Journal of Bioenergetics and Biomembranes, Vol. 33, No. 6, December 2001 (© 2001)

MRP Subfamily Transporters and Resistance to Anticancer Agents

Gary D. Kruh,[1,4] Hao Zeng,[1,3] Philip A. Rea,[2] Guosheng Liu,[2] Zhe-Sheng Chen,[1] Kun Lee,[1] and Martin G. Belinsky[1]

The MRP subfamily of ABC transporters from mammals consists of at least seven members, six of which have been implicated in the transport of amphipathic anions. MRP1, MRP2, and MRP3 bear a close structural resemblance, confer resistance to a variety of natural products as well as methotrexate, and have the facility for transporting glutathione and glucuronate conjugates. MRP1 is a ubiquitously expressed efflux pump for the products of phase II of xenobiotic detoxification, while MRP2, whose hereditary deficiency results in Dubin–Johnson syndrome, functions to extrude organic anions into the bile. MRP3 is distinguished by its capacity to transport the monoanionic bile constituent glycocholate, and may function as a basolateral back-up system for the detoxification of hepatocytes when the usual canalicular route is impaired by cholestatic conditions. MRP4 and MRP5 resemble each other more closely than they resemble MRPs 1–3 and confer resistance to purine and nucleotide analogs which are either inherently anionic, as in the case of the anti-AIDS drug PMEA, or are phosphorylated and converted to anionic amphiphiles in the cell, as in the case of 6-MP. Given their capacity for transporting cyclic nucleotides, MRP4 and MRP5 have also been implicated in a broad range of cellular signaling processes. The drug resistance activity and physiological substrates of MRP6 are unknown. However, its hereditary deficiency results in pseudoxanthoma elasticum, a multisystem disorder affecting skin, eyes, and blood vessels. It is hoped that elucidation of the resistance profiles and physiological functions of the different members of the MRP subfamily will provide new insights into the molecular basis of clinical drug resistance and spawn new strategies for combating this phenomenon.

KEY WORDS: MRP; drug resistance; ABC transporter.

MRP1

Pgp has served as a paradigm for the role of plasma membrane efflux pumps in resistance to anticancer agents and for development of the idea that pump inhibitors may be deployed to increase the efficacy of chemotherapeutic agents (Gottesman and Pastan, 1993). However, following the initial identification of Pgp, analyses of a variety of drug resistant cell lines indicated that other ATP-dependent efflux pumps might also confer resistance to a broad range of natural products. In the specific case of amphiphilic anion transporters, investigations of the anthracycline resistant leukemia cell line, HL60/Adr, disclosed an energy-dependent drug efflux system that was not associated with Pgp overexpression but instead with overexpression of another membrane protein, a 190 kDa resistance-associated protein (Marquardt *et al.*, 1990; Marsh *et al.*, 1986; Marsh and Center, 1987; McGrath *et al.*, 1989; McGrath and Center, 1987). Based upon its susceptibility to photoaffinity labeling by AzATP, a nucleotide-binding fold label, and its immunoreaction with peptide antisera directed at the conserved nucleotide-binding sites of Pgp, the 190 kDa species was concluded to be a distinct ABC transporter. The isolation of the *MRP1*

[1] Medical Sciences Division, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111.
[2] Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104.
[3] Present address: Department of Pharmacology, Yale University School of Medizine, 333 Codar Street, New Haven, CT 06520.
[4] To whom correspondence should be addressed; e-mail: gd_kruh@fccc.edu.

0145-479X/01/1200-0493$19.50/0 © 2001 Plenum Publishing Corporation

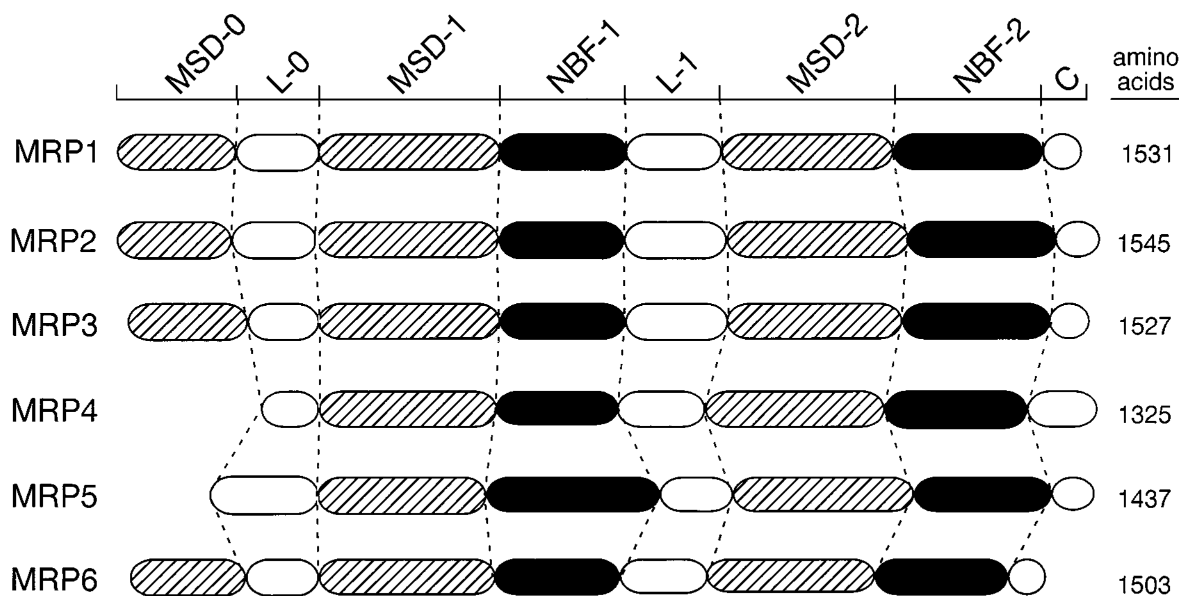

Fig. 1. Schematic comparing the structures of MRP subfamily transporters. Membrane spanning domains are indicated by striped ovals and nucleotide binding folds are indicated by black ovals. Cytoplasmic segments at the C-terminus (C), between MSD-0 and MSD-1, and between NBF-1 and MSD-2 are indicated by open ovals. Note that the conserved nucleotide binding folds are located at the C-termini of the black ovals but do not span the entire segment. The structures were brought into register by alignment of MSD-1. Membrane spanning domains (MSD-0, MSD-1, MSD-2); nucleotide binding folds (NBF1, NBF2); cytoplasmic segments between MSD-0 and MSD-1 (L-0) and between NBF1 and MSD-1 (L-1).

cDNA from a drug resistant lung cancer cell line (Cole *et al.*, 1992) revealed the molecular identity of the 190 kDa protein (Krishnamachary and Center, 1993) and subsequent transfection studies confirmed its drug resistance activity and defined its resistance profile (Breuninger *et al.*, 1995; Cole *et al.*, 1994; Grant *et al.*, 1994; Kruh *et al.*, 1994; Zaman *et al.*, 1994).

The MRP1 drug resistance phenotype overlaps with that of Pgp with regard to natural product drugs, in that it is associated with resistance to anthracyclines, etoposide, and vinca alkaloids. There are, however, two notable differences. First, MRP1 does not confer resistance to Taxol, a clinically important agent that is part of the Pgp resistance profile. Second, MRP1 confers high-level resistance to methotrexate in short term but not continuous drug exposure assays (Hooijberg *et al.*, 1999). The latter feature reflects the capacity of MRP1 to transport methotrexate but not its polyglutamylated metabolites (Zeng *et al.*, 2001). The in vivo resistance activity of the transporter is apparent in MRP1-deficient mice, which are hypersensitive to etoposide (Lorico *et al.*, 1997; Wijnholds *et al.*, 1997). MRP1-deficient mice also exhibit markedly increased damage of bone marrow, oropharyngeal mucosal surfaces and the testes by cytotoxic drugs (Lorico *et al.*, 1997; Wijnholds *et al.*, 1998), and impaired inflammatory responses (Wijnholds *et al.*, 1997), the latter of which is likely attributable to reduced extrusion of LTC_4 from mast cells (Bartosz *et al.*, 1998).

MRP1 and Pgp share only modest amino acid sequence identity (15%) and possess distinct in vitro activities despite their overlapping resistance profiles. MRP1 contains a third, N-terminally disposed membrane spanning domain (MSD-0) and a cytoplasmic loop (L-0) (Tusnady *et al.*, 1997), both of which are lacking from Pgp (Fig. 1). Detailed in vitro transport measurements, using membrane vesicles purified from both HL60/Adr and MRP1-transfected cells have shown that MRP1 is a lipophilic anion transporter capable of transporting glutathione conjugates, such as LTC_4 and DNP-SG (Jedlitschky *et al.*, 1994; Leier *et al.*, 1994), as well as glucuronate and sulfate conjugates, such as $E_217\beta G$ and dianionic bile salts, respectively (Jedlitschky *et al.*, 1996; Loe *et al.*, 1996a). By contrast, Pgp does not have the capacity to transport amphipathic anions but instead has a preference for amphipathic cations.

The current model for how an amphipathic anion transporter effluxes natural product anticancer agents, which are uncharged or mildly cationic and not known to be converted to anionic conjugates in the cell, is that the drugs are cotransported with free GSH. This model is supported by the observation that etoposide-stimulated GSH export is abolished in MRP1-deficient ES cells,

and by experiments on membrane vesicles demonstrating that transport of vincristine is GSH dependent (Loe *et al.*, 1996b; Lorico *et al.*, 1996). That MRP1-deficient animals have elevated levels of GSH provides additional support for an association between the activity of the pump and GSH (Lorico *et al.*, 1997). In accord with the notion that MRP1 lies at the intersection between the GSH-dependent cellular machinery of phase II detoxification and drug efflux, BSO modulates drug resistance in non–Pgp-expressing multidrug-resistant cell lines (Lutzky *et al.*, 1989) and in MRP1-overexpressing cells (Schneider *et al.*, 1995; Versantvoort *et al.*, 1995; Zaman *et al.*, 1995), and MRP1 together with the pacemaker enzymes for GSH synthesis and *S*-conjugation, γGCS and GSTs, respectively, are subject to coordinate expression regulation (Ishikawa *et al.*, 1996; Kuo *et al.*, 1996; Lacave *et al.*, 1998). That forced overexpression of MRP1 with γGCS and/or GSTs confers significantly enhanced levels of drug resistance compared to expression of single components alone indicates that coordinate expression regulation in tumors may be of clinical significant (Morrow *et al.*, 1998; O'Brien *et al.*, 2000).

MRP2

The second member of the MRP family, MRP2 (*alias* cMOAT) (Buchler *et al.*, 1996; Ito *et al.*, 1997; Paulusma *et al.*, 1996; Taniguchi *et al.*, 1996) bears a close resemblance to MRP1 in terms of both structure (Table I and Fig. 1) and substrate selectivity, but has a markedly different expression pattern. In contrast to MRP1, which is widely distributed (Kruh *et al.*, 1995) and has a basolateral plasma membrane localization in polarized cells (Evers *et al.*, 1996; Mayer *et al.*, 1995), MRP2 is primarily expressed in canalicular (apical) hepatocyte membranes—where it functions as a biliary GSH and glucuronate conjugate transporter—and at lower levels in small intestine and renal proximal tubules (Buchler *et al.*, 1996; Paulusma *et al.*, 1996).

Many of the properties of this transporter were deduced from studies of rat strains (TR- and EHBR) that are deficient in hepatobiliary excretion of organic anions (Ishikawa *et al.*, 1990; Kitamura *et al.*, 1990) and which were later defined to have hereditary defects in MRP2, as do patients with Dubin–Johnson syndrome, an hereditary disorder characterized by modest elevations in serum conjugated bilirubin (Kartenbeck *et al.*, 1996; Paulusma *et al.*, 1997). Biochemical studies using the cloned cDNA indicate that MRP2, like MRP1, directly mediates the transport of glucuronate and GSH conjugates (Cui *et al.*, 1999; Evers *et al.*, 1998; Ito *et al.*, 1998; Kawabe *et al.*, 1999; Madon *et al.*, 1997; van Aubel *et al.*, 1998) albeit at affinities significantly lower than those of MRP1.

MRP2 confers resistance to a variety of natural product agents (anthracyclines, vinca alkaloids, etoposide) as well as camptothecins and methotrexate (Cui *et al.*, 1999; Hooijberg *et al.*, 1999; Kawabe *et al.*, 1999; Koike *et al.*, 1997). In addition, MRP2 is notable for its capacity to confer resistance to cisplatin. Were it not for the fact that MRP1 does not confer resistance to this agent (Breuninger *et al.*, 1995; Cole *et al.*, 1994), this feature of the MRP2 resistance profile would not be surprising, as cisplatin is known to form toxic GSH complexes in the cell (Ishikawa and Ali-Osman, 1993). The biochemical mechanism whereby MRP2 confers resistance to natural product agents appears to be similar to that of MRP1, in

Table I. Amino Acid Identity Among MRP Subfamily Members

	MRP1	MRP2	MRP3	MRP4	MRP5	MRP6
MRP1	—	**49.8**	**58.0**	**40.8**	**37.9**	**46.1**
	—	(66.0/73.1)	(70.7/73.8)	(57.3/61.6)	(60.0/59.4)	(61.3/61.9)
MRP2	**49.8**	—	**48.0**	**38.8**	**38.0**	**40.0**
	(66.0/73.1)	—	(67.3/70.0)	(53.3/55.3)	(61.3/60.6)	(55.3/59.4)
MRP3	**58.0**	**48.0**	—	**37.8**	**35.6**	**44.0**
	(70.7/73.8)	(67.3/70.0)	—	(55.3/54.1)	(57.3/56.9)	(59.3/59.4)
MRP4	**40.8**	**38.8**	**37.8**	—	**38.3**	**35.5**
	(57.3/61.6)	(53.3/55.3)	(55.3/54.1)	—	(49.3/59.1)	(52.0/56.6)
MRP5	**37.9**	**38.0**	**35.6**	**38.3**	—	**33.7**
	(60.0/59.4)	(61.3/60.6)	(57.3/56.9)	(49.3/59.1)	—	(50.0/52.5)
MRP6	**46.1**	**40.0**	**44.0**	**35.5**	**33.7**	—
	(61.3/61.9)	(55.3/59.4)	(59.3/59.4)	(52.0/56.6)	(50.0/52.5)	—

Note. Overall percent amino acid identity is indicated in boldface. Percent identity of nucleotide binding folds 1 and 2 is indicated in parentheses (NBF1/NBF2). Percent identity was obtained using the GAP command in the GCG package. The Blosum 62 scoring matrix was used with a gap creation penalty of 8 and a gap extension penalty of 2. Adapted from Belinsky *et al.* 1999.

that it is GSH dependent (Evers *et al.*, 2000; van Aubel *et al.*, 1999). However, unlike MRP1, MRP2 may be competent in the transport of GSH alone without the need for simultaneous cotransport or binding of other compounds (van Aubel *et al.*, 1999). The capacity of MRP2 for GSH export is consistent with the notion that it is the component or one of the components responsible for the extrusion of GSH into bile (Elferink *et al.*, 1989; Takikawa *et al.*, 1991).

The Extended MRP Subfamily

As first suspected from the results of biochemical investigations (Saxena and Henderson, 1995), and confirmed by analyses of expressed sequence tags and other partial sequences (Allikmets *et al.*, 1996; Kool *et al.*, 1997; Longhurst *et al.*, 1996), it is now clear that there at least five MRP subfamily members in addition to MRP1 and MRP2. The predicted domain organizations and topologies of four of these transporters have been determined (Belinsky *et al.*, 1998; Belinsky and Kruh, 1999; Hirohashi *et al.*, 1998; Kool *et al.*, 1999a; Lee *et al.*, 1998; Suzuki *et al.*, 1997; Uchiumi *et al.*, 1998) and on this basis they appear to fall into two groups: those that contain (MRP1, MRP2, MRP3, MRP6) and those that lack (MRP4, MRP5) the N-terminal MSD-0 transmembrane domain (Fig. 1) (Belinsky *et al.*, 1998; Belinsky and Kruh, 1999). All, however, contain the L-0 linker domain which in retrospect might be expected given the functional dispensability of the MSD-0 but not the L-0 domain of MRP1 (Bakos *et al.*, 1998). MRP1, MRP2, MRP3 (48–50% identity), and to a lesser extent MRP6 (40–46% identity) resemble each other considerably at the sequence level but MRP4 and MRP5 are more divergent from each other and from the other MRPs (34–41% identity) (Table I). Although there is a possibility that MRP4 and MRP5 form their own subgroup they are nevertheless more related to the other MRPs than they are to other known ABC transporters as indicated by the results of phylogenetic cluster analyses (Belinsky *et al.*, 1998; Belinsky and Kruh, 1999). This conclusion is substantiated by investigations of their activities. (The properties of MRP subfamily members are summarized in Table II and Table III).

Table II. Chromosomal Localizations and Expression Patterns of MRP Subfamily Transporters

	Chromosomal localization	Tissue expression
MRP1	16p13.1	Widespread
MRP2	10q24	Liver, kidney, gut
MRP3	17q21.3	Pancreas, kidney, gut, liver, adrenal
MRP4	13q32	Prostate, testes, ovary, gut, pancreas, lung, muscle
MRP5	3q27	Widespread
MRP6	16p13.1	Liver, kidney

MRP3

Of the MRPs for which complete coding sequences have been determined, MRP3 shares the highest degree of structural resemblance with MRP1 (58%). However, by contrast with MRP1, the known drug resistance profile of MRP3 is narrow and restricted to epipodophyllotoxins, vincristine, and methotrexate (Kool *et al.*, 1999b; Zeng

Table III. Properties of MRP Subfamily Transporters

	Glucuronate and GS-conjugate transport	Notable physiological substrates	Proposed physiological functions
MRP1	+	Glutathione, LTC_4	Ubiquitous GS-X pump; efflux of LTC_4 from mast cells
MRP2	+	Glutathione, bilirubin glucuronide	Biliary excretion of amphipathic anions (hereditary deficiency results in Dubin-Johnson syndrome)
MRP3	+	Glycocholate	Efflux of amphipathic anions from cholestatic liver; enterohepatic circulation of bile acids
MRP4[a]	+	cGMP, cAMP	Modulation of cyclic nucleotide signaling pathways
MRP5[b]	–	cGMP, cAMP	Modulation of cGMP signaling pathways
MRP6	–	?	Connective tissue homeostasis (hereditary deficiency results in PXE)

[a]Transports E_2 17βG.
[b]Conjugate transport was not detected in membrane vesicle transport assays. However, DNP-SG transport was observed in cellular kinetic studies.

Table IV. Summary of Kinetic Parameters for MgATP-Dependent Amphipathic Anion Transport by Membrane Vesicles Prepared From MRP3-Transfected HEK293 Cells

Substrate	K_m(μM)	V_{max}(pmol/mg/min)	V_{max}/K_m
$E_2 17\beta G$	25.6 ± 5.4	75.6 ± 5.9	3.0
DNP-SG	5.7 ± 1.7	3.8 ± 0.1	0.7
LTC_4	5.3 ± 2.6	20.2 ± 5.9	3.8
Methotrexate	776 ± 319	288 ± 54	0.4
Glycocholate	248 ± 113	183 ± 34	0.7

Note. Adapted from Zeng *et al*. 2000. Values shown are means ± SE.

et al., 1999). Moreover, the potency of its resistance activity for etoposide and vincristine is considerably less than that of MRP1, as assessed by measurements of resistance levels in transfected cells. These in vivo properties are in part reflected in the in vitro transport characteristics of MRP3.

Membrane vesicles prepared from *MRP3*-transfected HEK293 cells transport the prototypical MRP1 substrates LTC_4, DNP-SG, and E217βG but at relatively low affinity (Table IV and Table V) (Zeng *et al.*, 2000). The cysteinyl leukotriene LTC_4 is a low affinity substrate of MRP3 (K_m 5.3 μM), in comparison to MRP1, for which it is a high affinity substrate (K_m 0.097 μM) (Jedlitschky *et al.*, 1996; Leier *et al.*, 1994; Loe *et al.*, 1996b) and MRP2, for which it is an intermediate affinity substrate (K_m 0.24–1.0 μM) (Cui *et al.*, 1999; Kawabe *et al.*, 1999). Similarly, the K_m for $E_2 17\beta G$ transport by MRP3 (25.6 μM) is considerably higher than the values reported for MRP1 (1.5–2.5 μM) (Jedlitschky *et al.*, 1996; Loe *et al.*, 1996a) and MRP2 (7.2 μM) (Cui *et al.*, 1999). The exception is DNP-SG for which only a small difference between MRP3 (K_m 5.7 μM) and MRP1 (K_m 3.6 μM) (Jedlitschky *et al.*, 1996) was detectable. Both MRP3 and MRP1 transport this conjugate at considerably higher affinities than does MRP2 (K_m 70 μM) (Paulusma *et al.*, 1999). As expected based upon its capacity to confer methotrexate resistance, MRP3 is also able to transport this monoglutamate at high capacity (Table IV). Of the differences from MRP1 and MRP2 one of the most striking was MRP3's activity toward the conjugated monoanionic bile acid glycocholate but not taurocholate. MRP3 transports glycocholate at high rates (Table IV). Similar studies of rat MRP3 have disclosed a similar pattern except that this transporter does not appear to transport GS-conjugates and does not distinguish glycocholate from taurocholate (Hirohashi *et al.*, 2000; Hirohashi *et al.*, 1998).

Table V. Comparison of Kinetic Parameters for Glutathione and Glucuronate Conjugate Transport by MRP1, MRP2, and MRP3

	K_m(μM)		
Transporter	LTC_4	DNP-SG	$E_2 17\beta G$
MRP3, human	5.3	5.7	25.6
MRP3, rat	ND	ND	67
MRP1, human	0.097	3.6	1.5, 2.5
MRP2, human	0.24, 1.0	70	7.2

Note. Adapted from Zeng *et al*., 2000. ND: not detected.

The in vitro transport characteristics of MRP3 provide potential insights into its limited drug resistance activity and physiological functions. It is not known whether GSH is involved in MRP3-mediated resistance to natural products, but the reduced affinity of human MRP3 for LTC_4 may be an indicator of MRP3's relatively restricted resistance profile if the capacity of this transporter for GSH cotransport is low by comparison with that of MRP1 or MRP2. With regard to physiological functions, the similarity of the MRP3 substrate selectivity to that of MRP2 together with its localization in basolateral membranes of hepatocytes (Konig *et al.*, 1999; Kool *et al.*, 1999b) and substantial induction in cholestatic conditions (Hirohashi *et al.*, 1998; Konig *et al.*, 1999; Ortiz *et al.*, 1999), suggest that in these conditions MRP3 may function to efflux into sinusoidal blood the amphipathic anions (e.g., bilirubin glucuronide) that are ordinarily exported by canalicular MRP2 into bile. Likewise, MRP3 may function in cholestatic conditions to efflux bile constituents, such as monoanionic bile salts, which are substrates of other canalicular pumps. There is also a possibility that in the gut, where MRP3 transcript is abundant, this transporter participates in the enterohepatic circulation of bile salts by transporting across the basolateral membranes of enterocytes those bile salts that have been imported from the gut lumen by the (apical) ileal bile salt transporter.

MRP4 AND MRP5

MRP4 and MRP5 confer a unique drug resistance phenotype and transport representatives of a different class of amphipathic conjugates in that both transporters have been implicated in resistance to and transport of purine and nucleotide analogues. The MRP4 gene was found to be amplified and overexpressed in a cell line resistant to the anti-AIDS nucleotide analogue PMEA (Schuetz *et al.*, 1999), and investigations of the MRP4 drug resistance profile of transfected NIH3T3 cells showed that, in addition to PMEA, MRP4 also confers resistance to methotrexate (Lee *et al.*, 2000). However, MRP4-transfected cells were not resistant to a variety of other anticancer agents, including natural product drugs. PMEA, an acyclic nucleoside

phosphonate, is an amphipathic anion by virtue of its nonhydrolyzable phosphonate group, and may therefore represent an MRP4 substrate itself. Alternatively, the mono- or diphosphorylated metabolites of PMEA are effluxed by MRP4.

Analyses of transfected cells indicate that MRP5 is also capable of conferring resistance to PMEA, as well as the anticancer purine analogues 6-mercaptopurine and 6-thioguanine (Wijnholds *et al.*, 2000). The resistance conferred by MRP5 is considered to derive from efflux of the phosphorylated metabolites of these anticancer agents. Recently MRP4 has also been shown to confer resistance to 6-mercaptopurine and 6-thioguanine (Chen *et al.*, 2001).

An important insight into how the potential physiological functions of MRP5 may impinge on drug resistance was recently gained from the discovery that this MRP is a primary active transporter of cGMP and cAMP (Jedlitschky *et al.*, 2000). Not only does this activity establish that MRP5 is a transporter of physiological phosphate conjugates but it also raises the possibility that it plays a role in at least some of the signal transduction pathways in which cyclic nucleotides participate. While assays of the transport of anionic fluorochromes indicate that the substrate selectivity of MRP5 is not limited to phosphorylated compounds (McAleer *et al.*, 1999) it remains to be determined if it is competent in the direct transport of typical MRP1 substrates. On the one hand, efflux of DNP-SG and GSH, concomitant with a diminution of intracellular GSH levels, has been observed in MRP5-transfected cells (Wijnholds *et al.*, 2000). On the other hand, transport of LTC_4 or $E_2 17\beta G$ has yet to be detected in MRP5-enriched membrane vesicles (Jedlitschky *et al.*, 2000).

MRP4 is also able to transport cyclic nucleotides (Chen *et al.*, 2001). However, its affinity for cAMP is 9-fold greater than that of MRP5. By contrast, its affinity for cGMP is ∼5-fold lower than that of MRP5. These features of MRP4 suggest that it may play a more prominent role in modulating cAMP signaling than does MRP5. Another feature that distinguishes the substrate selectivity of MRP4 from that of MRP5 is that MRP4 is able to transport glucuronides such as $E_2 17\beta G$.

MRP6

MRP6 has the properties of an amphipathic anion transporter that does not transport glutathione or glucuronate conjugates. Membrane vesicles containing the rat protein transport the anionic cyclopentapeptide BQ123 but not typical MRP1 substrates (Madon *et al.*, 2000). Critically, hereditary deficiency of MRP6 is the basis of pseudoxanthoma elasticum (PXE), a multisystem disease of the skin, eyes, and blood vessels (Bergen *et al.*, 2000; Le Saux *et al.*, 2000; Ringpfeil *et al.*, 2000). However, the relationship between MRP6, its high levels of expression in kidney and liver (Belinsky and Kruh, 1999; Kool *et al.*, 1999a), BQ123 transport and PXE remain to be elucidated.

Changing Perspectives on ABC Transporters and the Drug Resistant Phenotype

In spite of intense efforts, most attempts to establish a strict correlation between Pgp expression in tumors and clinical resistance to chemotherapeutic agents have met with little success in the case of adult solid tumors, and only modest success in the case of childhood tumors and adult hematopoietic tumors (Ling, 1997). At least one potential reason for this lack of correlation is now apparent: there are many ABC transporters with overlapping capabilities that confer resistance to this class of agents. Table VI summarizes the resistance profiles of ABC transporters known to be involved in

Table VI. Drug Resistance Profiles of ABC Transporters

Agent	Pgp	MRP1	MRP2	MRP3	MRP4	MRP5	BCRP	ABC2
Taxol	+	−		−	−	−	−	
Anthracyclines	+	+	+	−	−	−	+	
Vinca alkaloids	+	+	+	+	−	−	−	
Epipodophyllotoxins	+	+	+	+	−	−	−	
Mitoxantrone	+	−		−		−	+	
Camptothecins	+		+			−	+	
Methotrexate	−	+	+	+	+	−	−	
Cisplatin	−	−	+	−	−	−	−	
Nucleotide anal.					+	+		
Estramustine								+

resistance to anticancer drugs. Included in this table are the profiles of BCRP/MXR (Allikmets *et al.*, 1998; Doyle *et al.*, 1998; Miyake *et al.*, 1999) and ABC2 (Laing *et al.*, 1998), two ABC transporters that confer resistance to anticancer agents, but are not members of the Pgp or MRP subfamilies. Particularly noteworthy is the fact that there are at least four transporters that confer resistance to anthracyclines, vincristine, and etoposide. The only known exception to this pattern is Taxol, for which Pgp is the only ABC transporter that demonstrably confers resistance. The problem of redundant activities is further compounded by the extended resistance profiles, such as those for methotrexate and camptothecins, of some MRP subfamily members. This suggests that expression of a single transporter could simultaneously confer resistance to both natural product and nonnatural product agents in a given chemotherapy regimen. For example, methotrexate and anthracyclines are components of regimens for the treatment of lymphomas and childhood leukemias, and expression of either MRP1 or MRP2 could confer resistance to both of these agents.

The overlapping resistance profiles of drug pumps also have important ramifications for the clinical use of modulators designed to inhibit the action of transporters and thereby increase intracellular drug concentrations. Given the number of transporters and the possibility that it may not be possible to find a single compound that is capable of inhibiting all of them, detailed information concerning the de novo and posttreatment expression levels of drug transporters in specific tumors may be required in order to determine which inhibitors to use and when to use them during the course of a treatment regimen. The expression of some MRPs at excretory sites, for example the high levels of MRP2 and MRP3 expression in liver and kidney, may introduce the added complication of potentially profound pharmacokinetic perturbations. Reduced hepatobiliary clearance of drugs and hepatotoxicity have already been observed with the use of some Pgp inhibitors and one of the most obvious characteristics of MRP2-deficient rats is their reduced biliary clearance of methotrexate (Masuda *et al.*, 1997). Finally, and perhaps most dramatically, the surprising functions of some MRPs, for example cyclic nucleotide transport by MRP4 and MRP5, may presage completely unanticipated difficulties consequent on the use of pump inhibitors. Having made the case for caution and concern, there is also reason for optimism. Recent insights into the nature of plasma membrane defenses against anticancer agents have increased our understanding of the drug resistant phenotype and should facilitate the implementation and testing of strategies designed to reduce the potential clinical impact of ABC transporters on a more rational basis.

REFERENCES

Allikmets, R., Gerrard, B., Hutchinson, A., and Dean, M. (1996). *Hum. Mol. Genet.* **5**, 1649–1655.

Allikmets, R., Schriml, L. M., Hutchinson, A., Romano-Spica, V., and Dean, M. (1998). *Cancer Res.* **58**, 5337–5339.

Bakos, E., Evers, R., Szakacs, G., Tusnady, G. E., Welker, E., Szabo, K., de Haas, M., van Deemter, L., Borst, P., Varadi, A., and Sarkadi, B. (1998). *J. Biol. Chem.* **273**, 32167–32175.

Bartosz, G., Konig, J., Keppler, D., and Hagmann, W. (1998). *Biol. Chem.* **379**, 1121–1126.

Belinsky, M. G., Bain, L. J., Balsara, B. B., Testa, J. R., and Kruh, G. D. (1998). *J. Natl. Cancer Inst.* **90**, 1735–1741.

Belinsky, M. G., and Kruh, G. D. (1999). *Br. J. Cancer.* **80**, 1342–1349.

Bergen, A. A., Plomp, A. S., Schuurman, E. J., Terry, S., Breuning, M., Dauwerse, H., Swart, J., Kool, M., van Soest, S., Baas, F., ten Brink, J. B., and de Jong, P. T. (2000). *Nat. Genet.* **25**, 228–231.

Breuninger, L. M., Paul, S., Gaughan, K., Miki, T., Chan, A., Aaronson, S. A., and Kruh, G. D. (1995). *Cancer Res.* **55**, 5342–5347.

Buchler, M., Konig, J., Brom, M., Kartenbeck, J., Spring, H., Horie, T., and Keppler, D. (1996). *J. Biol. Chem.* **271**, 15091–15098.

Chen, Z. S., Lee, K., and Kruh, G. D. (2001). *J. Biol. Chem.* **276**, 33747–33754.

Cole, S. P., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M., and Deeley, R. G. (1992). *Science* **258**, 1650–1654.

Cole, S. P., Sparks, K. E., Fraser, K., Loe, D. W., Grant, C. E., Wilson, G. M., and Deeley, R. G. (1994). *Cancer Res.* **54**, 5902–5910.

Cui, Y., Konig, J., Buchholz, J. K., Spring, H., Leier, I., and Keppler, D. (1999). *Mol. Pharmacol.* **55**, 929–937.

Doyle, L. A., Yang, W., Abruzzo, L. V., Krogmann, T., Gao, Y., Rishi, A. K., and Ross, D. D. (1998). *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15665–15670.

Elferink, R. P., Ottenhoff, R., Liefting, W., de Haan, J., and Jansen, P. L. (1989). *J. Clin. Invest.* **84**, 476–483.

Evers, R., de Haas, M., Sparidans, R., Beijnen, J., Wielinga, P. R., Lankelma, J., and Borst, P. (2000). *Br. J. Cancer.* **83**, 375–383.

Evers, R., Kool, M., van Deemter, L., Janssen, H., Calafat, J., Oomen, L. C., Paulusma, C. C., Oude Elferink, R. P., Baas, F., Schinkel, A. H., and Borst, P. (1998). *J. Clin. Invest.* **101**, 1310–1319.

Evers, R., Zaman, G. J., van Deemter, L., Jansen, H., Calafat, J., Oomen, L. C., Oude Elferink, R. P., Borst, P., and Schinkel, A. H. (1996). *J. Clin. Invest.* **97**, 1211–1218.

Gottesman, M. M., and Pastan, I. (1993). *Annu. Rev. Biochem.* **62**, 385–427.

Grant, C. E., Valdimarsson, G., Hipfner, D. R., Almquist, K. C., Cole, S. P., and Deeley, R. G. (1994). *Cancer Res.* **54**, 357–361.

Hirohashi, T., Suzuki, H., Chu, X. Y., Tamai, I., Tsuji, A., and Sugiyama, Y. (2000). *J. Pharmacol. Exp. Ther.* **292**, 265–270.

Hirohashi, T., Suzuki, H., Ito, K., Ogawa, K., Kume, K., Shimizu, T., and Sugiyama, Y. (1998). *Mol. Pharmacol.* **53**, 1068–1075.

Hooijberg, J. H., Broxterman, H. J., Kool, M., Assaraf, Y. G., Peters, G. J., Noordhuis, P., Scheper, R. J., Borst, P., Pinedo, H. M., and Jansen, G. (1999). *Cancer Res.* **59**, 2532–2535.

Ishikawa, T., and Ali-Osman, F. (1993). *J. Biol. Chem.* **268**, 20116–20125.

Ishikawa, T., Bao, J. J., Yamane, Y., Akimaru, K., Frindrich, K., Wright, C. D., and Kuo, M. T. (1996). *J. Biol. Chem.* **271**, 14981–14988.

Ishikawa, T., Muller, M., Klunemann, C., Schaub, T., and Keppler, D. (1990). *J. Biol. Chem.* **265**, 19279–19286.

Ito, K., Suzuki, H., Hirohashi, T., Kume, K., Shimizu, T., and Sugiyama, Y. (1998). *J. Biol. Chem.* **273**, 1684–1688.

Ito, K., Suzuki, H., Hirohashi, T., Kume, K., Shimizu, T., and Sugiyama, Y. (1997). *Am. J. Physiol.* **272**, G16–G22.

Jedlitschky, G., Burchell, B., and Keppler, D. (2000). *J. Biol. Chem.* **275**, 30069–30074.
Jedlitschky, G., Leier, I., Buchholz, U., Barnouin, K., Kurz, G., and Keppler, D. (1996). *Cancer Res.* **56**, 988–994.
Jedlitschky, G., Leier, I., Buchholz, U., Center, M., and Keppler, D. (1994). *Cancer Res.* **54**, 4833–4836.
Kartenbeck, J., Leuschner, U., Mayer, R., and Keppler, D. (1996). *Hepatology* **23**, 1061–1066.
Kawabe, T., Chen, Z. S., Wada, M., Uchiumi, T., Ono, M., Akiyama, S., and Kuwano, M. (1999). *FEBS Lett.* **456**, 327–331.
Kitamura, T., Jansen, P., Hardenbrook, C., Kamimoto, Y., Gatmaitan, Z., and Arias, I. M. (1990). *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3557–3561.
Koike, K., Kawabe, T., Tanaka, T., Toh, S., Uchiumi, T., Wada, M., Akiyama, S., Ono, M., and Kuwano, M. (1997). *Cancer Res.* **57**, 5475–5479.
Konig, J., Rost, D., Cui, Y., and Keppler, D. (1999). *Hepatology* **29**, 1156–1163.
Kool, M., de Haas, M., Scheffer, G. L., Scheper, R. J., van Eijk, M. J., Juijn, J. A., Baas, F., and Borst, P. (1997). *Cancer Res.* **57**, 3537–3547.
Kool, M., van der Linden, M., de Haas, M., Baas, F., and Borst, P. (1999a). *Cancer Res.* **59**, 175–182.
Kool, M., van der Linden, M., de Haas, M., Scheffer, G. L., de Vree, J. M., Smith, A. J., Jansen, G., Peters, G. J., Ponne, N., Scheper, R. J., Elferink, R. P., Baas, F., and Borst, P. (1999b). *Proc. Natl. Acad. Sci. U.S.A.* **96**, 6914–6919.
Krishnamachary, N., and Center, M. S. (1993). *Cancer Res.* **53**, 3658–3661.
Kruh, G. D., Chan, A., Myers, K., Gaughan, K., Miki, T., and Aaronson, S. A. (1994). *Cancer Res.* **54**, 1649–1652.
Kruh, G. D., Gaughan, K. T., Godwin, A., and Chan, A. (1995). *J. Natl. Cancer. Inst.* **87**, 1256–1258.
Kuo, M. T., Bao, J. J., Curley, S. A., Ikeguchi, M., Johnston, D. A., and Ishikawa, T. (1996). *Cancer Res.* **56**, 3642–3644.
Lacave, R., Coulet, F., Ricci, S., Touboul, E., Flahault, A., Rateau, J. G., Cesari, D., Lefranc, J. P., and Bernaudin, J. F. (1998). *Br. J. Cancer.* **77**, 694–702.
Laing, N. M., Belinsky, M. G., Kruh, G. D., Bell, D. W., Boyd, J. T., Barone, L., Testa, J. R., and Tew, K. D. (1998). *Cancer Res.* **58**, 1332–1337.
Le Saux, O., Urban, Z., Tschuch, C., Csiszar, K., Bacchelli, B., Quaglino, D., Pasquali-Ronchetti, I., Pope, F. M., Richards, A., Terry, S., Bercovitch, L., de Paepe, A., and Boyd, C. D. (2000). *Nat. Genet.* **25**, 223–227.
Lee, K., Belinsky, M. G., Bell, D. W., Testa, J. R., and Kruh, G. D. (1998). *Cancer Res.* **58**, 2741–2747.
Lee, K., Klein-Szanto, A., and Kruh, G. D. (2000). *J. Natl. Cancer. Inst.* **92**, 1934–1940.
Leier, I., Jedlitschky, G., Buchholz, U., Cole, S. P., Deeley, R. G., and Keppler, D. (1994). *J. Biol. Chem.* **269**, 27807–27810.
Ling, V. (1997). *Cancer Chemother. Pharmacol.* **40**, S3–S8.
Loe, D. W., Almquist, K. C., Cole, S. P., and Deeley, R. G. (1996a). *J. Biol. Chem.* **271**, 9683–9689.
Loe, D. W., Almquist, K. C., Deeley, R. G., and Cole, S. P. (1996b). *J. Biol. Chem.* **271**, 9675–9682.
Longhurst, T. J., O'Neill, G. M., Harvie, R. M., and Davey, R. A. (1996). *Br. J. Cancer* **74**, 1331–1335.
Lorico, A., Rappa, G., Finch, R. A., Yang, D., Flavell, R. A., and Sartorelli, A. C. (1997). *Cancer Res.* **57**, 5238–5242.
Lorico, A., Rappa, G., Flavell, R. A., and Sartorelli, A. C. (1996). *Cancer Res.* **56**, 5351–5355.
Lutzky, J., Astor, M. B., Taub, R. N., Baker, M. A., Bhalla, K., Gervasoni, J. E., Jr., Rosado, M., Stewart, V., Krishna, S., and Hindenburg, A. A. (1989). *Cancer Res.* **49**, 4120–4125.
Madon, J., Eckhardt, U., Gerloff, T., Stieger, B., and Meier, P. J. (1997). *FEBS Lett.* **406**, 75–78.
Madon, J., Hagenbuch, B., Landmann, L., Meier, P. J., and Stieger, B. (2000). *Mol. Pharmacol.* **57**, 634–641.
Marquardt, D., McCrone, S., and Center, M. S. (1990). *Cancer Res.* **50**, 1426–1430.
Marsh, W., and Center, M. S. (1987). *Cancer Res.* **47**, 5080–5161.
Marsh, W., Sicheri, D., and Center, M. S. (1986). *Cancer Res.* **46**, 4053–4057.
Masuda, M., I'izuka, Y., Yamazaki, M., Nishigaki, R., Kato, Y., Ni'inuma, K., Suzuki, H., and Sugiyama, Y. (1997). *Cancer Res.* **57**, 3506–3510.
Mayer, R., Kartenbeck, J., Buchler, M., Jedlitschky, G., Leier, I., and Keppler, D. (1995). *J. Cell. Biol.* **131**, 137–150.
McAleer, M. A., Breen, M. A., White, N. L., and Matthews, N. (1999). *J. Biol. Chem.* **274**, 23541–23548.
McGrath, T., and Center, M. S. (1987). *Biochem. Biophys. Res. Commun.* **145**, 1171–1176.
McGrath, T., Latoud, C., Arnold, S. T., Safa, A. R., Felsted, R. L., and Center, M. S. (1989). *Biochem. Pharmacol.* **38**, 3611–3619.
Miyake, K., Mickley, L., Litman, T., Zhan, Z., Robey, R., Cristensen, B., Brangi, M., Greenberger, L., Dean, M., Fojo, T., and Bates, S. E. (1999). *Cancer Res.* **59**, 8–13.
Morrow, C. S., Smitherman, P. K., Diah, S. K., Schneider, E., and Townsend, A. J. (1998). *J. Biol. Chem.* **273**, 20114–20120.
O'Brien, M., Kruh, G. D., and Tew, K. D. (2000). *J. Pharmacol. Exp. Ther.* **294**, 480–487.
Ortiz, D. F., Li, S., Iyer, R., Zhang, X., Novikoff, P., and Arias, I. M. (1999). *Am. J. Physiol.* **276**, G1493–G1500.
Paulusma, C. C., Bosma, P. J., Zaman, G. J., Bakker, C. T., Otter, M., Scheffer, G. L., Scheper, R. J., Borst, P., and Oude Elferink, R. P. (1996). *Science* **271**, 1126–1128.
Paulusma, C. C., Kool, M., Bosma, P. J., Scheffer, G. L., ter Borg, F., Scheper, R. J., Tytgat, G. N., Borst, P., Baas, F., and Oude Elferink, R. P. (1997). *Hepatology* **25**, 1539–1542.
Paulusma, C. C., van Geer, M. A., Evers, R., Heijn, M., Ottenhoff, R., Borst, P., and Oude Elferink, R. P. (1999). *Biochem. J.* **338**, 393–401.
Ringpfeil, F., Lebwohl, M. G., Christiano, A. M., and Uitto, J. (2000). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6001–6006.
Saxena, M., and Henderson, G. B. (1995). *J. Biol. Chem.* **270**, 5312–5319.
Schneider, E., Yamazaki, H., Sinha, B. K., and Cowan, K. H. (1995). *Br. J. Cancer.* **71**, 738–743.
Schuetz, J. D., Connelly, M. C., Sun, D., Paibir, S. G., Flynn, P. M., Srinivas, R. V., Kumar, A., and Fridland, A. (1999). *Nat. Med.* **5**, 1048–1051.
Suzuki, T., Nishio, K., Sasaki, H., Kurokawa, H., Saito-Ohara, F., Ikeuchi, T., Tanabe, S., Terada, M., and Saijo, N. (1997). *Biochem. Biophys. Res. Commun.* **238**, 790–794.
Takikawa, H., Sano, N., Narita, T., Uchida, Y., Yamanaka, M., Horie, T., Mikami, T., and Tagaya, O. (1991). *Hepatology* **14**, 352–360.
Taniguchi, K., Wada, M., Kohno, K., Nakamura, T., Kawabe, T., Kawakami, M., Kagotani, K., Okumura, K., Akiyama, S., and Kuwano, M. (1996). *Cancer Res.* **56**, 4124–4129.
Tusnady, G. E., Bakos, E., Varadi, A., and Sarkadi, B. (1997). *FEBS Lett.* **402**, 1–3.
Uchiumi, T., Hinoshita, E., Haga, S., Nakamura, T., Tanaka, T., Toh, S., Furukawa, M., Kawabe, T., Wada, M., Kagotani, K., Okumura, K., Kohno, K., Akiyama, S., and Kuwano, M. (1998). *Biochem. Biophys. Res. Commun.* **252**, 103–110.
van Aubel, R. A., Koenderink, J. B., Peters, J. G., Van Os, C. H., and Russel, F. G. (1999). *Mol. Pharmacol.* **56**, 714–719.
van Aubel, R. A., van Kuijck, M. A., Koenderink, J. B., Deen, P. M., van Os, C. H., and Russel, F. G. (1998). *Mol. Pharmacol.* **53**, 1062–1067.
Versantvoort, C. H., Broxterman, H. J., Bagrij, T., Scheper, R. J., and Twentyman, P. R. (1995). *Br. J. Cancer.* **72**, 82–89.
Wijnholds, J., Evers, R., van Leusden, M. R., Mol, C. A., Zaman, G. J., Mayer, U., Beijnen, J. H., van der Valk, M., Krimpenfort, P., and Borst, P. (1997). *Nat. Med.* **3**, 1275–1279.
Wijnholds, J., Mol, C. A., van Deemter, L., de Haas, M., Scheffer, G. L., Baas, F., Beijnen, J. H., Scheper, R. J., Hatse, S., De Clercq, E.,

Balzarini, J., and Borst, P. (2000). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7476–7481.

Wijnholds, J., Scheffer, G. L., van der Valk, M., van der Valk, P., Beijnen, J. H., Scheper, R. J., and Borst, P. (1998). *J. Exp. Med.* **188**, 797–808.

Zaman, G. J., Flens, M. J., van Leusden, M. R., de Haas, M., Mulder, H. S., Lankelma, J., Pinedo, H. M., Scheper, R. J., Baas, F., Broxterman, H. J., and Borst, P. (1994). *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8822–8826.

Zaman, G. J., Lankelma, J., van Tellingen, O., Beijnen, J., Dekker, H., Paulusma, C., Oude Elferink, R. P., Baas, F., and Borst, P. (1995). *Proc. Natl. Acad. Sci. U.S.A.* **92**, 7690–7694.

Zeng, H., Bain, L. J., Belinsky, M. G., and Kruh, G. D. (1999). *Cancer Res.* **59**, 5964–5967.

Zeng, H., Liu, G., Rea, P. A., and Kruh, G. D. (2000). *Cancer Res.* **60**, 4779–4784.

Zeng, H., Chen, Z. S., Belinsky, M. G., Rea, P. A., and Kruh, G. D. (2001). *Cancer Res.* **61**, 7225–7232.