 mM diethylenetriaminepentaacetic acid, 100 μM doxorubicin, and microsomal protein (500 μg for human liver and 200 μg for human lymphoblastoid cell lines). Reactions were initiated by the addition of 0.125 mM NADPH, and the rate of NADPH oxidation was determined spectrophotometrically at 340 nm based on ε = 6.22 mmol⁻¹ cm⁻¹. Microsomal consumption of NADPH in the absence of doxorubicin was calculated by subtracting the rate of the reaction observed in samples lacking both microsomes and doxorubicin from the rate of the reaction observed in samples lacking doxorubicin. Microsomal consumption of NADPH in the presence of doxorubicin was calculated by subtracting the rate of the reaction observed in samples lacking microsomes from the rate of the reaction observed in the complete incubation mixture. Finally, doxorubicin-stimulated microsomal NADPH consumption was calculated by subtraction of microsomal NADPH consumption in the absence of doxorubicin from microsomal NADPH consumption in the presence of doxorubicin, and this final parameter is referred to as doxorubicin reduction. Product formation was linear with respect to protein concentration and incubation time.

RESULTS

To study the role of human CYPRED in doxorubicin reduction under aerobic conditions, we used a combination of “reaction phenotyping” approaches. As described previously (22, 36), we measured doxorubicin-
stimulated NADPH consumption as an indirect indicator of doxorubicin reductive bioactivation to the semiquinone radical. First, microsomes from human lymphoblastoid cells overexpressing human CYPRED displayed 17-fold higher CYPRED catalytic activity compared with microsomes from vector-transfected cells (1075 ± 126 versus 63 ± 3 nmol/min/mg microsomal protein; P ≤ 0.01, Student’s t test) and 11-fold higher doxorubicin reduction activity compared with microsomes from vector-transfected cells (22.0 ± 5.0 versus 2.0 ± 1.0 nmol/min/mg microsomal protein; P ≤ 0.01, Student’s t test).

Next, experiments were conducted to determine whether human CYPRED is an important contributor to doxorubicin reduction in a complex subcellular fraction such as human liver microsomes, which contain several enzymes active in drug biotransformation. Using a bank of 17 human liver microsome samples, CYPRED catalytic activity varied approximately 2.4-fold (range, 66–155 nmol/min/mg microsomal protein), and doxorubicin reduction varied approximately 4.4-fold (range, 2.04–8.94 nmol/min/mg protein). Doxorubicin reduction correlated significantly with both CYPRED catalytic activity (Fig. 1A) and CYPRED immunoreactivity (Fig. 1B). DPIC, a mechanism-based inactivator of CYPRED (37), caused concentration-dependent inhibition of CYPRED activity and doxorubicin reduction in human liver microsomes (Fig. 2). Both reactions showed a similar maximal inhibition by DPIC (∼80%), and the sensitivity of both reactions to DPIC was similar (IC50 ≈ 0.6 mM).

To determine whether CYPRED overexpression enhances the sensitivity of human breast cancer cells to the aerobic cytotoxicity of doxorubicin, we used two previously characterized stably transfected clones of MDA231 overexpressing human CYPRED. The Rd-16 and Rd-42 lines were originally found to display 16- and 42-fold elevations, respectively, in CYPRED catalytic activity compared with parental MDA231 cells, as assessed in cell lysates (29). Under the conditions used in the present study, both Rd-16 and Rd-42 showed microsomal CYPRED catalytic activity that was 9-fold higher than parental MDA231 cells (Fig. 3A) and pronounced overexpression of CYPRED immunoreactive protein (Fig. 3A).

Based on MTT assays, doxorubicin produced a concentration-dependent cytotoxic response in parental MDA231 cells with an IC50 ≈ 0.4 μM (Fig. 4A). CYPRED transfectants, Rd-16 and Rd-42, displayed sensitivities to doxorubicin that were similar to the parental line (Fig. 4A). As a positive control, we also tested the MDA231 cell lines for their sensitivity to tirapazamine, a drug that is known to be bioactivated via CYPRED-catalyzed one-electron reduction (29). Tirapazamine produced a concentration-dependent cytotoxic response in parental MDA231 cells with an IC50 ≈ 50 μM (Fig. 4B), and the Rd-16 and Rd-42 lines were approximately 8-fold more sensitive to the cytotoxic effects of this bioreductive agent (Fig. 4B).

Fig. 1. Correlation between doxorubicin reduction and CYPRED catalytic activity (A) and CYPRED immunoreactivity (B), using a bank of 17 human liver microsome samples. CYPRED activity was determined spectrophotometrically as the rate of cytochrome P reductase (PR) reduction. Doxorubicin reduction was assayed spectrophotometrically as the rate of doxorubicin-stimulated microsomal NADPH consumption. Relative CYPRED immunoreactivity was determined by immunoblot analysis. The equations of the lines of best fit were generated by least square linear regression analysis. Statistical significance of the correlations was determined by the Pearson correlation test.

Fig. 2. Concentration-dependent inhibition of CYPRED activity and doxorubicin reduction by DPIC in human liver microsomes. CYPRED activity was determined spectrophotometrically as the rate of cytochrome P reductase (PR) reduction. Doxorubicin reduction was assayed spectrophotometrically as the rate of doxorubicin-stimulated microsomal NADPH consumption. Enzyme activity is expressed as a percentage of the activity observed in the absence of DPIC. Human liver microsome sample K15 was used in this experiment, and control values (100%) for CYPRED activity and doxorubicin reduction were 103.3 and 5.1 nmol/min/mg microsomal protein, respectively. Each point represents the mean ± SD of triplicate determinations. * = significantly different from enzyme activity in the absence of DPIC, based on a randomized design one-way ANOVA and post hoc Newman-Keuls test (P ≤ 0.01). The chemical structure of DPIC is shown as an inset.
keul's test (H11349) P-ified design one-way ANOVA and ent from MDA231 parental cells, based on a random-
quintuplicate determinations. Cytotoxicity was assessed using a microplate MTT colorimetric assay. Fractional absorbance is expressed as a percentage of the absorbance displayed by cells exposed only to vehicle. Each point represents the mean ± SD of duplicate 96-well microplates with sextuplicate determinations per plate. Similar results were obtained in a total of two to three independent experiments. The chemical structure of tirapazamine is shown as an inset.

Fig. 3. Overexpression of CYPRED immunoreactive protein (A) and catalytic activity (B) in microsomes from human breast cancer cell lines stably transfected with human CYPRED cDNA. MDA231 human breast cancer cell lines were either not transfected (Parental) or transfected with vector containing the human CYPRED cDNA (Rd). A, immunoblot analysis of microsomal protein (5 μg) using a polyclonal antibody directed against rat CYPRED. The number on the left indicates the size of a molecular mass marker, and the arrow indicates the position of immunoreactive CYPRED protein (~77 kDa). B, microsomal CYPRED activity was determined spectrophotometrically as the rate of cytochrome c reduction. Data are expressed as mean ± SD of quintuplicate determinations. *, significantly different from MDA231 parental cells, based on a randomized design one-way ANOVA and post hoc Newman-Keuls test (P ≤ 0.001).

**DISCUSSION**

We have shown that human CYPRED is an important contributor to the reductive biotransformation of doxorubicin; however, overexpression of human CYPRED does not sensitize human breast cancer cells to the aerobic cytotoxicity of doxorubicin.

A limitation of the in vitro biochemical component is that we used an indirect assay of doxorubicin reduction, which measures the rate of doxorubicin-stimulated microsomal NADPH oxidation. Although we did not directly measure the drug metabolites produced, in particular the doxorubicin semiquinone radical, the indirect assay was validated as a simple surrogate indicator of the reductive bioactivation of doxorubicin to the redox-cycling radical species (22, 36). This validation was performed by electron spin resonance measurements of doxorubicin semiquinone production in rat liver microsomes and reconstituted systems under anaerobic conditions (22).

“Reaction phenotyping” involves the use of a combination of complementary experimental approaches to determine the enzyme(s) that make important contributions to the biotransformation of a chemical. In addition to our direct demonstration that heterologously expressed human CYPRED has the intrinsic ability to catalyze doxorubicin reduction, we also used multiple approaches to characterize the biotransformation of doxorubicin in human liver microsomes. Using a bank of 17 human liver microsome samples, CYPRED catalytic activity varied approximately 2.4-fold, and the amount of immuno-reactive CYPRED protein varied approximately 3-fold (Fig. 1). These findings are consistent with those of other investigators who reported very little variation (i.e., less than 3-fold) in the range of hepatic CYPRED activity, protein expression, and mRNA levels in human populations (38–41). Despite this rather limited variation in CYPRED levels/activity across human liver samples, we found a clear correlation between CYPRED and doxorubicin reduction (Fig. 1), providing strong evidence that human CYPRED is an important catalyst of the reductive biotransformation of doxorubicin in hepatic microsomes. This conclusion is further supported by our chemical inhibition studies using DPIC (Fig. 2), a mechanism-based inactivator of CYPRED, and other flavoenzymes that function as one-electron donors (37). CYPRED reduces DPIC to a reactive phenyl radical, which binds covalently to the flavin cofactor or adjacent amino acids important in catalysis, resulting in inhibition of CYPRED that is irreversible and time and concentration dependent. Although DPIC can inhibit several other flavoenzymes that catalyze one-electron reduction (e.g., NADPH oxidase, NADH dehydrogenase, xanthine oxidase, and nitric oxide synthase; Ref. 37), CYPRED is the major target for inhibition by this compound in hepatic microsomes.

Our data are supportive of a direct role for CYPRED in doxorubicin reduction; in addition, Goeptar et al. (22) showed that CYPRED can also participate in the reduction of doxorubicin to the semiquinone radical catalyzed by CYP enzymes in rat liver microsomes. It will be
important to assess the contributions of various human CYP enzymes to the reductive biotransformation of doxorubicin under aerobic and anaerobic conditions.

We also found that doxorubicin inhibits CYPRED activity in a concentration-dependent manner in human liver microsomes (data not shown), supporting a direct interaction of doxorubicin with CYPRED. However, the pharmacological significance of this is not clear because high concentrations of doxorubicin (200–600 μM) were required, and only partial CYPRED inhibition was achieved. In summary, many enzymes in addition to CYPRED can catalyze the reductive bioactivation of doxorubicin to the semiquinone radical: xanthine oxidase; NADH dehydrogenase; ferredoxin reductase; NADH-cytochrome b₅ reductase; and nitric oxide synthase (reviewed in Ref. 42). Based on the subcellular localization and cofactor requirements for these enzymes, none is expected to contribute to the NADPH-dependent microsomal reduction of doxorubicin examined in this study. Collectively, our data demonstrate that human CYPRED is an important catalyst of doxorubicin reduction, and therefore, this enzyme became the focus of our attempts to enhance the aerobic cytotoxicity of doxorubicin in human breast cancer cell lines.

It is generally accepted that bioactivation of doxorubicin to free radicals and subsequent generation of ROS are involved in cardiotoxicity (8, 9). Thus, under specific cellular conditions where free radical generation overwhelms antioxidant defenses, enzymatic bioactivation of doxorubicin to the semiquinone radical can clearly contribute to cytotoxicity. Although the role that this free radical mechanism plays in tumor cell killing by doxorubicin is debated (2), the above suggests that it should be possible to manipulate the tumor cell environment in ways that permit the free radical mechanism to augment the cytotoxic effects of doxorubicin. Together with the important role of CYPRED as a catalyst of doxorubicin reduction, this provided the rationale for our studies of human breast cancer cells stably transfected with CYPRED cDNA.

Many studies using human breast cancer cell lines, in particular the estrogen receptor-positive MCF-7 line and doxorubicin-resistant clones, suggest that doxorubicin redox cycling and ROS generation are an important mechanism of tumor cell cytotoxicity. Cellular changes such as elevation of selenium-dependent glutathione peroxidase, which lead to enhanced antioxidant defense, contribute to doxorubicin resistance (43–46). To examine the role of human CYPRED in this cytotoxic mechanism in breast cancer cells, we made use of the parental MDA231 cell line and transfectants stably overexpressing CYPRED protein (29).

Despite demonstrating human CYPRED protein overexpression in the MDA231 transfectants (Fig. 3), enhanced aerobic doxorubicin toxicity was not observed (Fig. 4A). The one-electron reduction potential of doxorubicin is −292 mV (47), and this is a key determinant of the rate of enzymatic reduction of the drug. This reduction potential is sufficiently high that we anticipated that reductive metabolism and redox cycling would occur in the breast cancer cells. There are several possible reasons why CYPRED overexpression did not enhance doxorubicin cytotoxicity. First, MTT assays are less sensitive indicators of cell survival than clonogenic assays, and thus small changes in drug sensitivity may be overlooked. Second, the CYPRED expression level achieved in the cells may not be adequate to promote significant free radical generation. Both explanations seem unlikely because our MTT assay could clearly detect enhanced tirapazamine cytotoxicity with the levels of CYPRED expression achieved in the MDA231 transfectants (Fig. 4B). Third, the subcellular localization of the overexpressed CYPRED protein in the endoplasmic reticulum membranes may not be optimal in relation to critical cellular targets for doxorubicin. Fourth, these cell lines may possess adequate antioxidant defenses to counteract any doxorubicin free radicals and ROS that may be generated; measurement of doxorubicin metabolite formation, covalent binding to macromolecules, ROS generation, and biological responses such as lipid peroxidation in the CYPRED transfectants would assist with interpretation of future studies.

Finally, a possible interpretation of our work is that reductive bioactivation of doxorubicin catalyzed by human CYPRED does not enhance the aerobic cytotoxicity of doxorubicin in breast cancer cells. As summarized by Gewirtz (2), it is likely that interaction of doxorubicin with topoisomerase II is the primary mechanism of tumor cell kill at drug concentrations in the nanomolar range that are likely to be achieved clinically. In contrast, many in vitro investigations of doxorubicin-induced free radical formation have been carried out at excessively high drug concentrations (≥100 μM) that are not pharmacologically relevant. Thus, free radical generation is likely to be of secondary importance compared with topoisomerase II inhibition in anthracycline cytotoxicity in tumor cells. Our cytotoxicity studies were validated by use of tirapazamine, a bioreductive drug that undergoes one-electron reduction to a cytotoxic free radical (reviewed in Ref. 48). The tirapazamine radical exhibits selective toxicity toward hypoxic cells; however, aerobic cytotoxicity results from redox cycling of the tirapazamine radical with subsequent ROS generation. Although several cellular reductases participate in the bioactivation of tirapazamine (48, 49), CYPRED is a key determinant of cytotoxicity in human breast cancer cells under both hypoxic and aerobic conditions (29, 50).

The conclusion of this study is that although human CYPRED is an important catalyst of doxorubicin reduction, overexpression of this enzyme does not confer enhanced sensitivity of human breast cancer cells to the aerobic cytotoxicity of doxorubicin. This has important implications for our understanding of anthracycline cytotoxic mechanisms and for future directions in enzyme-based prodrug activation strategies. It will be desirable to characterize both CYPRED- and CYPD-dependent doxorubicin reduction under aerobic and anaerobic conditions as well as the doxorubicin sensitivity of breast cancer cells overexpressing CYPRED and/or other activating enzymes under aerobic and hypoxic conditions. It will also be important to assess the relative importance of the doxorubicin free radical mechanism versus topoisomerase II inhibition in cardiac myocytes, tumor cells in exponential growth, and tumor cells that have reached plateau phase (51); it is likely that the relative balance of these cytotoxic mechanisms may shift depending on the proliferative state of the target cells. Finally, the transfected cell lines used in this study will be of use in the characterization of novel bioreductive agents.

ACKNOWLEDGMENTS

We thank the following individuals for their contributions to this work: Dr. Mike Rauth for providing tirapazamine; Peter Beaumont for assistance with cytotoxicity assays; and Dr. Xu, Nancy Fischer, and Tommy Cheung for assistance with human liver microsome preparation.

REFERENCES

CYTOCHROME P450 REDUCTASE AND DOXORUBICIN CYTOTOXICITY


Human NADPH-Cytochrome P450 Reductase Overexpression Does Not Enhance the Aerobic Cytotoxicity of Doxorubicin in Human Breast Cancer Cell Lines

Shairoz Ramji, Chunja Lee, Tadanobu Inaba, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/63/20/6914

Cited articles
This article cites 50 articles, 19 of which you can access for free at:
http://cancerres.aacrjournals.org/content/63/20/6914.full.html#ref-list-1

Citing articles
This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/63/20/6914.full.html#related-urls

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.