[CANCER RESEARCH 61, 6635–6639, September 15, 2001]

Advances in Brief

Acquired Mutations in the *MXR/BCRP/ABCP* Gene Alter Substrate Specificity in MXR/BCRP/ABCP-overexpressing Cells

Yasumasa Honjo, Christine A. Hrycyna, Qing-Wu Yan, Wilma Y. Medina-Pérez, Robert W. Robey, Anne van de Laar, Thomas Litman, Michael Dean, and Susan E. Bates[1]

Cancer Therapeutics Branch, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892 [Y. H., Q-W. Y., W. Y. M-P., R. W. R., A. v. d. L., S. E. B.]; Department of Chemistry, Purdue University, West Lafayette, Indiana 47907-1393 [C. A. H.]; Department of Medical Physiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark [T. L.]; and Laboratory of Genomic Diversity, National Cancer Institute, Frederick, Maryland 21702 [M. D.]

Abstract

A disparity was noted in the transport of rhodamine 123 among nine MXR/BCRP/ABCP-overexpressing cells studied; all demonstrated mitoxantrone transport, whereas only two effluxed rhodamine 123. When the *MXR/BCRP/ABCP* gene was sequenced in the cell lines studied, differences were noted at amino acid 482, predicted to be at the start of the third transmembrane domain. Sequencing genomic DNA revealed wild-type MXR/BCRP/ABCP to have an arginine at position 482. Cells having a threonine or glycine at position 482 were able to efflux rhodamine 123, whereas cells having an arginine were not. A vaccinia virus expression system confirmed that rhodamine as well as doxorubicin efflux is observed with R482T or R482G but not with the wild-type R482; all three MXR/BCRP/ABCP forms transported mitoxantrone. Cross-resistance studies suggest that, compared with wild-type MXR/BCRP/ABCP, cells having an R482T mutation have higher anthracycline resistance, whereas an R482G mutation seems to confer relatively less resistance to SN-38 and topotecan. These results suggest that amino acid 482 has a crucial role in MXR/BCRP/ABCP function and that mutation of a single amino acid residue significantly changes substrate specificity, thus altering the drug resistance phenotype.

Introduction

The mitoxantrone resistance protein, MXR/BCRP/ABCP, has been shown to confer high levels of resistance to mitoxantrone as well as resistance to anthracyclines; selected camptothecins including topotecan and SN-38; and flavopiridol (1–5). MXR/BCRP/ABCP is a half-transporter that belongs in the ABCG subfamily containing the *Drosophila* white protein, which is involved in the transport of precursors of eye pigment (6). The Human Genome Nomenclature Committee recently changed the gene symbol to ABCG2.[2] Recently, two new members of the ABCG subfamily (ABCG5 and ABCG8) have been identified that are involved in sterol transport (7, 8). Because MXR/BCRP/ABCP is a half-transporter, it is believed to dimerize in order to function (6). MXR/BCRP/ABCP may be involved in the maternal-fetal xenobiotic barrier, because an inhibitor increased accumulation of topotecan in the fetus in mice lacking the multidrug transporter mdr1a/1b (9). Studies in drug-resistant cells with overexpression of MXR/BCRP/ABCP show localization to the plasma membrane and efflux of substrates. In developing a flow cytometric assay for the detection of MXR/BCRP/ABCP, a disparity was noted in the transport of rhodamine 123 in MXR/BCRP/ABCP-overexpressing cells. Whereas all these cells were found to transport mitoxantrone, some cells readily effluxed rhodamine 123, whereas others did not (10). Initially, we attributed this problem to low sensitivity of rhodamine; however, studies with mitoxantrone-resistant sublines of MCF-7 expressing MXR/BCRP/ABCP mRNA at high levels also demonstrated no apparent rhodamine efflux. In light of the fact that mutations in Pgp derived from drug selection have been shown to alter substrate specificity (11), the MXR/BCRP/ABCP mRNA from 11 cell lines was sequenced to determine whether mutations could explain the differing substrate specificities. In the present study, we describe an acquired mutation in the *MXR/BCRP/ABCP* gene that correlates with the observed differences in rhodamine 123 transport and is associated with shifts in the cross-resistance profile of drug-resistant cells.

Materials and Methods

Cell Lines. Nine MXR/BCRP/ABCP-overexpressing sublines and two parental cell lines were examined in this study. MCF-7 AdVp3000 cells were maintained in 5 μg/ml verapamil and 3000 ng/ml adriamycin. Three sublines independently selected in mitoxantrone were also used: MCF-7/MX (12), MCF-7 MX8, and MCF-7 MX100. These were maintained in 600 nM, 8 ng/ml, and 100 nM mitoxantrone, respectively. The MCF-7 FLV1000 cells were maintained in 1000 nM flavopiridol (5). The human colon carcinoma cell line S1 and its drug-resistant subline S1-M1-3.2 were obtained from Dr. Lee M. Greenberger (Wyeth-Ayerst; Ref. 13). The S1-M1-80 subline was generated in our lab by exposing the S1-M1-3.2 cell line to increasing concentrations of mitoxantrone eventually reaching 80 μM (1). The A549 MX10, NCI-H460 MX10, and SF295 MX10 cell lines were selected and maintained in 10 nM mitoxantrone (10). The MCF-7 parental cells and resistant sublines were maintained in Improved Minimal Essential Medium, and the remaining cells and sublines were maintained in RPMI; both were supplemented with 10% FBS,[3] 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. HeLa cells are grown in DMEM.

Immunoblot Analysis. Cellular membrane proteins were prepared, and immunoblotting was performed as described previously using the polyclonal antibody 87405 (14).

Mitoxantrone and Rhodamine Efflux Assays. The efflux assays were based on those described previously (10, 15). Briefly, cells were suspended in complete medium alone (phenol red-free IMEM with 10% FCS), complete medium containing rhodamine 123 (0.5 μg/ml), or mitoxantrone (20 μM) with or without 10 μM FTC and incubated at 37°C in 5% CO_2 for 30 min. FTC has been shown previously to block MXR/BCRP/ABCP-mediated drug transport (13). The cells were then washed with ice-cold complete media and either placed on ice or incubated for 1 h at 37°C in 5% CO_2 in complete media with or without 10 μM FTC. This 1-h incubation allows drug efflux to occur, after which time the remaining drug is quantitated by measuring fluorescence. Previous studies have shown that the histogram obtained after efflux in the presence or absence of FTC is a valid measurement of MXR/BCRP/ABCP function that correlates well with mRNA levels (10). A FACSort flow cytometer with a 488-nm argon laser and 530-nm bandpass filter was used to read the fluorescence of rhodamine, whereas a FACSCalibur flow cytometer equipped

Received 5/1/01; accepted 7/26/01.

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] To whom requests for reprints should addressed, at Cancer Therapeutics Branch, National Cancer Institute, NIH, Building 10, Room 12N226, 9000 Rockville Pike, Bethesda, MD 20892. Phone: (301) 496-4916; Fax: (301) 402-0172; E-mail: sebates@helix.nih.gov.

[2] Internet address: http://www.gene.ucl.ac.uk/users/hester/abc.html.

[3] The abbreviations used are: FBS, fetal bovine serum; FTC, fumitremorgin C.

with a 635-nm red diode laser and 670-nm bandpass filter was used to read the fluorescence of mitoxantrone. Vaccinia virus-infected cells were assayed similarly, except as noted in the legend to Fig. 2.

RNA Isolation, cDNA, and Genomic DNA Sequencing. RNA was isolated from cell lines using RNA STAT-60 (Tel-Test, Inc., Friendswood, TX). Genomic DNA was isolated using the Wizard Plus DNA purification system (Promega, Madison, WI). Sequencing was performed using the ABI PRISM Rhodamine Terminator Cycle Sequencing Ready Reaction kit with an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA).

Vaccinia Virus Expression System. Expression of MXR/BCRP/ABCP by an infection-transfection protocol was performed as described previously (15, 16). Cells were infected with recombinant vaccinia virus encoding bacteriophage T7 RNA polymerase (vTF7-3) and cotransfected with the expression plasmids pTM1-*MXR/BCRP/ABCP* (G482), pTM1-*MXR/BCRP/ABCP* (T482), and pTM1-*MXR/BCRP/ABCP* (R482) containing the human *MXR/BCRP/ABCP* gene variants at amino acid position 482. After 18–20 h, cells were harvested by trypsinization, washed in Opti-MEM containing 5% FBS, and resuspended in Opti-MEM containing 5% FBS at a density of ~3 million cells/ml. pTM1-MXR was constructed by ligating an *Nco*I-*Bam*HI fragment from a TA cloning vector containing the *MXR/BCRP/ABCP* gene variants into the vaccinia virus expression plasmid pTM1.

Cytotoxicity Assays. Cytotoxicity assays were performed using the sulforhodamine method described previously (4). Cells were exposed to mitoxantrone, topotecan, adriamycin, and SN-38 at various concentrations for 96 h. Each concentration was tested in triplicate, and controls were done in replicates of eight.

Results

Disparate Transport of Rhodamine 123 and Mitoxantrone in MXR/BCRP/ABCP-overexpressing Cells. Rhodamine and mitoxantrone accumulation and efflux assays were performed on nine MXR/BCRP/ABCP-overexpressing sublines and two parental cell lines, MCF-7 human breast cancer cells and S1 human colon cancer cells. The assays were performed in the presence or absence of the MXR/BCRP/ABCP inhibitor FTC. All resistant sublines demonstrated reduced mitoxantrone accumulation, whereas only two (MCF-7 AdVp3000 and S1-M1-80) were found to transport rhodamine 123. Six of the nine sublines studied are shown in Fig. 1*A*. When mitoxantrone was the substrate, fluorescence after a period of efflux in the absence of FTC (····) is less than after efflux in the presence of FTC (----). In contrast, when similar studies were performed with rhodamine, reduced fluorescence after a period of efflux was observed only in MCF-7 AdVp3000 and S1-M1-80 cells. In these two cell lines, rhodamine efflux was also inhibited by FTC, as shown by the differences in fluorescence between the two histograms (···· *versus* ----). That the differences in efflux capabilities were not a result of differences in protein levels is shown in the immunoblot in Fig. 1*B*. High protein levels are found in MCF-7 AdVp3000 and S1-M1-80 cells, both of which have reduced rhodamine accumulation, and also in MCF MX100 and MCF FLV1000, which do not. Somewhat lower

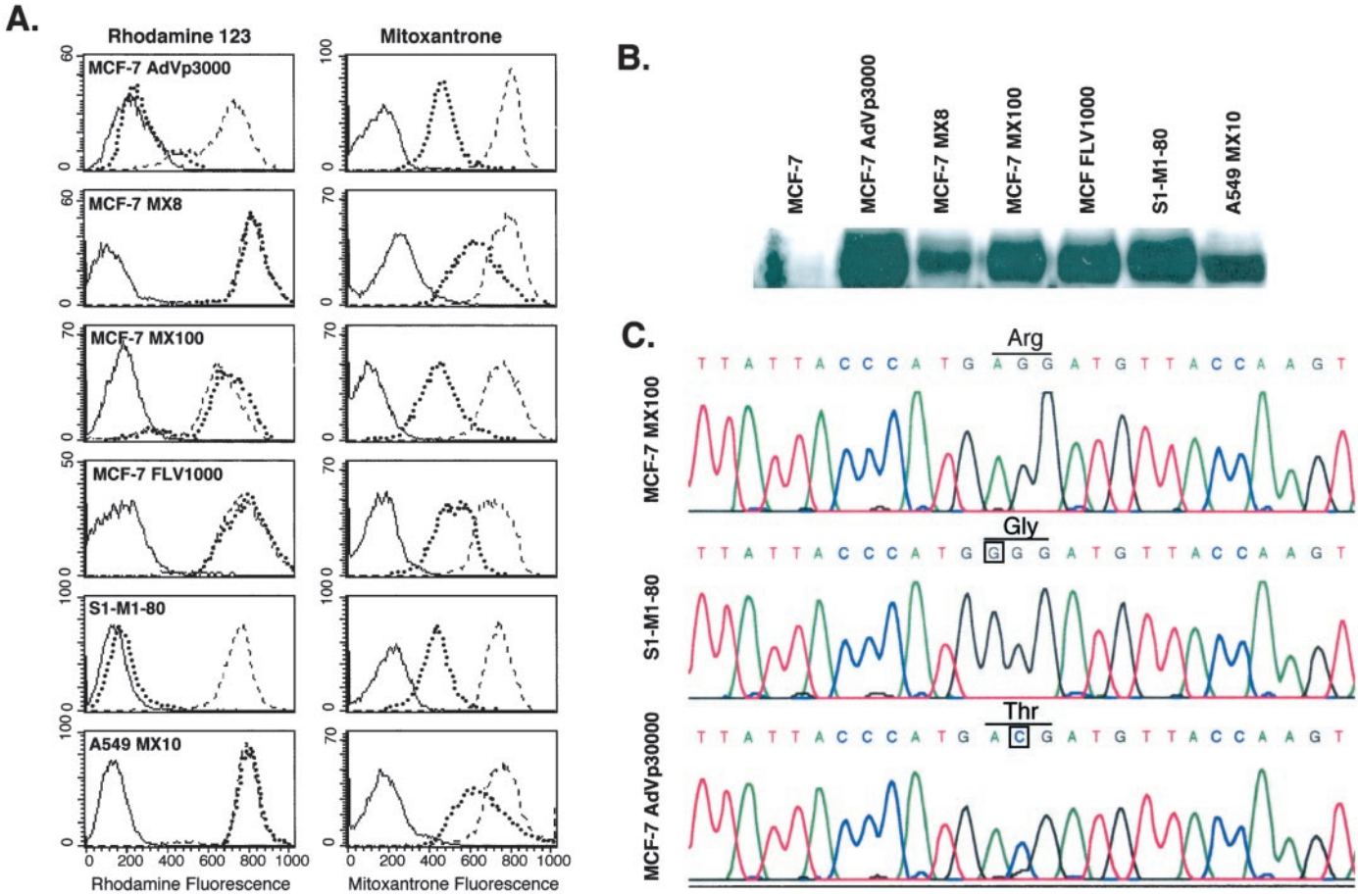

Fig. 1. Characterization of acquired mutations in MXR/BCRP/ABCP-overexpressing cells. *A*, disparate transport of mitoxantrone and rhodamine 123. Cells were incubated for 30 min with media alone (———); incubated with 0.5 μg/ml rhodamine or 20 μM mitoxantrone, washed, and then allowed to incubate for 60 min in media alone (····); or incubated in the presence of 10 μM FTC, with 0.5 μg/ml rhodamine or 20 μM mitoxantrone washed, and then allowed to incubate for 60 min in media containing only 10 μM FTC (----). *B*, Western blot analysis of MXR/BCRP/ABCP-overexpressing cells. Membrane protein from the six cell lines in *A* was incubated with the polyclonal MXR/BCRP/ABCP antibody 87405. *C*, electropherograms for MCF-7 MX100 (R482, *top*), S1-M1-80 (R482G, *middle*), and MCF-7 AdVp3000 (R482T, *bottom*) MXR/BCRP/ABCP forms.

levels were found in MCF-7 MX8 and A549 MX10 cells, consistent with the mitoxantrone efflux results.

To determine the cause of the differing ability of the resistant cells to efflux rhodamine, the MXR/BCRP/ABCP mRNA was sequenced. Differences were noted in residues corresponding to amino acids 12 and 482, and these were confirmed by DNA sequencing. Fig. 1*C* shows the differences at amino acid position 482. Arginine was found in the majority of cell lines, whereas glycine and threonine were found in S1-M1-80 and MCF-7 AdVp3000 cells, respectively, the cell lines that efflux rhodamine. In sequencing genomic DNA, parental MCF-7 and S1 cells were found to have an arginine at position 482, demonstrating that the glycine and threonine at position 482 were acquired mutations. An apparent polymorphism was found at amino acid 12, with alleles encoding either a methionine or a valine; there was no correlation with substrate specificity. A summary of results for all cells is shown in Table 1. Earlier steps of selection for MCF-7 AdVp3000 and MCF-7 FLV1000 were also sequenced. All steps of the FLV1000 selection were noted to be wild type (R482). Early steps of the MCF-7 AdVp selection were found to contain the mutant allele (*R482T*). The three MCF-7 sublines independently selected in mitoxantrone (MCF-7 MX8, MCF-7 MX, and MCF-7 MX100) were all found to contain the wild-type sequence (R482).

Expression of Wild-Type and Mutant MXR/BCRP/ABCP Proteins Using a Transient Vaccinia Virus Expression System. To confirm that the differences in sequence were responsible for the differences in rhodamine 123 and mitoxantrone efflux, HeLa cells were infected-transfected with VTF-7 vaccinia virus and either a control plasmid or one of the three MXR/BCRP/ABCP constructs containing an arginine, glycine, or threonine at position 482. Western blots demonstrated comparable amounts of protein expressed in total cell lysates (data not shown). As seen in Fig. 2*A*, mitoxantrone fluorescence was reduced in cells infected-transfected with any of the three MXR/BCRP/ABCP plasmids compared with HeLa cells infected-transfected with a control plasmid. These data are consistent with reduced intracellular mitoxantrone. In contrast, when these cells were examined for their ability to transport rhodamine 123 (Fig. 2*B*) and doxorubicin (Fig. 2*C*), only cells infected-transfected with either T482 or G482 had reduced rhodamine and doxorubicin fluorescence. Cells infected-transfected with R482 had rhodamine levels similar to those in cells infected-transfected with a control plasmid, corroborating the earlier observations made in the resistant cells. All cells were also found to transport topotecan to a slight degree (data not shown).

Differential Sensitivity to Chemotherapy Drugs. To determine whether the mutations identified in the two drug-resistant cell lines could affect the cross-resistance profile, we compared the sensitivity of the mutant sublines with that of MCF-7 MX100 (R482) to various chemotherapy agents. These results are summarized in Fig. 3. All three cell lines were comparably resistant to mitoxantrone, with relative resistance values differing by only 2- to 4-fold. However, the MCF-7 AdVp3000 cells with the R482T mutation were more resistant to adriamycin than the R482 wild type ($P = 0.001$), whereas S1-M180 cells with the R482G mutation were also more resistant to adriamycin ($P = 0.004$) but appeared to be less resistant to topotecan and SN-38. However, this latter difference did not reach statistical significance. Because no difference in topotecan transport could be detected be-

Table 1 *Amino acid variations in MXR/BCRP/ABCP and corresponding transport phenotype*

Efflux of rhodamine 123 (Rhod) and mitoxantrone (Mitox) assayed by flow cytometry.

Cell line	Amino acid 482	Rhod	Mitox
S1-M1-80	G	+++	+++
MCF AdVp3000	T	+++	+++
MCF-7/MX	R	−	+++
MCF MX100	R	−	+++
MCF FLV1000	R	−	+++
MCF MX8	R	−	++
A549 MX10	R	−	++
H460 MX10	R	−	++
SF295 MX10	R	−	++
MCF-7	R	−	+
S1	R	−	−

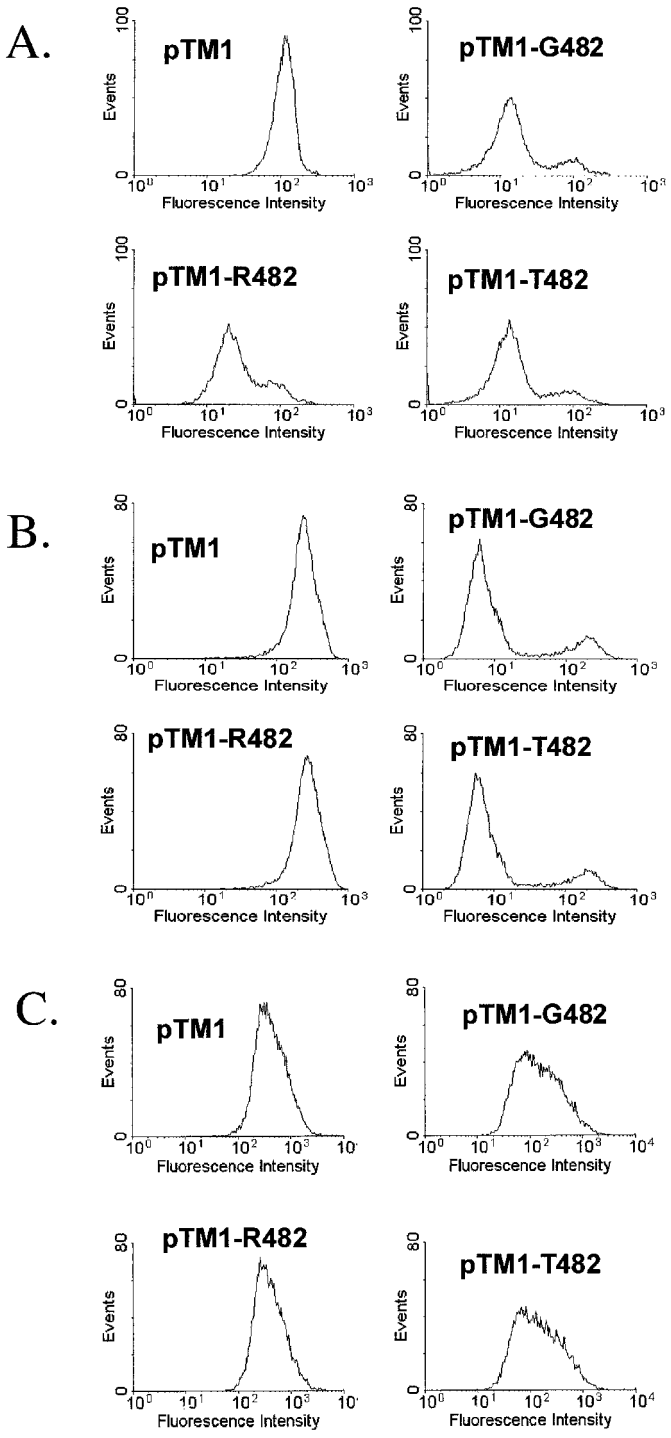

Fig. 2. Functional expression of MXR/BCRP/ABCP in transiently infected-transfected HeLa cells. HeLa cells were infected with vTF7-3 and transfected with pTM1-G482, pTM1-R482, or pTM1-T482 for 18 h. Mitoxantrone (20 μM) accumulation (*A*), rhodamine (0.5 μg/ml) accumulation (*B*), and doxorubicin (3 μM) accumulation (*C*) were determined in these cells after a 40-min incubation at 37°C in the presence of fluorescent substrate followed by a 20-min (mitoxantrone samples) or 40-min (rhodamine and doxorubicin samples) incubation in substrate-free media at 37°C. vTF7-3 infected cells transfected with pTM1 vector DNA were included as a negative control.

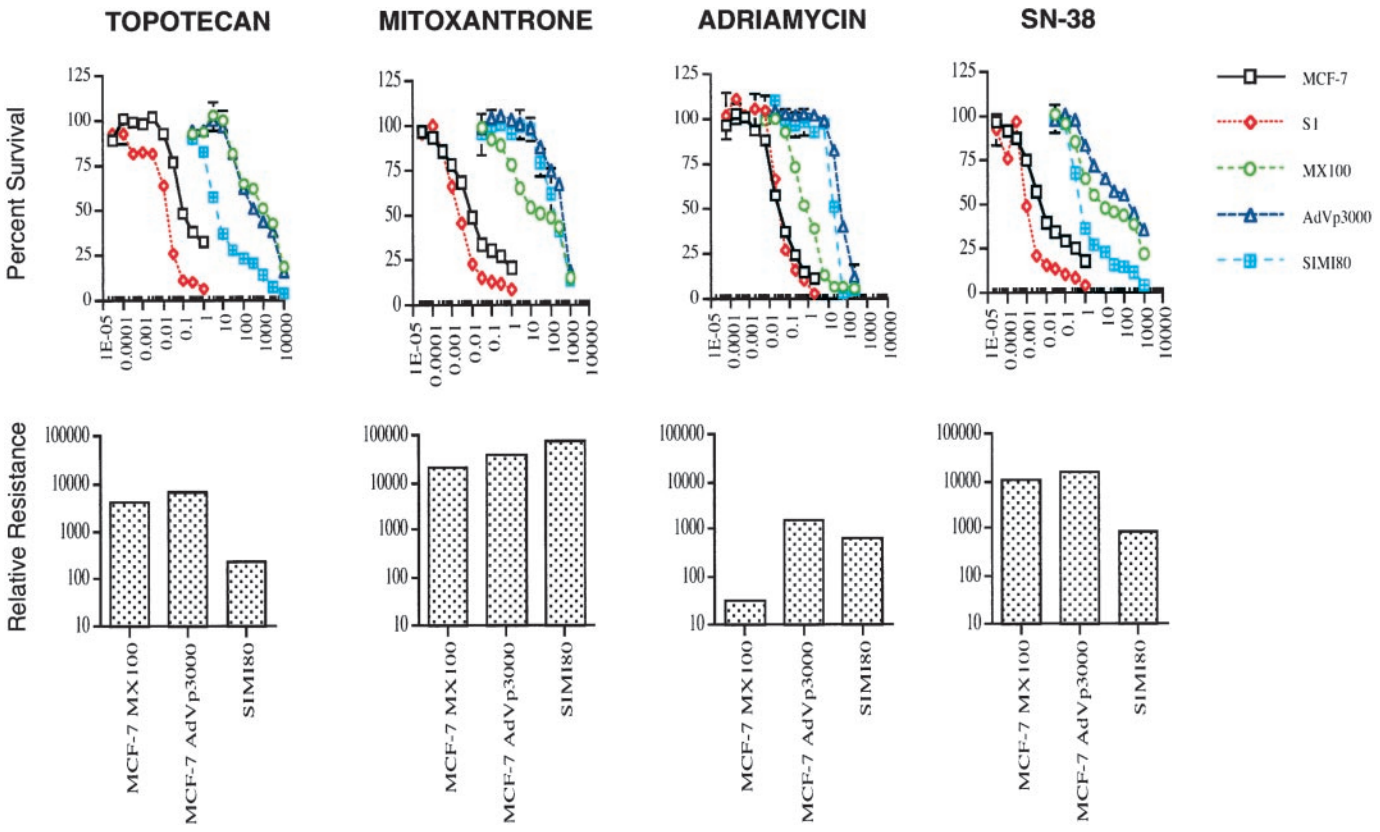

Fig. 3. Differential sensitivity of cell lines overexpressing wild-type and mutant MXR/BCRP/ABCP forms. Four-day cytotoxicity assays were performed on the MCF-7 MX100 (R482), MCF-7 AdVp3000 (R482T), and S1-M1-80 (R482G) cells with topotecan, mitoxantrone, adriamycin, and SN-38. Results of the cytotoxicity assay are shown in the *top row*, with the relative resistance of the cell lines given in the *bottom row*. Relative resistance values were obtained by dividing the IC_{50} value of the resistant subline by the IC_{50} value of the parent cell line and were calculated from the average IC_{50} obtained from three separate experiments performed in quadruplicate. Using an unpaired Student's *t* test, *P*s were obtained to evaluate significant differences between cell lines containing the mutation and the wild-type MXR/BCRP/ABCP forms. Only differences with adriamycin were statistically significant.

tween the three MXR forms in infected-transfected HeLa cells, it is possible that these trends may be attributable to mechanisms other than or related to MXR.

Discussion

This manuscript presents the characterization of two mutations in the *MXR/BCRP/ABCP* gene that were acquired during the course of drug selection and are shown to be responsible for differential drug efflux and sensitivity; this is one of the first examples of an ABC transporter exhibiting a gain of function mutation upon selection. Drug-resistant sublines overexpressing wild-type MXR/BCRP/ABCP with an arginine at position 482 can efflux mitoxantrone but not rhodamine or doxorubicin and have high resistance to topotecan and SN-38. The absence of rhodamine and doxorubicin efflux is a sharp contrast from the phenotype observed in S1-M1-80 cells, with an R482G mutation, and in MCF-7 AdVp cells with an R482T mutation. These latter two drug-resistant sublines efflux mitoxantrone, rhodamine, and doxorubicin. Cross-resistance studies with cell lines overexpressing the wild type and the two mutant genes found differences in cross-resistance profiles. Although the presence of other transporters or other mechanisms of resistance could explain these differences, amino acid 482 appears to be important in the function of the MXR/BCRP/ABCP protein.

When transmembrane domains for MXR/BCRP/ABCP were identified using the TMpred program, the third transmembrane domain was predicted to span amino acids 483–499 for wild-type MXR/BCRP/ABCP (R482) but was predicted to span amino acids 478–497 for the R482G mutation and 478–499 for the R482T mutation. Thus, the observed differences in drug efflux and resistance may result from a change in the size and/or conformation of the third transmembrane domain. This in turn may result in disruption of the interactions with other transmembrane domains. This view envisions the changes in amino acid 482 as resulting in structural changes beyond its immediate vicinity and considers the transmembrane domain to be adaptable. Alternately, residue 482 may be the site of drug interaction, and mutations at this site may result in altered affinity for different drugs. Although a dimerization partner has not been identified for MXR/BCRP/ABCP, another possibility is that the mutation impacts on the dimerization or oligomerization process.

Mutations in other half-transporters are also known to alter substrate presentation. The human *MXR/BCRP/ABCP* gene is related to the *Drosophila white, brown,* and *scarlet* genes, which encode transporters for eye pigment precursors (1, 3, 17). Mutations in the *white* gene have been described that significantly impair guanine transport by the White-Brown dimer (I581 del, G589E, and F590G), leaving intact tryptophan transport by the White-Scarlet dimer (6, 18). Both G589E and F590G are located near the extracellular surface of transmembrane segment five.

Spontaneous mutations are also known to alter the resistance profile of other drug transporters. Colchicine-selected KB cells were found to overexpress a Pgp that was preferentially resistant to colchicine because of a G185V mutation. Amino acid 185 is predicted to be within the first hydrophobic domain of Pgp and may be at the site of drug binding (11). Additionally, Loo and Clarke (19) have made multiple substitutions in various transmembrane segments, affecting substrate specificity. Some of these mutations altered the interaction of antag-

onists with Pgp, affecting drug resistance reversal. A similar situation could be envisioned for the mutations described herein. Studies are currently underway to determine whether these mutations can affect the action of MXR/BCRP/ABCP inhibitors.

The results obtained with the vaccinia virus expression system also suggest that MXR/BCRP/ABCP may function as a homodimer or multimer because expression of the half-transporter alone yields a functional protein. This confirms results obtained with the BCRP cDNA in stable transfectants (3). Results with the vaccinia virus also imply that the transporter is localized to the cell surface, as reported for drug-selected lines (20, 21). Both of these interpretations remain to be substantiated by experimental data.

In summary, we describe two mutations in the *MXR/BCRP/ABCP* gene acquired during the course of drug selection. Cells with these mutations have different cross-resistance profiles and categorical differences in rhodamine efflux, confirmed in a vaccinia virus transient expression system. Amino acid 482 may be part of the drug-binding site, or, more likely, mutations in this residue may lead to changes in the third transmembrane domain, resulting in structural alterations and consequent changes in substrate specificity.

References

1. Miyake, K., Mickley, L., Litman, T., Zhan, Z., Robey, R., Cristensen, B., Brangi, M., Greenberger, L., Dean, M., Fojo, T., and Bates, S. E. Molecular cloning of cDNAs which are highly overexpressed in mitoxantrone-resistant cells: demonstration of homology to ABC transport genes. Cancer Res., *59:* 8–13, 1999.
2. Yang, C. H., Schneider, E., Kuo, M. L., Volk, E. L., Rocchi, E., and Chen, Y. C. BCRP/MXR/ABCP expression in topotecan-resistant human breast carcinoma cells. Biochem. Pharmacol., *60:* 831–837, 2000.
3. Doyle, L. A., Yang, W., Abruzzo, L. V., Krogmann, T., Gao, Y., Rishi, A. K., and Ross, D. D. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc. Natl. Acad. Sci. USA, *95:* 15665–15670, 1998.
4. Brangi, M., Litman, T., Ciotti, M., Nishiyama, K., Kohlhagen, G., Takimoto, C., Robey, R., Pommier, Y., Fojo, T., and Bates, S. E. Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Res., *59:* 5938–5946, 1999.
5. Robey, R. W., Medina-Perez, W. Y., Nishiyama, K., Lahusen, T., Miyake, K., Litman, T., Senderowicz, A. M., Ross, D. D., and Bates, S. E. Overexpression of the ATP-binding cassette half-transporter, ABCG2 (MXR/BCRP/ABCP1), in flavopiridol-resistant human breast cancer cells. Clin. Cancer Res., *7:* 145–152, 2001.
6. Ewart, G. D., and Howells, A. J. ABC transporters involved in transport of eye pigment precursors in *Drosophila melanogaster*. *In:* S. Ambudkar and M. M. Gottesman (eds.), Methods in Enzymology, Vol. 292, pp. 213–224. San Diego, CA: Academic Press, Inc., 1998.
7. Berge, K. E., Tian, H., Graf, G. A., Yu, L., Grishin, N. V., Schultz, J., Kwiterovich, P., Shan, B., Barnes, R., and Hobbs, H. H. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science (Wash. DC), *290:* 1771–1775, 2000.
8. Lee, M. H., Lu, K., Hazard, S., Yu, H., Shulenin, S., Hidaka, H., Kojima, H., Allikmets, R., Sakuma, N., Pegoraro, R., Srivastava, A. K., Salen, G., Dean, M., and Patel, S. B. Identification of a gene, *ABCG5*, important in the regulation of dietary cholesterol absorption. Nat. Genet., *27:* 79–83, 2001.
9. Jonker, J. W., Smit, J. W., Brinkhuis, R. F., Maliepaard, M., Beijnen, J. H., Schellens, J. H., and Schinkel, A. H. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. J. Natl. Cancer Inst. (Bethesda), *92:* 1651–1656, 2000.
10. Robey, R. W., Honjo, Y., van de Laar, A., Miyake, K., Regis, J. T., Litman, T., and Bates, S. E. A functional assay for detection of the mitoxantrone resistance protein, MXR (ABCG2). Biochim. Biophys. Acta, *1512:* 171–182, 2001.
11. Choi, K., Chen, C. J., Kriegler, M., and Roninson, I. B. An altered pattern of cross-resistance in multidrug resistant human cells results from spontaneous mutations in the *mdr-1* (P-glycoprotein) gene. Cell, *53:* 519–529, 1988.
12. Yang, C. J., Horton, J. K., Cowan, K. H., and Schneider, E. Cross-resistance to camptothecin analogues in a mitoxantrone-resistant human breast carcinoma cell line is not due to DNA topoisomerase I alterations. Cancer Res., *55:* 4004–4009, 1995.
13. Rabindran, S. K., He, H., Singh, M., Brown, E., Collins, K. I., Annable, T., and Greenberger, L. M. Reversal of a novel multidrug resistance mechanism in human colon carcinoma cells by fumitremorgin C. Cancer Res., *58:* 5850–5858, 1998.
14. Litman, T., Brangi, M., Hudson, E., Fetsch, P., Abati, A., Ross, D. D., Miyake, K., Resau, J. H., and Bates, S. E. The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). J. Cell Sci., *113:* 2011–2021, 2000.
15. Hrycyna, C. A., Ramachandra, M., Pastan, I., and Gottesman, M. M. Functional expression of human P-glycoprotein from plasmids using vaccinia virus-bacteriophage T7 RNA polymerase system. Methods Enzymol., *292:* 456–473, 1998.
16. Hrycyna, C. A., Airan, L. E., Germann, U. A., Ambudkar, S. V., Pastan, I., and Gottesman, M. M. Structural flexibility of the linker region of human P-glycoprotein permits ATP hydrolysis and drug transport. Biochemistry, *37:* 13660–13673, 1998.
17. Allikmets, R., Schriml, L. M., Hutchinson, A., Romano-Spica, V., and Dean, M. A human placenta-specific ATP-binding cassette gene (ABCP) on chromosome 4q22 that is involved in multidrug resistance. Cancer Res., *58:* 5337–5339, 1998.
18. Mackenzie, S. M., Brooker, M. R., Gill, T. R., Cox, G. B., Howells, A. J., and Ewart, G. D. Mutations in the white gene of *Drosophila melanogaster* affecting ABC transporters that determine eye colouration. Biochim. Biophys. Acta, *1419:* 173–185, 1999.
19. Loo, T. W., and Clarke, D. M. Determining the structure and mechanism of the human multidrug resistance P-glycoprotein using cysteine-scanning mutagenesis and thiol-modification techniques. Biochim. Biophys. Acta, *1461:* 315–325, 1999.
20. Rocchi, E., Khodjakov, A., Volk, E. L., Yang, C. H., Litman, T., Bates, S. E., and Schneider, E. The product of the ABC half-transporter gene *ABCG2* (BCRP/MXR/ABCP) is expressed in the plasma membrane. Biochem. Biophys. Res. Commun., *271:* 42–46, 2000.
21. Scheffer, G. L., Maliepaard. M., Pijnenborg, A. C. L. M., van Gastelen, M. A., de Jong, M. C., Schroeijers, A. B., van der Kolk, D. M., Allen, J. D., Ross, D. D., van der Valk, P., Dalton, W. S., Schellens, J. H. M., and Scheper, R. J. Breast cancer resistance protein is localized at the plasma membrane in mitoxantrone- and topotecan-resistant cell lines. Cancer Res., *60:* 2589–2593, 2000.

Acquired Mutations in the *MXR/BCRP/ABCP* Gene Alter Substrate Specificity in MXR/BCRP/ABCP-overexpressing Cells

Yasumasa Honjo, Christine A. Hrycyna, Qing-Wu Yan, et al.

Cancer Res 2001;61:6635-6639.