Extensive Contribution of the Multidrug Transporters P-Glycoprotein and Mrp1 to Basal Drug Resistance

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ABSTRACT

Despite accumulating evidence that multidrug resistance transporter proteins play a part in drug resistance in some clinical cancers, it remains unclear whether the relatively low levels of multidrug resistance transporter expression found in most untreated tumors could substantially affect their basal sensitivity to antineoplastic drugs. To shed light on this problem, the drug sensitivities of wild-type mouse cell lines were compared with those of lines in which the Mdr1a and Mdr1b genes encoding P-glycoprotein (P-gp) were inactivated and lines in which the Mrp1 gene was inactivated in addition to Mdr1a and Mdr1b. These models permit a clean dissection of the contribution of each transporter to drug resistance at expression levels similar to those in normal tissues and avoid complications that might arise from previous exposure of cell lines to drug selection. For substrate drugs, we found that these contributions could in fact be very substantial. Lines lacking functional P-gp were, on average, markedly more sensitive to paclitaxel (16-fold), anthracyclines (4-fold) and Vinca alkaloids (3-fold). Lines lacking both P-gp and Mrp1 were (compared with wild-type lines) hypersensitive to an even broader array of drugs, including epipodophyllotoxins (4–7-fold), anthracyclines (6–7-fold), camptothecins (3-fold), arsenite (4-fold) and Vinca alkaloids, especially vincristine (28-fold). Thus, even very low levels of P-gp and Mrp1 expression that may be difficult to detect in tumors could significantly affect their innate sensitivity to a wide range of clinically important substrate drugs. An implication is that the use of resistance reversal agents to sensitize drug-naive tumors may be appropriate in more cases than is presently appreciated.

INTRODUCTION

The usual modus operandi for identifying mechanisms of resistance to antineoplastic drugs has been to select cell lines for high levels of drug resistance and then analyze changes in gene expression that might account for the resistance. A consequence of this strategy is that only changes able to confer high levels of resistance are likely to be detected. These include elevated expression of drug efflux pumps or metabolizing enzymes and mutations and/or changes in expression of genes encoding the targets of drug action. The genes encoding the efflux pumps P-gp (1–3), the MRPL (4) and BCRP/MXR/ABCP (5–7) were all identified in this manner.

A reasonable criticism of this approach is that high levels of drug resistance are atypical of clinical tumors. Chemotherapy is usually a marginal proposition in the sense that the maximum dose tolerated by the patient is often barely sufficient to kill a useful percentage of the tumor cells. Relatively small increases in drug resistance in tumor cells are thus sufficient to render the drug ineffective. Such small increases may be achieved by a much wider variety of mechanisms than those able to confer high levels of drug resistance. The relevance of the (elevated) drug transporter expression seen in drug-selected cell lines to clinical drug resistance cannot, therefore, be taken for granted.

A growing body of evidence links P-gp expression in untreated tumors to poor prognosis for chemotherapy with substrate drugs (for instance, see Refs. 8, 9). The connection is firm for several hematological cancers but, thus far, remains tentative for most other malignancies. Part of the reason is that reliable quantitation of gene expression in heterogeneous patient material is technically problematic (e.g., see the meta-analysis of P-gp expression in breast cancer in Ref. 10). Data concerning the contribution of MRPL to clinical drug resistance are still sparse (8, 9, 11), and the issue is complicated by the widespread expression of MRPL in normal tissues. In general, it is still unclear whether the (often low) basal levels of multidrug transporter expression significantly affect the innate drug sensitivity of most types of tumors.

We have therefore tested the effects of eliminating normal (i.e., not drug-selected) P-gp and Mrp1 expression on drug sensitivity by comparing mouse cell lines carrying either functional or targeted null alleles of the genes encoding these transporters. This model circumvents issues surrounding the heterogeneity of gene expression in tumor cells and permits a direct assessment of the contribution of multidrug transporters to basal drug sensitivity at levels of expression that can be more readily related to those in normal tissues or untreated tumors. We found that these contributions can be very substantial for drugs that are transporter substrates, implying that basal expression of multidrug transporters could significantly affect innate drug sensitivity of clinical tumors and that even small increases (in absolute terms) in their expression may well mediate acquired resistance. The remarkable sensitivity of cell lines lacking both P-gp and Mrp1 to antineoplastic drugs also makes them valuable in other contexts as tools for analysis of drug resistance mechanisms.

MATERIALS AND METHODS

Fibroblast Cell Lines. Most of the cell lines used in this study were derived by us from MEFs by 3T3-like procedures (12). The source embryos were of mixed genetic background (Fv3, or later crosses between 129/Ola and FVB strains). Lines lacking functional P-gp were obtained from Mdr1a<sup>−/−</sup>Bcrp<sup>−/−</sup> embryos (13), and lines lacking both P-gp and Mrp1 were obtained from Mdr1a<sup>−/−</sup>Bcrp<sup>−/−</sup>Mrp1<sup>−/−</sup> embryos (14). The MEF cultures went through crises at variable passage numbers and were cloned between passages 25 and 40, as allowed by their growth characteristics. NIH/3T3 cells (15), passage 126, were obtained from the American Type Culture Collection (Manassas, VA; CRL 1658). Note that this passage is no longer strongly contact inhibited, growing to approximately six times the density originally reported. All lines and MEF cultures were maintained in DMEM plus 10% FCS (Life Technologies, Breda, The Netherlands) and were passaged by treatment with trypsin-EDTA. Mean volumes of log phase cells were measured by 7-day colony assays stained with crystal violet.

P-gp and Mrp1 Protein Levels. Total tissue and cell lysates for immunoblots were prepared by homogenizing and sonicating at 10<sup>7</sup>/ml or tissues at 50 mg wet weight/ml in 10 mM Tris-HCl (pH 7.4), 10 mM KCl, 1.5 mM MgCl<sub>2</sub> 1% w/v SDS, 1% v/v 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride and Complete protease inhibitor mixture (Boehringer Mannheim, Mannheim, Germany). Samples were mixed with SDS-PAGE loading buffer.

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3 The abbreviations used are: P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MDR, multidrug resistance; MRP, multidrug resistance-associated protein; MEF, mouse embryo fibroblast; SN-38, (4S)-11-ethylcamptothecin-ol; Sggp, sister of P-gp.
and heated to 65°C for 5 min, this being adequate to dissociate immunoglobulins in the tissue samples without causing significant aggregation of either P-gp or Mrp1. Samples were analyzed on 8% SDS-PAGE gels and electroblotted to nitrocellulose. Blots were divided at the 97-kDa marker. The high molecular weight halves were probed for either P-gp or Mrp1 using the C219 (Centocor, Leiden, The Netherlands) or MRPr1 (16) monoclonal antibodies, respectively. These antibodies were raised against the human and Chinese hamster proteins but cross-react with the mouse homologues. The low molecular weight halves of the blots were probed with the anti-a-tubulin monoclonal YL 1/2 (17) as a loading control. Target proteins were visualized with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL). Replicate blots of total RNA from the cell lines and mouse tissues were hybridized with 32P-labeled antisense RNA probes for Bcrp1 (18) or Mdr2 (19) or random-primer DNA probes for Spgp (IMAGE clone 313236, GenBank accession number W11894) or Cyp3a16 (a complete coding sequence amplified by PCR from liver cDNA, kindly provided by J. W. Smit, The Netherlands Cancer Institute). Hybridization was performed in Ultrahyb buffer (Ambion, Austin, TX) under the recommended conditions, except for Cyp3a16, for which the hybridization and wash temperatures were reduced by 5°C to ensure detection of other homologous Cyp3a mRNAs. One blot was rehybridized with a CDNA probe for the mouse 18S rRNA as a loading control.

Drugs. Paclitaxel, vinblastine sulfate, doxorubicin (Adriamycin), cytarabine, melphanal, and sodium arsenite were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Formulations of vincristine sulfate, cisplatin, daunorubicin, mitoxantrone, etoposide, and teniposide were obtained from local pharmaceutical suppliers; the topotecan formulation was from SmithKline Beecham (Brentford, Middlesex, England); and Taxotere (docetaxel) and SN-38 were from Rhône-Poulenc Rorer (Vitry-Alfortville, France).

Drug Sensitivity Assays. Growth inhibition (IC_{50}) assays were performed by seeding 200 cells per well in 96-well plates in complete medium and, after cell attachment, applying drugs in a dilution series of 2-fold concentration steps, with each concentration in quadruplicate wells. After 4–4.5 days, when control wells were still subconfluent, cells were lysed in situ, and nucleic acids were stained with a proprietary dye (Cyquant; Molecular Probes, Eugene, OR) and quantified by fluorescence (485 nm excitation, 530 nm emission).

Descriptive and Inferential Statistics. The SDs shown in Table 2 represent variability in absolute IC_{50} determinations across independent experiments. Sensitivity factors in Table 3 are computed directly from the means shown in Table 2. Because distributions of drug resistance data are, by nature, positively skewed, IC_{50} values were transformed to logarithms for the purposes of statistical comparisons (20). All tests were two-tailed t tests assuming unequal variances.

RESULTS

Derivation and Characteristics of Fibroblast Cell Lines. Mouse cell lines of three genotypes were generated and compared: wild-type, P-gp knockout (Mdr1a^{−/−} Mdr2^{−/−}), and combined P-gp-Mrp1 knockout (Mdr1a^{−/−} Mdr2^{−/−} Mrp1^{−/−}). The lines were obtained by spontaneous immortalization of MEFs (see "Materials and Methods" for details). Their genetic background is a mix of 129/Ola and FVB strains, except for the NIH/3T3 line included for comparison, which was derived from an NIH/Swiss strain embryo (15). The MEF lines to be analyzed were chosen from a larger set on the basis of favorable growth characteristics—stability, vigorous growth to high density, and single-cell cloning efficiency—but differ somewhat in these respects (Table 1). Although growth properties might be expected to affect drug sensitivity, in practice no systematic effects from this source were observed.

Expression of Multidrug Transporters in the Cell Lines and Mouse Tissues. The wild-type cell lines are useful as models of innate drug sensitivity provided their expression of P-gp and Mrp1 is not substantially greater than that seen in normal tissues. P-gp and Mrp1 levels in wild-type and knockout cell lines and a panel of mouse whole tissue homogenates were therefore compared by immunoblot analysis (Fig. 1, A and B). P-gp levels in the wild-type cell lines were comparable with or lower than those seen in normal mouse tissues. The C219 antibody cross-reacts with Mdr2 P-gp (homologue of human MDR3) and Spgp, but their expression is mostly confined to liver (21, 22). Two observations exclude significant levels of either of these proteins in the fibroblast cell lines: Mdr2 and Spgp mRNAs were undetectable by Northern analysis of the cell lines but readily detectable in mouse liver (Fig. 2, A and B), and the C219 antibody detected no Mdr2 or Spgp protein in the six fibroblast lines null for Mdr1a/or (Fig. 1A). Thus, the C219 signal in the wild-type cell lines reliably reflects their expression of P-gp.

The substrate specificity of the drug-metabolizing cytochrome P450–3A family of enzymes overlaps that of P-gp; therefore, differences in their activity between different cell lines could, in principle, affect drug sensitivity. However, Northern analysis of the fibroblast lines with a Cyp3a16 probe at reduced stringency to recognize the major Cyp3a mRNAs did not reveal detectable expression in any of the lines, whereas a strong signal was observed in liver and intestine, as expected (Fig. 2C).

Mrp1 levels in the cell lines were somewhat higher than in most tissues examined. This result is adequate for the present purpose, given that Mrp1 tends to be concentrated in epithelial layers having substantially higher protein levels than the tissue taken as a whole (23–25). This also holds true for P-gp (26).

Sensitivity of the Lines to Antineoplastic Drugs. The relative sensitivities of the cell lines to an extensive panel of antineoplastic drugs were determined by growth inhibition assay (Table 2). The drug panel contained representatives from the major families of clinically important antineoplastic agents that are known substrates for P-gp or Mrp1, including taxanes, Vinca alkaloids, anthracyclines, epipodophyllotoxins, and camptothecin derivatives, as well as an arsenical. Control drugs were also included from several classes that are poor or

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Name</th>
<th>Passage</th>
<th>Doubling time (h)</th>
<th>Mean cell volume (fl)</th>
<th>Cloning efficiency (%)</th>
<th>Maximum cell density (10^3cm^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>NIH/3T3</td>
<td>137</td>
<td>17</td>
<td>2.0</td>
<td>55</td>
<td>&gt;3.5</td>
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<tr>
<td>2ac.1</td>
<td>32</td>
<td>21</td>
<td>2.8</td>
<td>18</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>2ac.2</td>
<td>32</td>
<td>22</td>
<td>4.8</td>
<td>20</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>WT1.2</td>
<td>30</td>
<td>28</td>
<td>7.0</td>
<td>34</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Mdr1a^{−/−} Mdr2^{−/−}</td>
<td>77.1</td>
<td>37</td>
<td>19</td>
<td>3.0</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>Mrp1^{−/−}</td>
<td>88.6</td>
<td>37</td>
<td>18</td>
<td>2.0</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Mrp1^{−/−}</td>
<td>55.2</td>
<td>12</td>
<td>25</td>
<td>6.6</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>MEF3.8</td>
<td>11</td>
<td>24</td>
<td>3.0</td>
<td>30</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>4B.65</td>
<td>8</td>
<td>25</td>
<td>3.0</td>
<td>30</td>
<td>1.9</td>
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</table>

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insignificant substrates of these proteins but for which acquired resistance is also a significant clinical problem: cisplatin, cytarabine (a nucleoside analogue), and melphalan (an alkylating agent). Mitoxantrone was included because it is an excellent substrate for the recently identified multidrug transporter BCRP/Bcrp1 (5, 6, 18).

The data show that basal expression of P-gp and Mrp1 can have a major impact on innate resistance to substrate drugs. Table 2 presents the mean IC50 values, and Table 3, to aid comparisons, shows the sensitivity factors plus results of statistical tests of the differences between genotypes. The Mdr1a+/−/Mrp1+/− cell lines were substantially more sensitive than the wild-type lines to all drugs tested that are good P-gp substrates—the taxanes, antracyclines, and Vinca alkaloids. The differences were surprisingly large given the relatively low levels of P-gp in the wild-type cell lines; the averages were 16-fold for P-gp substrates—the taxanes, antracyclines, and Vinca alkaloids. It may be noted that the cell lines lacking P-gp expressed somewhat higher levels of Mrp1 than the wild-type lines (Fig. 1). It is not clear whether this is a coincidental or a compensatory effect. Either way, it might partially mask the effect of inactivating P-gp for drugs that are substrates of both transporters, Vinca alkaloids, epipodophyllotoxins, and possibly antracyclines (see below).

The Mdr1a+/−/Mrp1+/− cell lines were markedly more sensitive than the Mdr1a+/−/Mrp1−/− cell lines to several Mrp1 substrate drugs, most notably vincristine (≥10-fold), sodium arsenite (≥7-fold), and the epipodophyllotoxins etoposide (7-fold) and teniposide (4-fold). Qualitatively, these differences in drug sensitivity are in agreement with those seen in previous studies of mouse embryonic stem cell lines and bone marrow mast cells in which only the Mrp1 gene had been inactivated by gene targeting (27, 28). However, the differences we observed are generally greater due, at least in part, to the absence of masking P-gp activity on drugs that are common substrates. For such drugs, the combined effect of eliminating the function of two transporters can sometimes be very striking, as exemplified by vincristine, for which the difference in sensitivity between Mdr1a+/−/Mrp1+/− and wild-type lines averaged 28-fold.

Human MRP1 transports antracyclines effectively and can confer resistance to these drugs, but it is unclear to what extent mouse Mrp1 does likewise (28–32). We found that mouse fibroblast lines lacking both Mrp1 and P-gp were only slightly more sensitive to doxorubicin and daunorubicin than lines lacking only P-gp (−1.5-fold), suggesting that these drugs are relatively poor substrates for mouse Mrp1.

It is interesting that the Mdr1a+/−/Mrp1−/− cell lines were 2–3-fold more sensitive to the camptothecin derivatives topotecan and SN-38 than the Mdr1a+/−/Mrp1+/− lines. This result suggests that mouse Mrp1 can transport these drugs, in line with a recent report (33) that elevated expression of human MRP1 in drug-selected or MRP1-transfected cells mediates resistance to SN-38 and CPT-11 (another camptothecin derivative), which is associated with ATP-dependent drug efflux and reversed by MRP1 inhibitors.

Overexpression of P-gp is usually associated with only modest resistance to mitoxantrone (e.g., Refs. 34, 35), implying that this drug is a relatively poor substrate. Increases in resistance to mitoxantrone have also been observed in MRP1-transfected cells (11). In line with these results, we did observe moderately increased sensitivity to mitoxantrone in Mdr1a+/−/Mrp1−/− and Mdr1a+/−/Mrp1+/− fibroblast lines of 2.5- and 4.3-fold, respectively, compared with wild-type lines, but only the latter difference was statistically significant (P = 0.041, two tailed). This is in part due to the considerable variability in resistance to mitoxantrone between different cell lines within each genotype. Mitoxantrone is a good substrate of the BCRP/Bcrp1 transporter (5, 6, 18), and there is indeed a qualitative correlation between Bcrp1 mRNA levels (Fig. 2D) and resistance to mitoxantrone.
antrone. However, the relationship is not so good that Bcrp1 expression might account for all of the differences in sensitivity to this drug.

Within genotypes, substantial systematic differences in drug sensitivity were evident only in the wild-type group of cell lines; the WT1.2 and especially the NIH/3T3 lines were frequently more sensitive than the 2ac.1 and 2ac.2 lines. This pattern was most pronounced for P-gp substrate drugs and was not observed consistently for drugs that are neither P-gp nor Mrp1 substrates (cisplatin, cytarabine, and melphalan). The systematic differences are thus probably at least partly the result of the lower P-gp expression observed in the WT1.2 and NIH/3T3 lines (Fig. 1). The different mouse strain origin of the NIH/3T3 line might also contribute to the differences observed.

**DISCUSSION**

Our data show clearly that P-gp and Mrp1 can be major determinants of innate drug sensitivity, even when the level of expression is low. Fibroblast cell lines lacking P-gp were consistently and often

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Table 2. Sensitivity of wild-type and knockout fibroblast cell lines to antineoplastic drugs, by genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cell line</th>
<th>Taxanes</th>
<th>Vinca alkaloids</th>
<th>Anthracyclines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paclitaxel</td>
<td>Docetaxel</td>
<td>Vincristine</td>
</tr>
<tr>
<td>Wild-type</td>
<td>NIH/3T3</td>
<td>53 ± 22 (4)</td>
<td>13.0 ± 0.5 (3)</td>
<td>5.8 ± 1.8 (4)</td>
</tr>
<tr>
<td></td>
<td>2ac.1</td>
<td>103 ± 16 (3)</td>
<td>41 ± 1.5 (3)</td>
<td>7.3 ± 2.2 (4)</td>
</tr>
<tr>
<td></td>
<td>2ac.2</td>
<td>218 ± 28 (3)</td>
<td>61 ± 2.6 (3)</td>
<td>16 ± 3.6 (3)</td>
</tr>
<tr>
<td></td>
<td>wt1.2</td>
<td>48 ± 5 (3)</td>
<td>12.2 ± 1.4 (2)</td>
<td>13 ± 6.3 (4)</td>
</tr>
<tr>
<td>Mdr1a−/−/Bc−/−</td>
<td></td>
<td>57.1</td>
<td>8.2 ± 1.3 (4)</td>
<td>13.1 ± 4.5 (4)</td>
</tr>
<tr>
<td></td>
<td>88.6</td>
<td>4.2 ± 0.2 (3)</td>
<td>12.9 ± 4.8 (4)</td>
<td>4.5 ± 1.0 (4)</td>
</tr>
<tr>
<td></td>
<td>55.2</td>
<td>6.9 ± 0.6 (3)</td>
<td>9.9 ± 2.7 (3)</td>
<td>4.1 ± 1.3 (4)</td>
</tr>
<tr>
<td>Mdr1a−/−/Bc−/−/Mrp−/−</td>
<td></td>
<td>5c.31</td>
<td>5.0 ± 1.0 (3)</td>
<td>9.0 ± 2.9 (4)</td>
</tr>
<tr>
<td></td>
<td>4b.65</td>
<td>5.2 ± 1.1 (3)</td>
<td>6.5 ± 0.8 (4)</td>
<td>0.28 ± 0.06 (4)</td>
</tr>
<tr>
<td></td>
<td>MEF3.8</td>
<td>4.3 ± 0.4 (4)</td>
<td>5.2 ± 0.5 (3)</td>
<td>0.39 ± 0.07 (5)</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Northern analysis of Mdr2 (A), Spgp (B), Cyp3a (C), and Bcrp1 (D) expression in the fibroblast cell lines. Probes for these mRNAs were hybridized to replicate blots containing 10 µg of total RNA/lane. One blot was subsequently rehybridized with an 18S rRNA cDNA probe as a loading control (E).
markedly more sensitive than wild-type lines to substrate drugs, including taxanes, anthracyclines, and Vinca alkaloids. Lines also lacking Mrp1 were more sensitive still to anthracyclines and Vinca alkaloids (with extreme sensitivity to vincristine) and considerably more sensitive than wild-type lines to epipodophyllotoxins, camptothecin derivatives, and arsenite. No such increased sensitivity was seen for drugs that are not substrates of either of the transporters. The extent of the differences is remarkable in that the levels of P-gp and Mrp1 in the wild-type fibroblast cell lines were similar to or lower than those seen in many normal, bulk tissues. In addition to showing the effects of (genetically) eliminating P-gp function on the basal sensitivity of cells to antineoplastic drugs, the results demonstrate the contribution of Mrp1 to basal resistance in the absence of the potentially confounding influence of P-gp, extending previous results obtained with Mrp1−/− embryonic stem cells and mast cells (27, 28).

Although many other mechanisms may intrude to alter drug resistance, there can be little doubt that the increased sensitivities seen in the knockout fibroblast lines are due to the loss of P-gp and Mrp1. Multiple independently derived lines of each genotype were examined, with similar origins and genetic background. Results for the cell lines of each genotype are in good agreement, and the pattern of drug sensitivities observed matches the known substrate specificities of the two transporter proteins.

These observations strongly support the position that the normal levels of expression of MDR transporters in cancer (or normal) cells can be important for innate resistance to antineoplastic drugs. They also imply that low absolute levels of expression of drug transporters in tumors can still be significant for acquired resistance, in that a change to low expression from very low (or no) initial expression could produce a difference in drug sensitivity of the same scale as observed in the cell line model. Although our results concern P-gp and Mrp1, there is no obvious reason to suspect that other drug transporters, such as Bcrp1, will differ in this respect. In “Introduction” we alluded to attempts to predict the responses of primary tumors to specific chemotherapeutic regimens on the basis of P-gp expression, which have been generally disappointing. Our data suggest that such failures are not reason to conclude that P-gp (or Mrp1) is irrelevant to clinical drug resistance but rather that the task of assessing expression of these (and other) drug transporters, although difficult, is worthwhile. It seems clear that simultaneous assessment of expression of multiple transporters of a given drug will be beneficial.

The relevance of basal expression of MDR transporters to chemotherapy will be increased with the adoption of potent inhibitors of P-gp such as Valspodar (and, ultimately, inhibitors of other transporters) for coadministration during chemotherapy, now being tested in Phase III clinical trials (e.g., see Ref. 9). Our results lend credence to the idea that inhibitors of MDR transporters may be useful not only to reverse or prevent acquired drug resistance but also to sensitize drug-naïve, untreated tumors to substrate drugs, even when expression of the relevant transporter(s) is low. A caveat to these approaches is that coadministration of inhibitors with antineoplastic drugs might increase the risk of toxic side effects in normal cells that are otherwise protected by drug transporter expression (14, 25, 36). The importance of such side effects can only be decided in the clinical trials.
CONTRIBUTION OF MDR TRANSPORTERS TO BASAL RESISTANCE

It must be reiterated that we are not suggesting that P-gp, MRP1, or other drug transporters are the only important influences on basal resistance in our cell lines, let alone in clinical tumors. The influence of other, unknown factors is evident in the data, for example, in the marked sensitivity of NIH/3T3 cells to arsinite and the variability in mitoxantrone sensitivity, only part of which is attributable to Bcrp1 expression. This is not surprising, given that drug sensitivity may be influenced by many factors. (In fact, the cell lines lacking P-gp and MRP1 function will be useful tools for investigating these other factors.) Other cell types, including tumor cells, may have a more extensive repertoire of drug resistance mechanisms than fibroblasts. If so, the relative contribution of P-gp and MRP1 to drug resistance in tumors or normal cells thus may (or may not) be less than is suggested by our results. Nevertheless, acknowledging the limitations of the cell line model, our data indicate that this contribution can be very marked.

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