Vulnerability of Multidrug-resistant Tumor Cells to the Aromatic Fatty Acids Phenylacetate and Phenylbutyrate

Sonsoles Shack, Alexandra Miller, Lei Liu, Premakala Prasanna, Alain Thibault, and Dvoret Samid

Clinical Pharmacology Branch, National Cancer Institute, Bethesda, Maryland 20892 [S. S., L. L., P. P., A. T., D. S.]; Armed Forces Radiobiology Research Institute, Radiation Biochemistry Department, Bethesda, Maryland 20889 [A. M.]; and University of Virginia Cancer Center, Charlottesville, Virginia 22908 [L. L., A. T., D. S.]

ABSTRACT

Cytotoxic chemotherapies often give rise to multidrug resistance, which remains a major problem in cancer management. In pursuit of alternative treatments for chemoresistant tumor cells, we tested the response of multidrug-resistant (MDR) tumor cell lines to the aromatic fatty acids phenylacetate (PA) and phenylbutyrate (PB), two differentiation inducers currently in clinical trials. Both compounds induced cytostasis and maturation of multidrug-resistant breast, ovarian, and colon carcinoma cells with no significant effect on cell viability. In contrast to their poor response to doxorubicin, the MDR cells were generally more sensitive to growth arrest by PA and PB than their parental counterparts. The aromatic fatty acids, like the differentiation-inducing aliphatic fatty acid butyrate, up-regulated mdr-1 gene expression. However, while butyrate increased multidrug resistance, PA and PB potentiated the cytotoxic activity of doxorubicin against MDR cells. The latter was associated with time-dependent declines in glutathione levels and in the activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione S-transferase, the antioxidant enzymes implicated in cell resistance to free radical-based therapies. Taken together, our in vitro data indicate that PA and PB, differentiation inducers of the aromatic fatty acid class, may provide an alternative approach to the treatment of MDR tumors.

INTRODUCTION

Multidrug resistance presents a challenging obstacle to successful cancer therapy. The MDR1 phenotype is observed in tumors undergoing primary therapy and, more frequently, develops as a result of treatment with various cytotoxic agents, including anthracyclins, epipodophyllotoxins, and Vinca alkaloids (1). Of the mechanisms implicated in drug resistance (2), perhaps the most common are: (a) an increase in drug efflux associated with over expression of the mdr-1 gene product, a Mr 170,000 plasma membrane glycoprotein (P-gp) that functions as an energy (ATP)-dependent efflux pump for cytotoxic drugs (1–4) and (b) an abnormal redox status developing secondary to drug exposure, in which glutathione and antioxidant enzyme activities are elevated and protect the cell against free radical aggression (2, 5). Other mechanisms include reduced or altered forms of topoisomerase II and enhanced repair of drug-induced DNA damage.

In the search for compounds that may circumvent MDR, attention has focused on the effectiveness of differentiation inducers such as butyrate, DMSO, dimethylformamide, and retinoids against human cancer cell lines that are unresponsive to cytotoxic drugs (6–8). Some of these agents can, however, up-regulate the mdr-1 gene expression. Although an additional increase in P-gp production does not necessarily affect the MDR phenotype, it has been associated with increased drug resistance in certain human cancer cell lines treated with butyrate (8, 9).

We have recently identified PA and related AFAs as a new class of differentiation inducers. PA and its analogue PB induce cytostasis and differentiation in a variety of murine and human hematopoietic and solid tumor models (10–14). Phase I studies with PA in the treatment of adults with cancer revealed that drug concentrations effective in experimental models (1–4 mM) can be achieved with minimum toxicity and benefit some patients with high-grade gliomas and hormone-independent prostate cancer who failed conventional therapies (15, 16). Previous experience with adults and children suffering from hyperammonemia showed that, like PA, PB is well tolerated at high concentrations (17, 18). Although the mechanisms of antitumor action of the AFAs are unclear, there is evidence for alterations in protein prenylation (13), DNA hypomethylation (19), and activation of the peroxisome proliferator-activated nuclear receptor, a transcriptional factor known to control lipid metabolism and cell growth (20).

The promise of PA and PB as differentiation inducers with novel mechanisms of action prompted our interest in their effect on MDR tumor cells. In the present study, we used as a model MDR cell lines derived from breast, ovarian, and colon carcinomas. The purpose of our study was to test the response of MDR tumor cells to PA and PB and evaluate the effect of these compounds on the MDR phenotype.

MATERIALS AND METHODS

Cell Cultures and Reagents. MCF-7, MCF-7ADR, SW620, and HCT-15 were obtained from Dr. Richard Camalier, National Cancer Institute, Frederick, MD). SW620ADR was generously provided by Dr. Marianne Poruczynski, and 2780 and 2780ADR was provided by Dr. Stephania Scala (Medicine
Cytoxicity, Viability, and Colony Survival Assays. To determine the antiproliferative effect of PA, PB, and butyrate, tumor cells were seeded at the density of $5 \times 10^5$ cells/dish in six-well plates (Costar Co., Cambridge, MA), and drugs were added 24 h later. On day 7 of treatment, cells were detached with trypsin/EDTA (Life Technologies, Inc.) and enumerated using a Coulter counter (Hialeah, FL) or a hemocytometer. IC$_{50}$ values were determined from a dose-response curve. Cell viability was assessed by trypan blue exclusion. The colony survival assay was used to evaluate the cellular response to doxorubicin and its modulation by the fatty acids. Exponentially growing MDR cells, plated onto 100-mm dishes, were incubated with either PA, PB, or butyrate for 72 h (unless otherwise indicated); doxorubicin was added during the last 30 min of treatment. Following exposure to doxorubicin, cells were washed with PBS, trypsinized, and counted with a Coulter counter. Cells ($10^5$–$10^6$/plate) were seeded onto 60-mm dishes and incubated for 10 days to allow for colony formation. Cells were then fixed with methanol/glacial acetic acid 3:1, stained with 1% crystal violet, and colonies composed of more than 50 cells were counted.

Isobologram Analysis. To determine whether two compounds given in combination act additively, synergistically or antagonistically, isobologram analysis was performed (21). The IC$_{50}$ values were determined for MCF-7ADR cells that were pretreated with PA (0–10 mM), PB (0–3 mM), or butyrate (0–3 mM) for 72 h prior to the addition of doxorubicin (0–15 mM), as described above. The effect of these combinations was compared to the IC$_{50}$ values for each compound used alone. The pattern of the interaction between doxorubicin and the fatty acids was examined by plotting IC$_{50}$ isoboles as described (21).

Statistical Analysis of Survival Data. Statistical significance was determined using the Kruskal-Wallis test, which is a nonparametric one-way ANOVA (22). Survival curves were analyzed using a computer program (22). There were no significant differences in the survival data when analyzed using several models; therefore, the data were fitted by linear regression. Unless otherwise noted, data points are the mean ± SD of three experiments with triplicates.

Northern Blot Analysis and DNA Probes. Total RNA was extracted from exponentially growing or treated or control cells with the RNA STAT-60 method (Tel Test “B,” Friendswood, TX) and separated by electrophoresis in 1.2% agarose-formaldehyde gels. RNA preparation, blotting onto nylon membranes (Schleicher & Schuell, Keene, NH), hybridization with radiolabeled DNA probes, and autoradiography were performed as described (14). The cDNA probes included: mdr-1 HaelIII/Sma 683-bp fragment (Dr. Lynn Mickley) and HLA-A3 HindIII/EcoRI DNA (12). The glyceraldehyde-3-phosphate dehydrogenase cDNA probe (American Type Culture Collection, Rockville, MD) was used as internal control to ensure equal loading of the samples. Radiolabeled probes were prepared with $[\alpha-^{32}P]dCTP$ (New England Nuclear) using the Ready-To-Go DNA Labeling kit (Pharmacia Biotech Inc., Piscataway, NJ).

Enzyme Assays. Cells were prepared for GSH, enzyme, and protein assays as described previously (22). Briefly, 8.75 × $10^6$ cells were collected from twenty-five 100-mm Petri dishes; each was maintained at a designated low cell density ($3.5 \times 10^5$ cells/dish) by trypsinization and cell dilution. The lowered cell density allowed us to avoid confluent-induced cell synchrony, which can affect antioxidant enzyme and GSH measurements. The cells were washed with PBS, pelleted, and stored at -20°C. Immediately before analysis, samples were thawed and 2.5 mg/ml digitonin was added, which did not affect enzyme activity. Samples were vortexed and cellular debris pelleted by centrifugation. The supernatant was stored at 4°C until enzyme activity and protein analyses were performed. Antioxidant enzyme activity, cellular GSH content, and total protein levels were determined as described previously (22). Spectrophotometric methods were used to assess catalase SOD, glutathione peroxidase, glutathione S-transferase, and glutathione reductase activity. Coomassie brilliant blue (Bio-Rad, Richmond, CA) dye binding was used to measure protein levels with bovine $\gamma$-globulin as the standard. Data from all biochemical assays represent the mean ± SD of triplicates from three independent experiments.

RESULTS

Effect of PA and PB on MDR Cell Growth, Viability, and Differentiation. Table 1 provides a summary of the cytostatic effect of the AFAs PA and PB in breast, colon, and ovarian carcinoma cell lines (MCF-7, SW620, and 2780) and their respective MDR subclones (MCF-7ADR, SW620ADR, and 2780ADR), documented after 7 days of continuous treatment. The effect of the aliphatic fatty acid butyrate is shown for comparison. The MDR subclones were found to be similarly or more sensitive to growth arrest by PA, PB, and butyrate than the parental lines. The increased vulnerability was most notable with PA, with IC$_{50}$ values in the three MDR cell lines being 2–5-fold lower than in the parental counterparts (Fig. 1). PA had no effect on cell viability when used at doses as high as 10 mM; there was over 95% survival, as determined by trypan blue exclusion. Moreover, PA (7.5 mM) did not significantly decrease the clonogenic survival of MCF-7ADR, which is consistent with previous observations showing a reversible inhibitory effect of the AFAs on tumor cell growth (11, 12, 14). A minor decline in cell viability was observed with 2 mM PB and 2 mM butyrate (92 ± 2% and 88 ± 3% survival, respectively).

Unpublished data.
Differential sensitivity of parental and MDR tumor lines to PA.

Breast, ovarian, and colon carcinoma cells were cultured with 2.5, 5.0, or 10 mM PA for 7 days, after which cells were trypsinized and counted with a Coulter counter. Inhibition of growth is expressed as percentage of growth with respect to untreated controls. The values presented are the means of duplicates from three independent experiments. Bars, SD. In all cases, cell viability was 95%, as determined by trypan blue exclusion.

**Table 1** Response of MDR and parental tumor cells to fatty acid versus doxorubicin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell growth (% of control)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF-7</td>
</tr>
<tr>
<td>PA, 5 mM</td>
<td>55</td>
</tr>
<tr>
<td>PB, 1 mM</td>
<td>17</td>
</tr>
<tr>
<td>Butyrate, 1 mM</td>
<td>10</td>
</tr>
<tr>
<td>DOX, 5 μM</td>
<td>&lt;0</td>
</tr>
<tr>
<td>Ver, 5 μg/ml</td>
<td>88</td>
</tr>
<tr>
<td>DOX, 5 μM, + Ver, 5 μg/ml</td>
<td>&lt;0</td>
</tr>
<tr>
<td>PA, 5 mM, + Ver, 5 μg/ml</td>
<td>44</td>
</tr>
</tbody>
</table>

* Percentage of values indicate the increment in number of cells above starting cell number compared to untreated controls, where 0 reflects complete growth arrest (but no cell death). Values are the mean of triplicates from three independent experiments. In all cases, SDs did not exceed 15% of the mean.

P, Parental; DOX, doxorubicin; Ver, verapamil.

The value <0 indicates less than the starting cell number (i.e., cell death confirmed by trypan blue exclusion and clonogenic potential); complete cell death was observed with 0.1 μM doxorubicin.

Additional studies focusing on the breast carcinoma lines revealed that, in addition to cytostasis, PA, PB, and butyrate all induced morphological changes characteristic of cell maturation, including an increased cytoplasm:nuclear ratio, cell flattening, and lipid accumulation (data not shown). Moreover, Northern blot analysis revealed up-regulation of HLA-A3 mRNA (see below), coding for a MHC class I antigen, a marker of differentiation associated with increased tumor immunogenicity in vivo (23).

Verapamil Restores Sensitivity of MDR Cells to Doxorubicin but Does Not Affect the Response to PA or PB. The tested MDR cells overexpress the mdr-1 gene coding for P-gp. The calcium channel blocker verapamil can partially reverse the MDR phenotype by stopping P-gp from expelling drugs such as doxorubicin out of the cell. To determine whether P-gp activity had any effect on the response of MDR cells to AFAs, we next evaluated the cytostatic effect of PA (5 mM) in the presence or absence of verapamil (5 μg/ml) (Table 1). Verapamil caused 10–15% growth inhibition on its own, but did not alter the sensitivity of MDR cells to PA (Table 1) or to PB (data not shown). It appears, therefore, that tumor cell response to these AFAs is independent of the functional status of the P-gp efflux pump.

Fig. 1 Differential sensitivity of parental and MDR tumor lines to PA. Breast, ovarian, and colon carcinoma cells were cultured with 2.5, 5.0, or 10 mM PA for 7 days, after which cells were trypsinized and counted with a Coulter counter. Inhibition of growth is expressed as percentage of growth with respect to untreated controls. The values presented are the means of duplicates from three independent experiments. Bars, SD. In all cases, cell viability was 95%, as determined by trypan blue exclusion.
PA and PB, Unlike Butyrate, Potentiate the Cytotoxic Effect of Doxorubicin against MDR Cells. The effect of PA, PB, and butyrate on the response of MDR cells to the cytotoxic agent doxorubicin was evaluated in two representative cell lines: MCF-7ADR, a breast carcinoma line with a high degree of multidrug resistance (24), and HCT-15, a colon carcinoma line with low drug resistance. The clonogenic survival assay was used to determine the survival of cells treated for 72 h with PA, PB, and butyrate alone or followed by a short doxorubicin exposure. A 72-h pretreatment with PA (7.5 mM, a nontoxic dose) or with PB (2 mM, causing about 10% toxicity) resulted in a 2–4-fold increase in cell killing by doxorubicin (Fig. 2). Increased chemosensitivity, although less pronounced, was observed in MCF-7ADR cells pretreated with 1–5 mM PA and 0.3–1 mM PB, i.e., doses completely devoid of cytotoxic effect. Similar pretreatments of the parental MCF-7 cells did not significantly alter their response to doxorubicin (IC₅₀ for doxorubicin used alone was 0.07 ± 0.01 μM, and 0.05 ± 0.01 μM when added following pretreatment with 7.5 mM PA). In marked contrast to the AFAs, pretreatment of the MDR cell lines with 2 mM butyrate (cytotoxicity comparable to that of 2 mM PB), was associated with increased resistance to doxorubicin (Fig. 2), as previously documented in HCT-15 cultures (8).

To further analyze the pattern of interaction between doxorubicin and the fatty acids, an isobole was constructed using the IC₅₀ values obtained when MCF-7ADR cells were treated with each drug used alone or in combination. The results suggest a synergy between doxorubicin and PA (Fig. 3A) or PB (data not shown), whereas the isobole for doxorubicin used in combination with butyrate confirms an antagonistic effect (Fig. 3B).

Modulation of mdr-1 Gene Expression by PA and PB. Augmentation of the MDR phenotype by butyrate has been associated with induction of mdr-1 gene expression (8). We speculated therefore that the increased sensitivity to doxorubicin caused by PA and PB may be related to a decline in mdr-1. Contrary to our hypothesis, treatment for 72 h of MCF-7ADR and HCT-15 cells with PA and PB increased the steady-state levels of mdr-1 mRNA (Fig. 4). The induction of mdr-1 expression was most marked (over 10-fold) in MCF-7ADR exposed to 2 mM PB, a treatment that partially restored cell sensitivity to doxorubicin (Fig. 2). In all cases, the changes in mdr-1 were accompanied by significant increases in the expression of HLA-A3, a marker of cell differentiation (Fig. 4).

PA and PB, Unlike Butyrate, Decrease the Antioxidant Capacity of MDR Cells. To further investigate the mechanisms by which the AFAs may potentiate tumor cell killing by doxorubicin, we considered the contribution of GSH, GSH-dependent enzymes, and other antioxidant enzymes to the maintenance of the MDR phenotype (2, 5). The levels of GSH as well as the activity of catalase, SOD, glutathione reductase, total glutathione S-transferase, and glutathione peroxidase were measured in MCF-7ADR and HCT-15 cells (Fig. 5). Interestingly, the effect of PA and PB was highly time dependent: a transient increase in the activity of all tested enzymes was observed within 24 h of treatment (P < 0.05), followed by a significant decline in enzyme activity that reached nadir levels at 72 h. As shown in Fig. 5, the changes in enzymatic activity were paralleled by a transient increase in GSH levels, followed by a reduction to 84% of control at 72 h. Similar results were obtained with PB (data not shown). After 72 h, levels of GHS and antioxidant enzyme activity slowly increased, but remained below control levels even after 14 days of continuous exposure to PA (1 mM, in the case of HCT-15; 0.5 mM in the case of MCF-7ADR, data not shown). In contrast, butyrate induced a steady increase in antioxidant enzyme activity and GSH levels in the cell lines tested, which appeared to plateau after 72 h of treatment (Fig. 5) and subsequently declined, yet remained above control levels after 14 days of treatment (0.5 mM butyrate for HCT-15 or 0.1 mM for MCF-7ADR; data not shown).

DISCUSSION

The need for effective treatment of MDR tumors is well recognized. Current treatment strategies, which rely primarily on high-dose cytotoxic chemotherapy or the use of MDR mod-
Fig. 3 Isobologram analysis of the interaction between doxorubicin and the fatty acids. A. Concentrations of PA (X-axis) are plotted against those of doxorubicin. Points corresponding to IC50 values of each drug alone are connected by a solid straight line. The IC50 values of doxorubicin used in combination with several concentrations of PA are plotted and joined to form an isobol (- - -). B, concentrations of butyrate (X-axis) are plotted against those of doxorubicin, and the isobol was constructed as for PA.

Fig. 4 Modulation of mdr-1 and HLA-A3 gene expression by the fatty acids. Cells in exponential growth were incubated with various doses of PA, PB, or butyrate. Following 72 h of incubation, total RNA was extracted and blotted onto Nytran membranes. Northern blots were hybridized with radioactively 32P-labeled HLA-A3 (a) and mdr-1 (b) cDNA probes. Equal loading was confirmed by ethidium bromide staining of the gel and by hybridization with the glyceraldehyde-3-phosphate dehydrogenase probe.

Modulators (e.g., verapamil), have often been hindered by unacceptable toxicities. Studies focusing on differentiating agents could lead to a better understanding of the biology of MDR tumors and, possibly, result in beneficial clinical applications. The data presented here point to the differentiation inducers of the AFA class, PA, and PB, as candidates for the treatment of MDR tumors. In contrast to their resistance to doxorubicin, MDR human breast, ovarian, and colon carcinoma cells were generally found to be more sensitive to PA and PB than their parental counterparts.
parts. The increased vulnerability was manifested by a more efficient growth arrest induced by the AFAs, with no significant change in cell viability. For example, PA IC_{50} values were 2.0 ± 0.3 mM versus 8.4 ± 0.5 mM in MCF-7ADR and MCF7, respectively. The effect was independent from the status of the P-g pump, as indicated in studies involving the calcium channel blocker verapamil. The decline in proliferative capacity, evident after 2 or more days of treatment, was accompanied by morphological and molecular changes consistent with cell maturation. The latter included an increase in the cytoplasm:nuclear ratio and in the levels of HLA-A3 mRNA, encoding a class I major histocompatibility antigen implicated in enhanced tumor immunogenicity in vivo (23). The changes in tumor biology, reminiscent of those noted in other responsive malignancies (10, 12–14), were documented with PA and PB concentrations that have been achieved in humans with no significant toxicities (15–18). In addition to differentiation, these noncytotoxic compounds caused a dose- and time-dependent increase in the sensitivity of MDR cells to killing by doxorubicin.

The mechanisms of action of the AFAs against MDR cells are not known. Both PA and PB cause major alterations in lipid metabolism. Treated breast MCF-7ADR carcinoma cells, like other tumor cells responsive to AFAs (12, 13), consistently accumulate fatty droplets. In addition, we now have evidence for reduced prenylation of proteins critical to signal transduction (13) as well as up-regulation of the human peroxisomal proliferator-activated receptor, a nuclear receptor controlling cholesterol and fatty acid metabolism (20). Cells exhibiting the MDR

Fig. 5 Changes in GSH levels and antioxidant enzyme activity. MCF-7ADR cells and HCT-15 cells were incubated with 7.5 mM PA or 2 mM butyrate (B) for 24, 48, and 72 hours, and the levels of GSH and antioxidant enzyme activity were measured at each time point. PB (2 mM) had a similar effect to PA (data not shown). Bars, SD. CT, catalase; GST, glutathione S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase.
phenotype are characterized, among other features, by marked abnormalities in lipid composition (25–28). Alterations in membrane cholesterol, triglycerides, and fatty acids in MDR cells may affect transmembrane transport, intercellular communication, and cell signaling. Targeting such vulnerable control points in the biology of MDR cells may underlie the effective cytostasis induced by the AFAs as well as their potentiation of doxorubicin cytotoxicity. Consistent with this hypothesis, lipid changes in MDR tumor cells treated with polyunsaturated fatty acids have been associated with effective growth arrest and partial reversal of the MDR phenotype (29–31).

The aliphatic fatty acid butyrate, also a modulator of lipid metabolism, induces tumor cytostasis and differentiation and is active against MDR tumor cells. However, in contrast to the AFAs, butyrate was found to increase chemoresistance in conjunction with up-regulation of mdr-1 expression (our studies and Refs. 8, 9). One possible explanation for the different effect on the MDR phenotype could be related to mdr-1 expression. Paradoxically, however, Northern blot analysis of representative MDR-positive and MDR-negative cell lines revealed that, like butyrate, both PA and PB up-regulated mdr-1 gene expression. The effect on P-gp production, posttranslational modifications (e.g., phosphorylation), and biological function have not yet been characterized. Nevertheless, our findings are consistent with previous reports showing that increases in mdr-1/P-gp expression do not necessarily lead to an increase in the MDR phenotype (8, 31). In our studies with PA and PB, as in others involving retinoids, butyrate, DMSO, and dimethylformamide (8, 32), overexpression of the mdr-1 gene may simply serve as a marker of cell differentiation.

It appears therefore that increased sensitivity of MDR cells to doxorubicin by the AFAs is independent of mdr-1 gene expression. Other cellular and molecular events have been considered, including changes in cell cycle, cytotoxicity, and differentiation, but these alone could not explain chemosensitization, as indicated by the following findings: (a) Butyrate, a fatty acid that shares in common with PA and PB the ability to arrest tumors in G1 and induce differentiation (6, 33), had an opposite effect on the MDR phenotype. Another differentiation inducer, retinoic acid, while increasing mdr-1 expression, did not affect cell responses to cytotoxic drugs (32). (b) Under the experimental conditions used, butyrate and PB caused similar cytotoxicity, yet the isofoles evaluating their interaction with doxorubicin indicate an antagonistic effect for the one and synergy for the other. (c) Chemosensitization by the AFAs, like that induced by polyunsaturated fatty acids is selective, affecting MDR cells but not their corresponding wild-type tumor cells exposed to similarly cytostatic/cytotoxic doses of the fatty acids (present study and Ref. 30).

Among the mechanisms that could significantly affect drug resistance is a change in cellular antioxidant capacity. Intracellular GSH, its related enzymes glutathione peroxidase, glutathione S-transferase, and glutathione reductase, as well as SOD and catalase all constitute an important defense mechanism against free radical-producing agents like doxorubicin (2, 5, 34). By depleting cellular glutathione contents and lowering the activity of GSH-dependent enzymes, MDR cells can be rendered more vulnerable to doxorubicin-induced damage. Exogenous SOD and catalase have been shown to protect MCF-7 cells from doxorubicin (35). The present study with the AFAs confirms that changes in the antioxidant defense can alter drug resistance, independent of mdr-1 gene expression. PA and PB induced a transient increase in both GSH and enzyme activity within 24 h of treatment, followed by a significant reduction below control levels. This diminished antioxidant capacity at 72 h of treatment was correlated with reduced cell resistance to doxorubicin. In contrast, butyrate induced a steady increase in GSH and antioxidant enzyme activity, which could account for the increased tolerance to doxorubicin (our studies and Ref. 9).

MDR breast, ovarian, and colon cancer lines show high sensitivity to treatment with the differentiation-inducing fatty acids PA, PB, and butyrate. However, substantial differences have been identified regarding the effect of the AFAs PA and PB versus the short aliphatic fatty acids butyrate on chemoresistance. Should these fatty acids prove useful clinically in the treatment of MDR tumors, combinations with cytotoxic drugs should be carefully designed based on the documented effect on the MDR phenotype to maximize antitumor activity.

ACKNOWLEDGMENTS

We thank Dr. William D. Perkins for his continuous support, William Houck for excellent technical assistance, and Stacy Ayres for manuscript preparation.

REFERENCES

12. Samid, D., Shack, S., and Myers, C. E. Selective growth arrest and phenotypic reversion of prostate cancer cells in vitro by nontoxic phar-
Vulnerability of multidrug-resistant tumor cells to the aromatic fatty acids phenylacetate and phenylbutyrate.

S Shack, A Miller, L Liu, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/2/5/865

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.