

A Family of Drug Transporters: the Multidrug Resistance-Associated Proteins

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The human multidrug resistance-associated protein (MRP) family currently has seven members. The ability of several of these membrane proteins to transport a wide range of anticancer drugs out of cells and their presence in many tumors make them prime suspects in unexplained cases of drug resistance, although proof that they contribute to clinical drug resistance is still lacking. Recent studies have begun to clarify the function of the MRP family members. MRPs are organic anion transporters; i.e., they transport anionic drugs, exemplified by methotrexate, and neutral drugs conjugated to acidic ligands, such as glutathione (GSH), glucuronate, or sulfate. However, MRP1, MRP2, and MRP3 can also cause resistance to neutral organic drugs that are not known to be conjugated to acidic ligands by transporting these drugs together with free GSH. MRP1 can even confer resistance to arsenite and MRP2 to cisplatin, again probably by transporting these compounds in complexes with GSH. MRP4 overexpression is associated with high-level resistance to the nucleoside analogues 9-(2-phosphonyl-methoxyethyl) adenine and azidothymidine, both of which are used as anti-human immunodeficiency virus drugs. MRPs may, therefore, also have a role in resistance against nucleoside analogues used in cancer chemotherapy. Mice without *Mrp1*, a high-affinity leukotriene C_4 transporter, have an altered response to inflammatory stimuli but are otherwise healthy and fertile. MRP2 is the major transporter responsible for the secretion of bilirubin glucuronides into bile, and humans without MRP2 develop a mild liver disease known as the Dubin-Johnson syndrome. The physiologic functions of the other MRPs are not known. Whether long-term inhibition of MRPs in humans can be tolerated (assuming that suitable inhibitors will be found) remains to be determined. [J Natl Cancer Inst 2000;92:1295-302]

Drug pumps are an important part of the defense of cells against carcinostatic drugs. This was first realized by the Danish physician Danø (1), who demonstrated in 1973 that multidrug-resistant (MDR) Ehrlich ascites cells were able to lower their intracellular daunorubicin concentration by active drug extrusion. In 1976, Juliano and Ling (2) discovered a large glycoprotein in the plasma membrane of MDR cells, the P-glycoprotein (P-gp), that looked like a good candidate pump. It took a long time, however, before most scientists in the field were convinced that P-gp was a so-called "primary active" drug pump, i.e., a protein able to bind and transport drugs against a drug concentration gradient and at the expense of adenosine triphosphate (ATP) hydrolysis (3,4).

In 1992, Cole et al. (5) discovered a second type of drug pump in MDR cancer cells, the multidrug resistance-associated protein (MRP). While the human genome encodes only two

P-gp's (6) [and a distant relative of P-gp, the bile salt transporter (7)], it is shown to contain many more genes related to MRP (8,9).

This MRP family is the subject of this review. We will focus on the ability of MRPs to transport anticancer drugs and the possible contribution of MRPs to drug resistance in patients. Other aspects of MRPs have been summarized in depth in recent reviews and review volumes that deal with multidrug resistance (10-12) or with ABC transporters (ATP-binding cassette transporters) in general (13,14).

OVERVIEW OF THE MEMBERS OF THE MRP FAMILY

Since several laboratories have contributed to the characterization of the MRP family, individual members may have multiple names (Table 1). MRP7 is a recent addition to the family and has not yet been characterized. The six MRPs studied thus far fall into one of two groups (Table 2 and Fig. 1). MRP1, MRP2, MRP3, and MRP6 all have the extra N-terminal domain (indicated as TMD₀ in Fig. 1) that is lacking in P-gp. The percent identity of MRP4 and MRP5 with MRP1 is below 40%; these two MRPs are also smaller than MRP1, and they appear to lack the TMD₀ domain. Nevertheless, MRP4 and MRP5 are much more homologous to the other MRPs than to P-gp or other classes of ABC transporters. Moreover, investigators (15) have shown that the TMD₀ part of MRP1 is not required for transport activity. The essential L₀ part of MRP1 (see Fig. 1) is, however, conserved in the long N-terminal intracellular parts of MRP4 and MRP5 (15,16).

Table 3 summarizes the tissue distribution of MRPs. It also includes what is known about the physiologic functions of these transporters (MRP1 and MRP2 only).

MRP1: TRANSPORTER OF A REMARKABLE RANGE OF DRUGS

The substrate specificity of MRP1 initially seemed to be similar to that of P-gp, as shown in Table 4, which compares the drug resistance profile of transfected cells overexpressing MDR1 P-gp or MRP1. Although MRP1 transports paclitaxel relatively poorly, other differences with P-gp initially seemed minor. Subsequent work showed, however, that the preferred substrates for MRP1 are organic anions (17-19), e.g., drugs conjugated to glutathione (GSH), glucuronate, or sulfate (10,11,20), whereas

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Table 1. The human multidrug resistance protein (MRP) family and some of the alternative names used in the literature for individual family members

Members	Other names used*
MRP1	ABCC1 and MRP
MRP2	ABCC2, cMOAT, and cMRP
MRP3	ABCC3, MOAT-D, and cMOAT-2
MRP4	ABCC4 and MOAT-B
MRP5	ABCC5, MOAT-C, and pABC11
MRP6	ABCC6, MOAT-E, MLP-1, and ARA†
MRP7	ABCC10

*The ABC (adenosine triphosphate-binding cassette) nomenclature comes from the ground-breaking paper by Allikmets et al. (8) and refers to ABC transporters; MOAT stands for multispecific organic anion transporter and comes from cMOAT, the canalicular MOAT (23). The pABC11 designation is from McAleer et al. (48). ABCC refers to the C group of ABC transporters; MLP-1 stands for MRP-like protein 1; ARA stands for anthracycline resistance associated. See Cole (86) for recent discussion about MRP nomenclature and the home page of Michaël Müller for a complete and up-to-date overview of ABC transporters (<http://www.med.rug.nl/Mdlhumanabc.htm>).

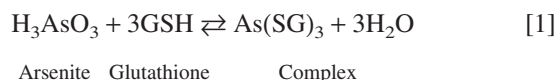
†Only 3' end sequence of the MRP6 gene.

Table 2. Percent amino acid identity between fully sequenced human multidrug resistance proteins (MRPs)*

	MRP1 1531 aa	MRP2 1545 aa	MRP3 1527 aa	MRP4 1325 aa	MRP5 1437 aa	MRP6 1503aa
MRP1	100					
MRP2	49	10				
		0				
MRP3	58	48	10			
			0			
MRP4	39	37	36	100		
MRP5	34	35	33	36	100	
MRP6	45	38	43	34	31	100

*Homology between human MRPs, expressed as percent amino acid (aa) identity; from (12). For the multiple sequence alignment the PILEUP program from the University of Wisconsin Genetics Group (GCG) package (version 9.1) was used. The following accession numbers were used: MRP1, L05628; MRP2, U49248; MRP3, AF009670; MRP4, AF071202; MRP5, AF104942; and MRP6, AF076622.

P-gp has a low affinity for such negatively charged compounds. In fact, MRP1 is one of the elusive glutathione-S-conjugate (GS-X) pumps (21), a transporter able to transport drugs conjugated to GSH out of the cell. This explains the ability of MRP1 to transport substrates, such as methotrexate (MTX) or arsenite (H_3AsO_3). MTX is an organic anion; H_3AsO_3 can form a complex with three GSH molecules as shown in equation 1:



Moreover, it is presumably this complex that is transported by MRP1, as indicated by the ability of H_3AsO_3 to induce increased GSH export from cells with elevated levels of MRP1 (22).

MRP2: TRANSPORTER OF ANTICANCER DRUGS

Long before MRP1 was discovered, biochemical and genetic studies (11,23,24) had demonstrated the presence of an organic anion transporter in the canalicular membrane of hepatocytes. This transporter was originally known as the canalicular multispecific organic anion transporter (cMOAT), but it is now called MRP2. Its substrate specificity was defined in detail with

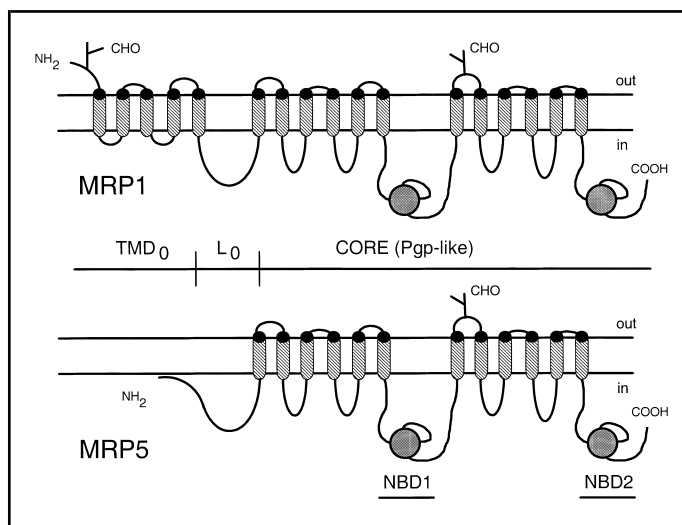


Fig. 1. Two-dimensional membrane topology models for P-glycoprotein (Pgp), MRP1, and MRP5. MRP1 is characterized by the presence of an extra N-terminal domain (TMD₀), which is absent in P-glycoprotein or MRP5. NBD = nucleotide-binding domain. Note that this figure presents highly schematic models only indicating the trans-membrane segments, adenosine triphosphate-binding sequences, and the location of carbohydrate (CHO) chains. In reality, the trans-membrane segments probably come together in the membrane to form a kind of pore closed off at the exoplasmic phase, as suggested for P-glycoprotein (87,88). L₀ = linker domain zero.

Table 3. Tissue distribution and physiologic functions of human multidrug resistance proteins (MRPs)

	Main location in body	Major physiologic function
MRP1	Ubiquitous (low in liver)	Major leukotriene C ₄ transporter
MRP2	Liver, kidney, and gut	Major transporter of bilirubin glucuronides and other organic anions from liver into bile
MRP3	Liver, adrenals, pancreas, kidney, and gut	?
MRP4	Prostate, lung, muscle, pancreas, testis, ovary, bladder, and gallbladder	?
MRP5	Ubiquitous	?
MRP6	Liver and kidney	?

the help of a rat strain (TR⁻/GY) lacking cMOAT. These TR⁻/GY rats are mainly deficient in bilirubin-glucuronide secretion, and they are now known to contain inactivating mutations in their cMOAT/MRP2 gene (25,26), just like their human counterparts, i.e., patients with the Dubin-Johnson syndrome (27,28).

Because MRP2 was known to handle a similar range of GSH conjugates as MRP1, it was to be expected that MRP2 would also be able to transport the carcinostatic agents transported by MRP1. This expectation is borne out by recent experiments with transfected cells. Introduction of an antisense construct containing a long segment of DNA complementary to MRP2 RNA into cultured liver HepG2 cells enhanced the sensitivity of these cells to cisplatin, vincristine, doxorubicin, and the camptothecin derivatives CPT11 and SN38 but not to etoposide (29). MRP2 was also shown to mediate vinblastine transport in polarized cells (30). In transfected cells, overexpression of MRP2 resulted in resistance to MTX (31), cisplatin, etoposide, doxorubicin, epirubicin (32), and mitoxantrone (unpublished results from our laboratory). This list is not yet complete because it has been

Table 4. Comparison of drug resistance profiles in cells with elevated levels of human multidrug resistance proteins MRP1 or MDR1 P-glycoprotein*

Drug	HeLa (MRP1)	2008 (MRP1)	S1 (MRP1)	S1 (MDR1)
Doxorubicin	6	8	3	4
Daunorubicin	5	6	3	4
Epirubicin	9	ND	3	3
Idarubicin	ND	ND	3	2
Mitoxantrone	1	7	2	2
Bisantrene	ND	ND	1	200
Vincristine	8	5	5	16
Vinblastine	4	2	2	10
Etoposide	12	19	5	3
Paclitaxel	2	1	1	7
Melphalan	ND	ND	1	1
Cisplatin	1	1	1	1
Arsenite	4	1	1	1
Trivalent antimony	5	2	2	1

*The resistance of the MRP1- or MDR1-overexpressing cells relative to the parental cells (expressed as relative resistance factor) was determined in clonogenic survival assays (S1 cells) or growth inhibition assays (HeLa and 2008 cells). The HeLa cell results are from (89); the human ovarian carcinoma 2008 cell line results are unpublished data from M. Kool and P. Borst; and the SW1573 (S1) human lung carcinoma results are from (90). ND = not done.

difficult to get transfected cells in which the MRP2 is routed efficiently to the plasma membrane (12,32). The spectrum of drug resistance induced in cells by MRP2 may eventually turn out to be similar to that shown for MRP1 (Table 4) with one exception: MRP2 induces cisplatin resistance (9,32,33), which has never been seen in cells overexpressing MRP1.

MRP1 AND MRP2: TRANSPORT OF VINCA ALKALOIDS AND ANTHRACYCLINES

Vinca alkaloids and anthracyclines are weak organic bases and are not known to be conjugated to acidic ligands in human cells. It is, therefore, puzzling that elevated levels of MRP1 or MRP2 result in resistance to these compounds. GSH is required for resistance, however. Depletion of cellular GSH abolished MRP1-mediated resistance against vinca alkaloids and anthracyclines (22,34); moreover, in vesicular transport experiments, transport of vincristine or daunorubicin occurred only in the presence of reduced GSH (35–38). The drugs are probably cotransported with GSH (36–38). In polarized kidney cells transfected with an MRP2 construct, the increased transport of vinblastine is associated with a stoichiometrically increased export of GSH (unpublished data from our laboratory). It, therefore, looks as if drug resistance mediated by MRP1 or MRP2 requires a continuing supply of GSH to allow export of unconjugated drug, as indicated in Fig. 2. Indeed, there is often a simultaneous increase in expression of MRP1 and gamma-glutamylcysteine synthetase in tumor cells (39,40).

MRP3

Recent work has shown that MRP3 is also an organic anion transporter (41,42); however, unlike MRP1 and MRP2, it prefers glucuronate conjugates as substrates over GSH conjugates (41). It has been difficult to get transfected, nonpolarized cells with high levels of MRP3; cells with low amounts of MRP3 are resistant to etoposide and teniposide but not to other drugs affected by multidrug resistance. Resistance to short-term exposure to MTX also has been observed in these cells (42), which is in agreement with the observation that MRP3 can transport

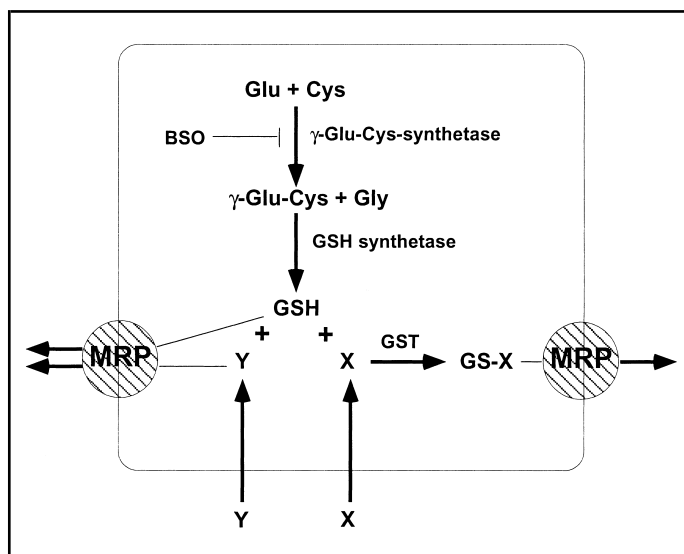


Fig. 2. Model showing interrelation between multidrug resistance-associated protein (MRP) and glutathione (GSH). Some drugs (X) can be conjugated to GSH by glutathione S-transferase (GST) and are then transported by MRP. Other drugs (Y) are cotransported with GSH. In both cases, drug transport is dependent on the continued synthesis of GSH, which can be blocked by DL-buthionine (S, R)-sulfoximine (BSO). Note that some compounds are turned into MRP substrates by conjugation to glucuronate or sulfate, whereas some other substrates are organic anions and do not require conjugation.

MTX in vesicular transport experiments (41). In view of the technical problems with transfected cells, the true range of resistance that can be induced by overexpression of MRP3 obviously remains to be sorted out.

The physiologic function of MRP3 remains to be established. The massive increase in the expression of MRP3 seen in the liver of cholestatic rats (43,44) and humans, quoted in (11,42), suggests that MRP3, located in the basolateral membrane of the hepatocyte (42,45), may allow efflux of organic anions from the liver into the blood when secretion into bile is blocked. A role for MRP3 in the normal uptake of bile salts from the gut has also been postulated. Why MRP3 expression is high in the adrenal cortex (42) remains to be determined.

MRP4, A NUCLEOTIDE ANALOGUE PUMP

Schuetz et al. (46) recently made the interesting discovery that MRP4 can function as a cellular efflux pump for the anti-human immunodeficiency virus drugs 9-(2-phosphonyl-methoxyethyl)adenine (PMEA) and azidothymidine monophosphate (AZTMP) in PME-resistant cells. High levels of MRP4 severely impaired the antiviral efficacy of several nucleoside analogues. MRP4 can also confer resistance against 9-(2-phosphonylmethoxyethyl)guanine, a compound with some anti-neoplastic activity. In view of the results obtained with MRP5 (see below), it seems likely that MRP4 may also be able to cause resistance to anticancer base analogues (e.g., 6-mercaptopurine and thioguanine), but this hypothesis has not yet been tested.

PMEA and AZTMP are organic anions; therefore, MRP4 can be considered to be an organic anion transporter, as is expected for an MRP family member. MRP4 might, however, be specific for phosphate conjugates, and it remains to be seen whether MRP4 can also transport GSH, glucuronide, or sulfate conjugates. The physiologic function of MRP4 is not known.

MRP5

Our work with transfected cells shows that MRP5 is an organic anion transporter of GSH conjugates and that MRP5 can be inhibited by typical organic anion transport inhibitors like sulfinpyrazone and benzbromarone but not by probenecid (47).

McAleer et al. (48) also found decreased accumulation of anionic fluorochromes in MRP5-transfected cells. It is interesting that we found that MRP5 overexpression results in low-level resistance to thiopurines (e.g., 6-mercaptopurine and thioguanine), as well as PMEA, but no notable resistance to other anticancer drugs tested (e.g., anthracyclines, vinca alkaloids, podophyllotoxins, or MTX). The transfected cells tend to accumulate less 6-mercaptopurine and PMEA and extrude increased amounts of 6-thioinosinemonophosphate and PMEA from the cell. Like MRP4, MRP5, therefore, appears to be a nucleotide analogue pump.

McAleer et al. (48) found that cells transfected with an MRP5 gene construct are resistant to heavy metals (e.g., cadmium chloride and potassium antimonyl tartrate). We did not detect such a resistance in our cells overproducing MRP5. Like MRP2, however, MRP5 does not route efficiently to the plasma membrane in nonpolarized cells, and most of the protein remains in intracellular membranes. More work is, therefore, required to determine the full range of drug resistance that can be caused by MRP5. Since 6-mercaptopurine and thioguanine are used in the treatment of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), it will be interesting to look at MRP5 and MRP4 in 6-mercaptopurine- or thioguanine-resistant ALLs or AMLs in which resistance cannot be attributed to mutations in the hypoxanthine phosphoribosyl transferase gene.

The physiologic function of MRP5 remains to be determined. Mice homozygous for a disrupted *Mrp5* gene are viable and healthy at least up to 1 year (47).

MRP6

Both the physiologic function and the potential involvement of MRP6 in drug resistance are still unclear. It has been shown recently (49,50) that the 3' end of the MRP6 protein is almost identical to the anthracycline resistance-associated (ARA) protein identified previously in epirubicin-selected leukemia cells (51–53). MRP6 is highly expressed in the liver and kidneys and to a low extent in a few other tissues (49). Overexpression and amplification of the complete or partial MRP6 gene in resistant tumor cells were found only in cell lines with high overexpression and amplification of the MRP1 gene. It seems likely that MRP6 does not play a role in the resistance of these cells and that MRP6, or part of it (ARA), is only coamplified with MRP1 because of its location immediately next to it on chromosome 16 (49).¹

INHIBITORS OF MRPs

The potential involvement of drug pumps in clinical drug resistance has led to a search for compounds that can be used to inhibit these transporters in cancer patients. In the case of MDR1 P-gp, this search has been successful. Examples of effective inhibitors are the nonimmunosuppressive cyclosporin A analogue PSC833 and the carboxamide derivative GG918 [reviewed in (54–56)]. Attempts to find inhibitors for MRPs have concentrated mainly on MRP1 and MRP2. Compounds that efficiently block MDR1 P-gp only have a low affinity for MRP1

or MRP2 (57); therefore, it is unlikely that these compounds will be useful for inhibiting MRPs in human cancer. Most high-affinity substrates for MRP1 and MRP2 are organic anions with a substantial hydrophobic moiety and at least one, but preferably two, negative charge(s). Examples of potent competitive inhibitors are high-affinity substrates, such as leukotriene C₄, S-decylglutathione, and the leukotriene D₄ antagonist MK571 (35,58). Other inhibitors for MRP1 are organic acids that were originally developed to inhibit transport of uric acid, like sulfinpyrazone, benzbromarone, and probenecid [see, for example, Holló et al. (59)]. Although MRP1 and MRP2 have a similar substrate specificity, inhibitors for MRP1 are not necessarily good inhibitors for MRP2. Sulfinpyrazone, for instance, does not inhibit transport of the model substrate dinitrophenyl S-glutathione by MRP2 (30).

Negatively charged compounds do not readily enter cells. They, therefore, do not provide obvious lead compounds for drug development. Good inhibitors probably have to be made as prodrugs in which the charged moiety is shielded.

MRPs AND PROTECTION OF NORMAL TISSUES AGAINST ANTICANCER DRUGS

The contribution of drug transporters to the protection of normal mammalian tissues has been investigated by disrupting the genes for these transporters in mice, resulting in “knockout,” null, or (–/–) mice. Experiments on *Mdr1a/b* (–/–) mice have shown that P-gp plays a major role in normal drug handling (60). The location of P-gp in the gut epithelium helps to prevent entry of drugs into the body (61); its location in renal tubules and in the canalicular membrane of the hepatocytes helps to clear drugs from the body; and its presence in strategic locations in brain (62), testis, and placenta helps to protect these organs and the fetus against drugs (63).

P-gp is invariably located in the apical membrane of epithelial cells, in the appropriate position for its protective role. MRP1, in contrast, is located basolaterally (64) and, therefore, tends to pump drugs into the body. Indeed, no decreased disposal of drugs has been observed in *Mrp1* (–/–) mice (65–67). Nevertheless, these mice are hypersensitive to etoposide (65), and we have found that MRP1 has nonredundant protective functions against etoposide in the bone marrow, the epithelium of the oropharynx, the testicular tubules, and the urinary-collecting duct cells (68).

Especially interesting is the protection of the testicular tubules. As illustrated in Fig. 3, the basolateral location of *Mrp1* in the Sertoli cells allows this pump to protect the contents of the testicular tubules, the germline cells, against drug damage. A similar situation appears to exist in the choroid plexus, where many substances enter the cerebrospinal fluid from the epithelial cells covering the plexus. The high level of *Mrp1* in these cells (69,70) plays a crucial role in preventing the entry of a drug, such as etoposide, into the cerebrospinal fluid (71).

Hardly anything is known about the possible protective role of MRPs other than MRP1. An *Mrp5* (–/–) mouse exists (47), but it has not yet been analyzed in detail.

MRPs AND CLINICAL MULTIDRUG RESISTANCE

P-gp was discovered in 1976 (2). Today, 24 years later, there is still no consensus on its contribution to drug resistance in cancer patients. The first MRP, MRP1, was discovered only 8

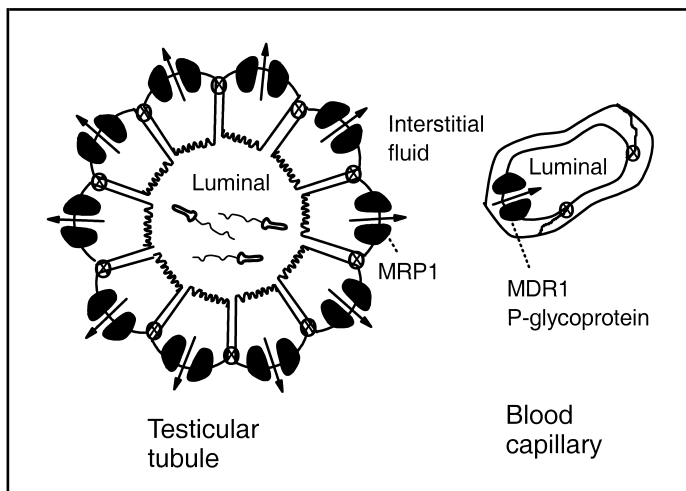


Fig. 3. Blood–testis barrier: schematic representation of the localization of multidrug resistance protein 1 (MRP1) and multidrug resistance 1 (MDR1) P-glycoprotein in a testicular tubule and blood capillary, respectively. The physical barrier between the luminal (apical) and basal plasma membrane is formed by tight junctions, indicated by ⊗.

years ago (5); therefore, it is not surprising that we still do not know much about its clinical significance. Because MRP1 is ubiquitous in human tissues (Table 3), it is potentially present in most tumors and could, therefore, play a role in resistance. Indeed, MRP1 has been detected in almost every tumor type examined, but no strong association has emerged between MRP1 levels and clinical resistance [reviewed in (10)]. In the absence of effective and specific MRP inhibitors, it is impossible to analyze the possible contribution of MRP1 to resistance by use of intervention studies in which anticancer drugs transported by MRP1 are combined with an inhibitor of MRP1.

No association between the expression of MRP2 and multidrug resistance was ever found in cell lines selected for multidrug resistance. However, MRP2 was found in 95% of renal clear-cell carcinomas, and MRP2 was also detected in lung, gastric, colorectal, and hepatocellular carcinomas [reviewed in (11)]. Initial studies on MRP3 (9) did not find any association between MRP3 and drug resistance in cell lines, but a more recent survey of lung cancer cell lines (72) showed a strong association between MRP3 and doxorubicin resistance and a weaker association between MRP3 and resistance to vincristine, etoposide, and cisplatin. The potential contribution of MRP4–7 to multidrug resistance remains to be studied.

MRPs AND CLINICAL MTX RESISTANCE

Biochemical experiments have identified several different GS-X pumps that are able to extrude MTX from erythrocytes and leukemic cells (31,73,74), and several lines of evidence support the idea that these pumps might be identical to MRPs. For example, MTX excretion into bile is diminished in TR⁻ rats lacking MRP2 (75), and vesicular transport of MTX is increased in membrane vesicles from cells with increased MRP1 levels (31) and decreased in erythrocytes from Mrp1 (-/-) mice (unpublished results from our laboratory). Nevertheless, no MTX resistance was found in cells overexpressing any MRP during continuous MTX exposure.

This paradox was resolved when Jansen and co-workers (31,42) found that MRP1, MRP2, and MRP3 do protect cells

against a 4-hour exposure to high MTX concentrations but not to a 96-hour continuous exposure to low-dose MTX. Apparently, there is a competition in the cell between export of MTX via MRPs and polyglutamylation, as indicated in Fig. 4. MTX polyglutamates are not detectably transported by MRP1, and the gradual accumulation of these polyglutamates in chronically exposed cells may explain the loss of resistance during long-term drug exposure. It is possible that polyglutamylation of folates also prevents depletion of cellular folate pools by MRP, but this hypothesis has not yet been analyzed.

The results now available suggest that at least some of the MTX-transporting GS-X pumps, identified in cultured cells by Henderson and co-workers (73,74), are actually MRP1, MRP2, and MRP3. Whether raised levels of MRPs could contribute to MTX resistance *in vivo* has not yet been studied.

MRPs AND CLINICAL CISPLATIN OR ARSENITE RESISTANCE

Ishikawa et al. (76) first pointed out that cisplatin can form complexes with GSH (equation 2):



Cisplatin Glutathione Complex

Moreover, they (76) showed that these complexes are themselves toxic and that they might be removed from the cells by a GS-X pump. All attempts to demonstrate transport of cisPt-(SG)₂ complexes by MRP1 have failed, but there is now good evidence that MRP2 could mediate cisplatin resistance, as discussed above. Whether there is any association between clinical cisplatin resistance and raised MRP2 levels in tumors remains to be studied.

Clinical interest in arsenite comes from observations showing that arsenite treatment can induce remissions in promyelocytic leukemia, probably by promoting apoptosis [see (77,78)]. Clinical resistance against arsenite arises rapidly during treatment, and it seems possible that overexpression of MRPs could be involved in some forms of resistance. Although vesicular trans-

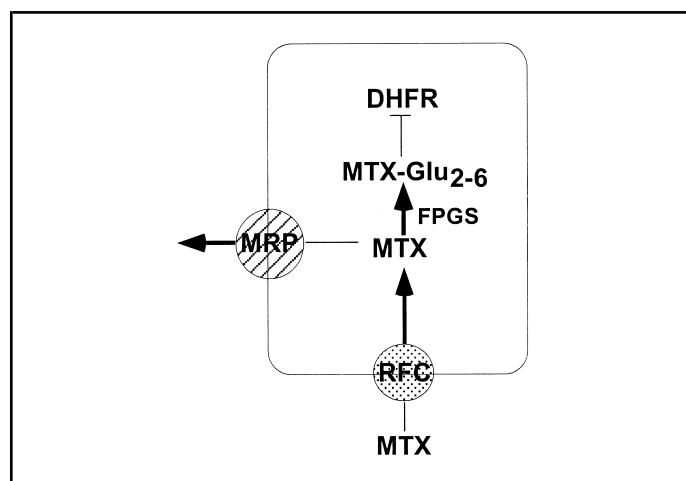


Fig. 4. Multidrug resistance proteins (MRPs) and folic poly-γ-glutamate synthase (FPGS) compete for methotrexate (MTX). MTX is taken up by the cells via the reduced folate carrier (RFC), and overexpression of MRP results in transport of unmodified MTX. Polyglutamated MTX (MTX-Glu₂₋₆) formed in a relatively slow reaction by FPGS, which results in the efficient inhibition of dehydrofolate reductase (DHFR).

port experiments demonstrating As(SG)₃ (see equation 1) transport by MRPs are lacking (and technically difficult), there is good indirect evidence for transport. Some cell lines overexpressing MRP1 are somewhat resistant to arsenite (79), embryonic stem cells with disrupted Mrp1 genes are arsenite hypersensitive (80), and arsenite increases the GSH efflux from cells overexpressing MRP1 (22), MRP2, or MRP3 (unpublished results from our laboratory). It will, therefore, be of interest to test whether increased expression of one of these MRPs can help cells escape death from arsenite.

WHY SO MANY PUMPS?

Many toxins found in nature and used by oncologists as natural-product drugs enter cells by passive diffusion. These amphipathic drugs are hydrophobic enough to diffuse through a lipid bilayer, but they are hydrophilic enough to be water soluble and to reach their target. Because of the hydrophilic parts in these drugs, they enter cells slowly. The rate of entry of doxorubicin, for instance, is measured in minutes rather than in milliseconds (81). Because doxorubicin does not require a protein to enter the cell, organisms cannot defend themselves against this drug by altering an import protein. This is an effective strategy to keep out water-soluble drugs, such as MTX, which is dependent on the reduced folate carrier for rapid cellular uptake.

Once inside, these amphipathic drugs can be inactivated by oxidation and/or conjugation. However, as Ishikawa (21) pointed out, conjugation by itself is not enough to get rid of the drug. The conjugated drug is now more hydrophilic because of the GSH attached to it and cannot leave the cell by passive diffusion. As drug continues to enter the cell, the GSH conjugate will accumulate to excessive concentrations (that will be toxic in themselves) unless exported by a dedicated export pump, a GS-X pump.

Genes for drug pumps and drug-conjugate pumps are prominent in all of the genomes of simple organisms sequenced. Three classes of drug (-conjugate) pumps have been found in the human genome [i.e., the P-gp's, the MRPs, and the breast cancer resistance proteins (82–85)], with a total of 12 members identified so far. It will be a formidable task to sort out which of these pumps contributes to resistance, to which anticancer drugs, and in which tumors.

The potential benefit of this knowledge to cancer treatment is, however, large. As the rapid analysis of the expression levels of thousands of genes in tumor samples is entering the phase of clinical application, it will become possible to reconstruct a resistance profile for the predominant cell types in each tumor and to adjust the chemotherapy accordingly. This should at least make it possible to spare some patients an aggressive therapy that does not work.

OUTLOOK

What makes the MRP family so remarkable is the range of anticancer drugs handled by its members. Whereas P-gp's have become deservedly famous for transporting a wide range of neutral or slightly basic organic compounds, the current members of the MRP family are even more versatile. In addition to the P-gp-transported neutral organic compounds, MRP1, MRP2, and MRP3 also transport drugs conjugated to GSH, glucuronate, or sulfate and other organic anions, such as MTX. Because of the ability of these transporters to handle compounds associated with GSH, they can even cause resistance to small molecules

that can form GSH complexes, such as cisplatin (MRP2) or arsenite. The newer family members, MRP4 and MRP5, are able to cause resistance to nucleotide analogues, such as PMEA and purine base analogues, such as 6-mercaptopurine and thioguanine. Transport by MRPs, therefore, affects a stunning range of anticancer drugs and provides a link between transporters and the GSH system previously associated with resistance to carcinostatics.

The potential importance of MRPs in drug resistance is, therefore, high. What remains to be done is to sort out which fraction of this awesome potential is actually used in cancer patients and how we could make use of this knowledge to treat patients more effectively.

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NOTES

¹Mutations in the MRP6 gene were very recently shown to cause a skin and eye disease known as pseudoxanthoma elasticum (91–93).

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