Review

Pharmacogenetics of ATP-binding Cassette Transporters in Cancer and Chemotherapy[1]

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Abstract

The ATP-binding cassette (ABC) transporters belong to the largest known transporter gene family and translocate a variety of substrates including chemotherapy agents. ABC multidrug transporter expression has been implicated in tumor cell resistance to anticancer therapy, altered disposition of chemotherapy drugs, and associated chemotherapy toxicity. More recently, genetic heterogeneity has been described in a number of the ABC transporter genes, including ABC transporters that contribute to the pharmacokinetics and/or pharmacodynamics of chemotherapy drugs. The role of these transporters and their naturally occurring genetic polymorphisms in cancer and chemotherapy is reviewed.

Introduction

Limiting cellular exposure to toxic xenobiotics is critical to the survival of the host organism. Membrane-bound transporter proteins have emerged as a key defense mechanism against potential toxins. Among the various uptake and efflux transporters expressed in prokaryotic as well as eukaryotic cells, members of the so-called ABC[3] superfamily of transporters are remarkably conserved across species and importantly involved in the transport of endobiotics as well as xenobiotics.

The ABC transporters belong to the largest known transporter gene family (1, 2). These intracellular and extracellular membrane-spanning proteins translocate a variety of substrates including sugars, amino acids, metal ions, peptides, proteins, and hydrophobic compounds across cellular compartments (3). Due to the multiplicity of transport functions and substrates, mutations in genes encoding these transporters frequently cause or contribute to human genetic disorders including cystic fibrosis, neurological disease, cholesterol and bile transport defects, anemia, and unanticipated drug toxicity (4). Importantly, expression of the ABC multidrug transporters has been implicated in tumor cell resistance to anticancer therapy, altered disposition of chemotherapy drugs, and associated chemotherapy toxicity. More recently, genetic heterogeneity has been described in a number of the ABC transporter genes, including ABC transporters that contribute to the pharmacokinetics and/or pharmacodynamics of chemotherapy drugs. In this review, the role of naturally occurring polymorphisms in the genes encoding ABC transporters and their potential relevance to cancer chemotherapy will be outlined.

ABC Transporters

The ABC transporters primarily function to transport substrates ranging from low molecular weight molecules to polypeptides across biological membranes (Fig. 1). Despite their diverse substrate specificities, members of the ABC transporter superfamily exhibit a number of structural similarities. They are composed of a combination of functional units (specifically, the MSDs, which are usually composed of six transmembrane helices, and the remarkably conserved NBDs). The NBD consists of two domains, a glycine rich P-loop also known as the Walker A domain that binds to ATP at a phosphate group and the Walker B domain containing an aspartate, which interacts with nucleotide-associated magnesium (5, 6). The structure of the ABC transporters tends to increase in complexity from prokaryotic systems to mammalian systems by increasing the number of these modular functional units. For example, the simplest prokaryotic ABC transporters contain one functional domain, whereas the eukaryotic MRPs have multiple functional units (7, 8). In general, eukaryotic ABC transporters contain two NBDs and two MSDs (9).

Based on phylogenetic analysis, the ABC transporters have been categorized into seven subfamilies designated as ABCA through ABCG (Table 1). There are common substrate characteristics shared by many members of particular ABC subfamilies; however, there is also considerable substrate and functional heterogeneity within families, making an assignment of general biological function based on subfamily membership inaccurate (3).

ABC Subfamily A: ABCA2 (ABC2)

Transporters in the ABCA subfamily have been primarily implicated in lipid homeostasis. The discussion here is lim-

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[3] The abbreviations used are: ABC, ATP-binding cassette; MSD, membrane-spanning domain; NBD, nucleotide-binding domain; MRP, multidrug resistance-associated protein; SNP, single nucleotide polymorphism; AML, acute myelogenous leukemia; MDR, multidrug resistance; GSH, reduced glutathione; PXE, pseudoxanthoma elasticum; LTC_4, leukotriene C4; DJS, Dubin-Johnson syndrome; P-gp, P-glycoprotein.

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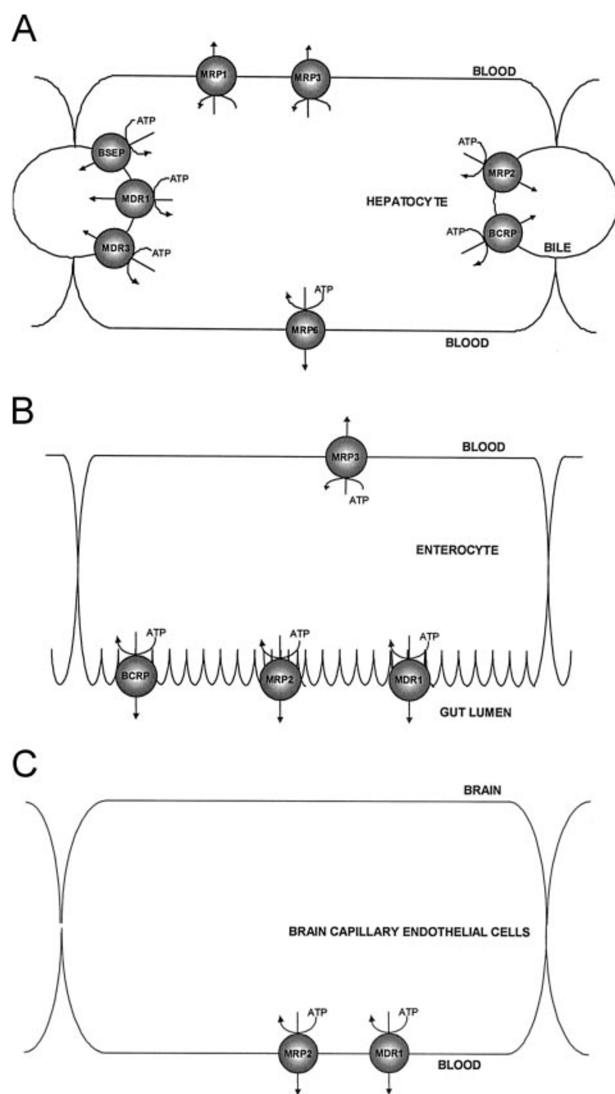

Fig. 1. Schematic diagram depicting ABC transporters and their localization in the human hepatocyte (*A*), small intestinal enterocyte (*B*), and blood-brain barrier (*C*).

ited to ABCA2 because this is the only known transporter in this subfamily involved in cancer or cancer chemotherapy.

Estramustine is a nitrogen mustard chemotherapy agent used in the treatment of hormone-refractory metastatic prostate cancer. However, resistance to estramustine in these cancers has been demonstrated despite a lack of overexpression of P-gp, the *MDR1* (*ABCB1*)-encoded gene product classically associated with resistance to chemotherapy agents (10). To explore the possibility that estramustine resistance was related to an overexpression of an alternate efflux pump, Laing *et al.* (11) developed an estramustine-resistant ovarian carcinoma cell line. Cytogenetic analysis of the resistant cell line revealed a homogeneously staining region at chromosome 9q34. *ABCA2*, a previously described ABC transporter, was localized to this site (12).

The *ABCA2* gene encodes a full-sized transporter protein consisting of 2436 amino acids with an apparent molecular weight of 270,000, notable for a tandem repeat of the hydrophobic domain with six transmembrane helices followed by an ABC domain (13). In subsequent studies of normal tissues, ABCA2 expression was noted to be highest in the brain and lower in the kidney and liver. Although chemotherapy resistant cell lines treated with antisense ABCA2 mRNA can result in resensitization of chemotherapy resistant cells to estramustine (13), the physiological role and clinical significance of ABCA2 is unknown. A potential role of ABCA2 in steroid or lipid disposition has been proposed (13, 14).

According to the SNP database, there are no reported polymorphisms in ABCA2.

ABC Subfamily B

ABCB1 (MDR1). MDR1 (ABCB1) was the first ABC transporter described and is the most extensively studied ABC transporter. The link between MDR1 expression and cancer has evolved since the initial observation that Ehrlich ascites cells actively decreased their intracellular concentration of daunorubicin and the subsequent discovery of P-gp in multidrug-resistant cells (15, 16).

The *MDR1* gene is located on chromosome 7 and encodes a M_r 170,000 transporter that regulates the transport of a large range of amphipathic hydrophobic substrates, including cytotoxic chemotherapeutic agents, hormones, and carcinogens as well as an array of structurally divergent drugs (17–22). The structure of MDR1 has been proposed as a single polypeptide consisting of two homologous halves, each containing a MSD and a NBD in a 6 + 6 hexagonal ring, surrounding a large aqueous pore (23, 24). This 6 + 6 model is widely accepted, although other studies have reported conflicting results (23–26).

MDR1 is expressed at high levels in some cancers and has been associated with clinical drug resistance, making modulation of this resistance pathway an attractive therapeutic strategy (27–29). However, this membrane efflux transporter is also found in normal tissues, such as the canalicular domain of hepatocytes, kidney, small intestine, colon, adrenal glands, and the capillary endothelium of the brain and testes (27, 30, 31).

Therapeutic agents targeted to exploit the role of MDR1 in cancer chemotherapy resistance, MDR1 modulators, have been developed and have entered clinical evaluation. Unfortunately Phase I and II trials of the early MDR1 modulators demonstrated unacceptable toxicity at doses required to achieve inhibitory plasma concentrations of the drugs (32). Furthermore, some trials have demonstrated a number of undesirable pharmacokinetic interactions with the anticancer drugs. The modulators tend to increase the serum half-life and thus the area under the curve of the concurrently prescribed cytotoxic agents, presumably by inhibiting their clearance (33–37). Despite these potential problems, a randomized clinical trial by the Southwest Oncology Group demonstrated that treatment with cyclosporine A, an inhibitor of MDR1, resulted in a statistically significant improvement in remission duration and survival in patients with acute myeloid leukemia (38). However, this effect may have been due to higher plasma concentrations of daunorubicin rather than the MDR1 inhibitory effects of cyclosporine A.

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Table 1 Localization and role of the ABC transporters involved in cancer and chemotherapy

Symbol	Transporter	Gene location	Tissue location	Role in cancer or chemotherapy
ABCA2	ABC2	9q34	Brain	Estramustine resistance
ABCB1	PGP, PGY1, MDR	7p21	Adrenal, kidney, brain, intestine, liver	Multidrug resistance, tumor prognostic factor
ABCC1	MRP1	16p13.1	Lung, testes, erythrocytes	Multidrug resistance, tumor prognostic factor
ABCC2	MRP2	10q24	Liver, kidney	Cannalicular organic ion transporter
ABCC3	MRP3	17q21.3	Lung, intestine, liver	Multidrug resistance in lung cancer and other tumors
ABCC4	MRP4	13q32	Prostate, liver, kidney	Nucleoside analogue transport
ABCC5	MRP5	3q27	Ubiquitous	Nucleoside analogue transport/resistance
ABCC6	MRP6	16p13.1	Kidney, liver	Anthracycline and epipodophyllotoxins resistance
ABCC11	MRP8	16q12.1	Breast, testes	Unknown role in breast cancer
ABCC12	MRP9	16q11	Breast, testes	Unknown role in breast cancer
ABCG2	ABCP, MXR, BCRP	4q22	Placenta, intestine, liver	Mitoxantrone, anthracycline, topotecan, methotrexate, and SN-38 resistance

Due to MDR1's importance as a determinant of chemotherapeutic drug resistance as well as drug bioavailability, there has been much interest in the study of functional polymorphisms in MDR1. A list of known polymorphisms is provided in Table 2. The most studied *MDR1* polymorphism, C3435T at exon 26, has been associated with variable P-gp expression in intestinal epithelial cells and in a subset of lymphoid cells (39, 40). Specifically, MDR1 expression is substantially lower in people with the T/T genotype than in those with the C/C genotype. Additionally, these genetic differences appear to vary with ethnicity, such that subjects of African origin had a frequency of 73–84% for the C allele compared with a frequency of 34–59% in subjects of European and Asian origin, inferring important prognostic and therapeutic implications for use of MDR1-dependent drugs in individuals of African descent (41). An examination of the relationships between these common naturally occurring polymorphisms and the pharmacokinetics of commonly prescribed drugs has indicated correlations between allelic variability and drug levels of digoxin, phenytoin, and fexofenadine (39, 42, 43). In a population of patients treated for HIV with the antiretroviral agents efavirenz and nelfinavir, plasma concentrations of these drugs varied with allelic status and as a consequence predicted immune recovery after treatment initiation (44). Conversely, no statistical differences in cyclosporine pharmacokinetics were found depending on the C or T allelic status (45). It should be noted that in most subjects, the exon 21 G2677T polymorphism is linked to the exon 26 C3435T polymorphism. Therefore, haplotypes in *MDR1* may have been better predictors of the functional consequences of polymorphisms in this transporter.

Similarly, the previously described G2677T polymorphism in exon 21 has been studied for its contribution to disease and therapeutic response. G2677T was not a major determinant of MDR1 function in hematopoietic stem cells or associated with altered MDR1 expression or function in AML (46, 47). However, AML patients homozygous for the T or G alleles had a shorter time to relapse and a shorted overall survival than their heterozygous counterparts (47). Recent investigations of the effects of common naturally occurring somatic polymorphisms in *MDR1* on the clinical outcomes in patients with AML demonstrated differences in the rate of relapse and the presence of poor prognostic features depending on allele expression. The observed outcomes did not correlate directly with MDR1 expression, indicating that allelic variants of the *MDR1* gene may influence therapy outcome by additional mechanisms, different from P-gp expression, such as the pharmacokinetic effects of P-gp (48).

Studies specifically evaluating allelic variations in *MDR1* and their relationship to chemotherapy disposition are under way, and preclinical and clinical trials of newer MDR1 modulating agents with greater specificity and potency are ongoing.

ABC Subfamily C

ABCC1 (MRP1). Subsequent to the discovery of P-gp, investigations of cancer cells displaying the MDR phenotype not associated with MDR1 expression led to the discovery of MRP1, the founding member of the MRP subfamily (49). The MRP1-mediated MDR cellular phenotype was confirmed through transfection studies (50, 51). Studies have shown that MRP1 preferentially transports negatively charged compounds often conjugated with glutathione (52, 53). Additionally, sulfate conjugates, glucuronides, and unmodified compounds in the presence of GSH are also substrates for MRP1 (54–57). With regard to drug resistance, the latter mechanism appears to predominate because there is no evidence for conjugation of GSH to drugs to which MRP1 confers resistance (58, 59). There are indications that MRP1 mediates GSH transport (59, 60) and may function as a cotransporter for GSH and the drug.

MRP1 is a M_r 190,000 membrane-spanning protein that shares 15% amino acid homology with MDR1, and the *MRP1* gene is located on chromosome 16. Based on experimental data and protein folding algorithms, MRP1 has five transmembrane segments in MSD_1, six transmembrane segments in MSD_2, and four or six transmembrane segments in MSD_3 (8, 61). *MRP1* mRNA and MRP1 protein are broadly expressed in the epithelial cells of multiple tissues including the digestive, urogenital, and respiratory tracts, in the endocrine glands, and in the hematopoietic system (62). MRP1 expres-

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Table 2 Summary of genetic polymorphisms in ABC transporters involved in cancer chemotherapy

Name	Polymorphism	Position	Effect	Function	Ref. no.
ABCA2	?	?	?	?[a]	
ABCB1	A61G	Exon	N21D	?	39–41, 43, 83, and 166–173
	T307C	Exon	F103L	?	
	A548G	Exon	N183S	?	
	A729G	Exon	Synonymous	↔	
	G1199A	Exon	S400N	↔	
	C1236T	Exon	Synonymous	↔	
	C1474T	Exon	R4892C	?	
	C2650T	Exon	Synonymous	↔	
	G2677T	Exon	A893S	↔	
	G2677A	Exon	A893T	?	
	A2956G	Exon	M986V	?	
	G2995A	Exon	A999T	?	
	A3320C	Exon	Q1107P	?	
	C3396T	Exon	Synonymous	?	
	T3421A	Exon	S1141T	?	
	C3435T	Exon	Synonymous	↓	
ABCC1	Gene deletion	Exon	No protein	ϕ	49, 81–83, 85, and 174
	G128C	Exon	C43S	?	
	C218T	Exon	T73I	?	
	C350T	Exon	T117M	?	
	T825C	Exon	Synonymous	↔	
	T1062C	Exon	Synonymous	↔	
	C1303A	Exon	R433S	↓	
	T1684C	Exon	Synonymous	↔	
	G1898A	Exon	R633Q	?	
	C2001T	Exon	Synonymous	↔	
	C2007T	Exon	Synonymous	↔	
	G2012T	Exon	G671V	?	
	G2168A	Exon	R723Q	?	
	C2665T	Exon	Synonymous	↔	
	T2694C	Exon	Synonymous	↔	
	G3173A	Exon	R1058Q	?	
	G3450A	Exon	Synonymous	?	
	G4002A	Exon	Synonymous	↔	
	C4524T	Exon	Synonymous	↔	
	C4535T	Exon	S1512L	?	
ABCC2	C56T	Exon	P19L	?	103, 105, 108, and 174–178
	A234G	Exon	Synonymous	↔	
	G299A	Exon	R100Q	?	
	G842A	Exon	S281N	?	
	G1249A	Exon	V417I	?	
	C1457T	Exon	T486I	?	
	T1815+2A	Intron	Splice	ϕ	
	C1967+2T	Intron	Splice	ϕ	
	C2302T	Exon	R768W	ϕ	
	C2366T	Exon	S789F	ϕ	
	T2439+2C	Intron	Splice	ϕ	
	G2647A	Exon	D833N	?	
	A2882G	Exon	K961R	?	
	G2934A	Exon	Synonymous	↔	
	C3039T	Exon	Synonymous	↔	
	G3057T	Exon	Q1019H	?	
	C3196T	Exon	R1066X	ϕ	
	G3321T	Exon	Synonymous	↔	
	G3449A	Exon	R1150H	↓	
	A3517T	Exon	I1173F	↓	
	G3521A	Exon	R1174H	?	
	T3563A	Exon	V1188E	?	
	T3732G	Exon	N1244K	?	
	A3897C	Exon	K1299Q	?	
	C3927T	Exon	Synonymous	?	
	C3972T	Exon	Synonymous	↔	
	C4100G	Exon	S1367C	?	
	A4145G	Exon	Q1382R	ϕ	
	Del 4170-5	Exon	Del R,M	ϕ	
	G4290T	Exon	Synonymous	↔	
	G4348A	Exon	A1450T	?	
	C4488T	Exon	Synonymous	↔	
	G4544A	Exon	C1515Y	?	
ABCC3	T124C	Exon	C42R	?	109, 111–113, 115, 174, and 179
	G258A	Exon	Synonymous	↔	
	C1031G	Exon	A344G	?	
	C1633T	Exon	Synonymous	↔	
	T1706A	Exon	F569Y	?	
	C3039T	Exon	Synonymous	?	
	C3932T	Exon	Synonymous	↔	
	C3942T	Exon	Synonymous	?	
	C4048G	Exon	L1362V	?	
	A4509G	Exon	Synonymous	↔	
ABCC4	T513G	Exon	C171G	?	174 and 180
	T669C	Exon	Synonymous	↔	
	G906T	Exon	K302N	?	
	G951A	Exon	Synonymous	?	
	G969A	Exon	Synonymous	?	
	C1497T	Exon	Synonymous	↔	
	G2271A	Exon	E757K	?	
	A2712G	Exon	Synonymous	?	
	C2844T	Exon	Synonymous	?	
	A3348G	Exon	Synonymous	?	
ABCC5	GC527-8CG	Exon	R176P	?	111, 126, 128, and 181
	A723G	Exon	Synonymous	↔	
	G1146A	Exon	Synonymous	↔	
	C1185T	Exon	Synonymous	↔	
	AGC1198-200GGT	Exon	S400G	?	
	C1200T	Exon	Synonymous	↔	
	A1741G	Exon	I581V	?	
	T1782C	Exon	Synonymous	↔	
	T3624C	Exon	Synonymous	↔	
	C4148A	Exon	T1383N	?	
ABCC6	Gene deletion	Exon	No protein	ϕ	79–81, 109, 132, 134, 136, 139, and 182–187
	Del 15	Exon	Frameshift	ϕ	
	Del 23–29	Exon	Frameshift	ϕ	
	Del 179–195	Exon	Frameshift	ϕ	
	G189C	Exon	Synonymous	↔	
	T190C	Exon	W64R	?	
	G549A	Exon	Synonymous	↔	
	C681G	Exon	Y227X	ϕ	
	938-939insT	Exon	Frameshift	ϕ	
	960 del C	Exon	Frameshift	ϕ	
	C1132T	Exon	Q378X	?	
	T1233C	Exon	Synonymous	↔	
	G1245A	Exon	Synonymous	↔	
	G1363C	Exon	A455P	ϕ	
	C1552T	Exon	R518X	ϕ	
	G1553A	Exon	R518Q	ϕ	
	T1703C	Exon	F568S	ϕ	
	T1841C	Exon	V614A	↔	
	C1890G	Exon	Synonymous	↔	
	C1896A	Exon	H632Q	?	
	1944 del 22	Exon	Frameshift	ϕ	
	Del 1967–89	Exon	Frameshift	ϕ	
	1995 del G	Exon	Frameshift	ϕ	
	T2018C	Exon	L673P	ϕ	
	G2294A	Exon	R765Q	ϕ	
	C2304A	Exon	Y768X	ϕ	
	2322 del C	Exon	Frameshift	ϕ	
	C2490T	Exon	Synonymous	↔	
	2542 del G	Exon	Frameshift	ϕ	
	GIVS21+1T	Intron	Splice	ϕ	
	C3088T	Exon	R1030X	ϕ	
	G3341C	Exon	R1114P	ϕ	
	C3362G	Exon	S1121W	ϕ	
	C3412T	Exon	R1138W	ϕ	
	G3413A	Exon	R1138Q	ϕ	
	G3413C	Exon	R1138P	ϕ	
	C3421T	Exon	R1141X	ϕ	
	C3490T	Exon	R1164X	ϕ	
	G3608A	Exon	G1203D	ϕ	

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Table 2 Continued

Name	Polymorphism	Position	Effect	Function	Ref. no.
	GIVS26-1A	Intron	Splice	ϕ	
	C3709T	Exon	Splice	ϕ	
	G3736-1A	Exon	Q1237X	ϕ	
	3775 del T	Exon	Frameshift	ϕ	
	3798 del T	Exon	Frameshift	$\leftrightarrow$	
	G3803A	Exon	R1268Q	$\downarrow$	
	G3892T	Exon	V1298F	$\downarrow$	
	C3902T	Exon	T1301I	ϕ	
	G3904A	Exon	G1302R	ϕ	
	G3907C	Exon	A1303P	ϕ	
	C3940T	Exon	R1314W	$\downarrow$	
	G3941A	Exon	R114Q	ϕ	
	G3961A	Exon	G1321S	ϕ	
	C3978T	Exon	Synonymous	$\leftrightarrow$	
	C4015T	Exon	R1339C	ϕ	
	G4041C	Exon	Q1347H	ϕ	
	G4081A	Exon	D1361N	ϕ	
	4104 del C	Exon	Frameshift	ϕ	
	C4192T	Exon	R1398X	ϕ	
	4220 ins AGAA	Exon	Frameshift	ϕ	
	4243 ins AGAA	Exon	Frameshift	ϕ	
	T4271C	Exon	I1424T	ϕ	
ABCC11	?	?	?	?	
ABCC12	?	?	?	?	
ABCG2	G34A	Exon	V12M	?	150, 151, and 188
	G71T	Exon	A24V	?	
	C376T	Exon	Nonsense	$\downarrow$	
	C421A	Exon	Q141K	$\downarrow$	
	C496G	Exon	Q166E	?	
	A616C	Exon	I206L	?	
	T623C	Exon	F208S	?	
	G1086A	Exon	Synonymous	$\leftrightarrow$	
	A1768T	Exon	N590Y	?	

[a] ?, unknown effect on transport function; $\leftrightarrow$, does not alter transport activity; $\downarrow$, decreased transport activity; ϕ, null function.

sion has been demonstrated in multiple tumor tissues and has been implicated as a component of the MDR phenomenon in cancers of the lung, colon, breast, bladder, and prostate as well as leukemias (63, 64). As a prognostic factor, however, clinical evidence is limited, but MRP1 expression has been associated with amplification of the N-*myc* oncogene and reduced survival in neuroblastoma patients and has been suggested as a potential prognostic indicator in M4 subtype AML with abnormal eosinophils and retinoblastoma (65–67).

With regard to the role of MRP1 as a drug transporter, the best-characterized substrate for MRP1 is LTC_4, the arachidonic acid-derived chemical mediator of inflammation (68–70). In terms of chemotherapeutic agents, MRP1 has been shown to transport glutathione conjugates of several drugs, including alkylating agents as well as etoposide and doxorubicin, but only confers resistance to the latter agents (71–74). Additional agents in the MRP1 resistance profile include the *Vinca* alkaloids, methotrexate, and certain arsenic and antimonial centered oxyanions (50, 75–77). The clinical significance of the ability of MRP1 to transport conjugated forms of certain chemotherapy agents has not been determined, nor has a direct role in the disposition of drugs during cancer chemotherapy been observed. The development of inhibitory modulators of multiple multidrug transporters is in progress (78).

Polymorphisms in *MRP1* have been described in studies linking PXE with mutations in MRP6 due to close proximity of the genes for both transporters on chromosome 16. In a population of PXE patients who were otherwise phenotypically normal, large chromosomal deletions encompassing the entire *MRP1* and *MRP6* genes on chromosome 16 have been described (79, 80). SNPs in *MRP1* have also been described in studies examining the genetic basis for PXE (79–82) as well as in another study that screened for *MRP1* polymorphisms in a Japanese population. However polymorphisms in *MRP1*, without the inclusion of variations in *MRP6*, do not appear to play a role in PXE (83).

The impact of polymorphisms in *MRP1* on drug disposition has not been studied extensively (Table 2). For the most part, MRP1 appears to be highly conserved, in terms of SNPs. G671V, a polymorphism resulting in amino acid substitutions near the first NBD, was evaluated using a transfection construct. Transport of the MRP1 substrates LTC_4, 17β-estradiol 17β-(D)-glucuronide, and estrone sulfate using membrane vesicles prepared from transfected cells was comparable with that of wild-type MRP1 (84). In a series of transport experiments with membrane vesicles, a second low-frequency ($<$1%) naturally occurring mutation in MRP1, R433S, resulted in a 2-fold reduction in the ATP-dependent transport of LTC_4 and estrone sulfate and, conversely, a 2-fold increase in resistance to doxorubicin, whereas resistance to tested *Vinca* alkaloids was unaffected (85). The studies on R433S provide an example of a naturally occurring mutation in *MRP1* that results in an altered transport phenotype. Additional studies are required to examine the impact of *MRP1* polymorphisms on clinical drug disposition as well as the response to chemotherapeutic agents.

ABCC2 (MRP2). Organic anion transporters in hepatocyte canalicular membranes were known to exist prior to their definitive molecular characterization (70, 86). The canalicular multispecific organic anion transporter, now referred to as MRP2, was previously studied using conventional biochemical techniques, but its substrate specificity and relationship to MRP1 were defined using the MRP2-deficient (TR−) animal model. In humans, deficiency of MRP2 results in DJS (87). Both TR− rats and humans with DJS characteristically demonstrate chronic conjugated hyperbilirubinemia as a result of loss of function mutations in *MRP2*.

Unlike MRP1, which is usually located on the basolateral surfaces of epithelial cells, MRP2 is located on the apical membranes of hepatocytes, renal proximal tubules, and intestinal epithelia (88). The *MRP2* gene has been localized to chromosome 10, spans 45 kb, and contains 32 exons. The human MRP2 protein consists of 1545 amino acids and serves to secrete exogenous and endogenous substances usually conjugated with glutathione, glucuronide, and sulfate to form anionic moieties and then secrete them across the canalicular membrane (89). Structurally, MRP2 is similar to MRP1 with two NBDs, three MSDs, and an extracellular NH_2 terminus. However, MRP2 and MRP1 differ in their tissue localization and their transport kinetic properties (90, 91).

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Drug resistance conferred by MRP2 has been examined in various systems to determine the role of MRP2 in chemotherapy resistance. Tumor cell lines expressing drug resistance phenotypes have been evaluated for their level of MRP2 expression. *MRP2* mRNA overexpression corresponded with cisplatin resistance (92–94). In recombinant systems, the expression of MRP2 enhanced resistance to etoposide, vincristine, cisplatin, doxorubicin, and epirubicin (90). In a third approach using antisense cDNA, sensitivity to cisplatin, vincristine, doxorubicin, and CPT-11 was restored in cell lines expressing MRP2 (95). In addition to the previously listed agents, it has also been reported that MRP2 plays a role in the excretion of methotrexate into the bile (96). Similarly, methotrexate resistance was conferred to ovarian cancer cell lines after short-term exposure to methotrexate and transfection with MRP2 (75). Interestingly, reversal of methotrexate resistance has been demonstrated *in vitro* when site-specific mutations were introduced into *MRP2* (97). In clinical specimens, MRP2 expression has been demonstrated in renal clear cell carcinomas at both the mRNA and protein level and has been demonstrated by RT-PCR, immunoblotting, and immunofluorescence microscopy in lung, gastric, hepatocellular, and colorectal cancer cells (98–101). MRP2 therefore appears to be involved in the resistance of many of these tumor types to various chemotherapies, especially cisplatin. To date, there has been no demonstrated correlation between MRP2 expression and clinical outcome.

A number of genetic polymorphisms in MRP2 are associated with the DJS, a condition resulting in hyperbilirubinemia. In the known cases, these mutations are associated with a complete absence of immunochemically detectable MRP2 in affected individuals, postulated to be due to rapid degradation of mutated *MRP2* mRNA, impaired MRP2 protein maturation, or inappropriate MRP2 trafficking (102–105). Additionally, the mutations are varied and range from point mutations to bp deletions leading to missense mutations, premature stop codons, and aberrant RNA splicing (Table 2). The relationship of mutations in *MRP2* to the disposition of chemotherapeutic agents in humans is unknown because only the disposition of sulfobromophthalein and synthetic sulfobromophthalein-glutathione has been studied in patients with DJS (106). However, *in vivo* evidence from animal studies indicates that the MRP2 transporter may play a role in the disposition of certain chemotherapeutic agents [specifically, CPT-11 and its metabolites (107)]. In rats carrying loss of function mutations in *MRP2*, biliary excretion of the four anionic forms of CPT-11 and its metabolites was reduced. It should be noted that a number of naturally occurring mutations in *MRP2* do not result in DJS (83). Interestingly, heterozygous carriers of certain *MRP2* mutations have been shown to have higher urinary levels of coproporphyrin isomer I, a metabolic byproduct of heme synthesis and a substrate for MRP2, despite comparable serum bilirubin concentrations (108).

ABCC3 (MRP3). The third member of this family, MRP3, shares 55% amino acid homology with MRP1. Similar to MRP1 and MRP2, MRP3 transports compounds conjugated with glutathione, glucuronide, or sulfate. However, MRP3 appears to preferentially transport glucuronidated compounds rather than those conjugated with glutathione (109, 110).

The *MRP3* gene has been localized to chromosome 17 and encodes a protein expressed in the normal adrenal gland, colon, small intestine, liver, and pancreas and, to a lesser extent, in the kidney and lung (93, 111–115). Structurally, MRP3 is very similar to MRP1 and MRP2 because it also consists of three MSDs and two NBDs (93, 116–118). There is an implied relationship between MRP2 and MRP3 because hyperbilirubinemic rats have elevated expression of MRP3 (110, 119, 120). Similar expression has been demonstrated in DJS patients and other patients with liver disease (113, 114) However, unlike MRP2, MRP3 is expressed on the basolateral domain of hepatocytes and may function to reduce intracellular bile acid concentrations when normal bile flow through the biliary tree is interrupted.

Using a MRP3 expression vector system, MRP3-transfected cells demonstrated resistance to etoposide, vincristine, and methotrexate when compared with control-transfected cells. Additionally, the resistance profile was distinct from and less extensive than those of MRP1 or MRP2 (121). In lung cancer cell lines and patient samples, MRP3 expression was associated with decreased sensitivity to etoposide, doxorubicin, vincristine, and cisplatin and was postulated to play a role in the intrinsic resistance of NSCLC cells (122, 123). However, no direct association between MRP3 expression and tumor prognosis has been observed.

The combination of GenBank cDNA sequence comparison and data from the public SNP database reveals the presence of several SNPs in *MRP3*, many of which involve nonsynonymous amino acid changes (Table 2). These SNPs have not been functionally characterized, and therefore the clinical impact of such *MRP3* variants is unknown.

ABCC4 (MRP4). MRP4 was identified as a result of screening human-expressed cDNA sequence tags in an effort to discover and characterize additional mammalian ABC transporters. In contrast to some of the more ubiquitously expressed transporters, MRP4 is expressed only at very low levels in a few tissues (specifically, lung, kidney, bladder, gall bladder, and tonsil) but expressed most abundantly in the prostate gland (93). The gene encoding for *MRP4* has been localized to chromosome 13 and yields a transporter protein that is distinct from MRP1, MRP2, and MRP3 in that it does not contain an NH_2-terminal MSD (1, 93).

The role that MRP4 plays in normal tissues or in cancer or chemotherapy has not been clearly defined. MRP4 was not overexpressed in resistant tumor cell lines, but a role for MRP4 as a cellular efflux pump for nucleoside analogues was postulated after MRP4 was implicated as a cause of non-virally mediated drug resistance for anti-HIV therapy. Overexpression of MRP4 was associated with impaired efficacy for antivirals and nucleoside analogues (124). Nucleoside analogues are prescribed for the treatment of some hematological malignancies, but treatment resistance to 6-mercaptopurine or thioguanine has yet to be investigated. Recent evidence using stably transfected cell lines overexpressing MRP4 indicates that this transporter may mediate resistance to purine analogues (125).

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Polymorphisms in *MRP4* are known to exist (Table 2). It remains to be determined whether these polymorphisms in *MRP4* impact on drug disposition or pharmacodynamics *in vivo*.

ABCC5 (MRP5). Similar to the discovery of MRP4, MRP5 was identified after screening databases of human-expressed cDNA sequence tags (1, 93). MRP5 is widely expressed in multiple tissues, with especially high expression in skeletal muscle and brain. However, *MRP5* RNA expression was modest in only two cell lines tested (ovarian and lung carcinomas), and expression did not correlate with resistance to doxorubicin or cisplatin (93).

MRP5 has been mapped to chromosome 3, and the transporter protein has been localized to the plasma membrane (126). Similar to MRP4, MRP5 does not contain an NH_2-terminal MSD, a characteristic distinguishing it from MRPs 1–3 (93).

Although not apparently associated with resistance to cytotoxic chemotherapy, MRP5 apparently functions as an efflux transporter of cyclic nucleotides based on the substrate specificity of the protein using isolated membrane vesicles from hamster lung fibroblasts expressing *MRP5* cDNA (127). Additionally, MRP5 may contribute to the resistance of leukemias to thiopurine anticancer drugs because transfected cells expressing *MRP5* cDNA demonstrated resistance against 6-mercaptopurine and thioguanine. Unfortunately, most MRP5 expression in transfected cells was intracellular, rather than in the cell membrane; thus, the role of MRP5 in mediating the MDR phenomenon has not been adequately assessed (128).

In comparing available *MRP5* cDNA sequences in GenBank, variations were found suggesting the existence of SNPs (Table 2). Functional characterization of these polymorphisms has not been performed.

ABCC6 (MRP6). MRP6 is a disease-associated ABC transporter in which alterations in the MRP6 gene are associated with PXE, a heritable disorder of the connective tissue characterized by ophthalmological, dermatological, and cardiovascular abnormalities (79). *MRP6* and the *PXE* gene have been mapped to chromosome 16 and families with deletions or mutations in the *MRP6* gene manifest the heritable form of PXE.

Tissue expression of *MRP6* mRNA levels was high in the liver and kidney and lower in other tissues (129). Immunohistochemistry staining to detect MRP6 at the protein level in normal tissues confirmed the expression of MRP6 in the liver and kidney but failed to show expression in other tissues, including those in which PXE abnormalities are manifested (130).

MRP6 is thought to be an amphipathic anion transporter, based on its ability to transport BQ-123, an anionic cyclopentapeptide (131). Based on a specific pattern of MRP6-mediated inhibition, MRP6 has substrate specificity for the transport of organic anions that is separate from ABCC1 and ABCC2 (132). To assess the contribution of MRP6 to chemotherapy, MRP6-transfected Chinese hamster ovary cells were generated, and their drug sensitivity was analyzed. Compared with control cells, the MRP6-transfected cells displayed modest levels of resistance to anthracyclines and epipodophyllotoxins (133).

The 3′ end of *ABCC6* is amplified and its mRNA is overexpressed in multidrug-resistant leukemia cell lines, implicating MRP6 in AML-associated MDR (134–136). This portion of *ABCC6* was previously referred to as the anthracycline resistance-associated gene. Patients with AML with an inversion at chromosome 16 associated with deletions of *MRP1* and the 3′ end of *MRP6* appeared to have an improved survival (137). The proximity of the 3′ end of *MRP6* to *MRP1* on chromosome 16 makes it more likely that the resistance would be attributed to MRP1 rather than a portion of MRP6 (138).

Not all detected polymorphisms in *MRP6* are causative for PXE, and their functional relevance remains to be determined (139).

ABCC11 (MRP8). Using a cluster of expressed sequence tags to screen cDNA libraries, Bera *et al.* (140) identified the *MRP8* gene that is highly expressed in many breast cancer samples. The MRP8 protein is a M_r 150,000 protein mapped to chromosome 16 and is moderately expressed in normal human testis and breast and, to a lesser extent, in the liver. Amino acid sequence analysis indicates that MRP8 is probably a full transporter with homology to MRP5 and has 2 NBDs and 12 transmembrane-spanning regions (140). To date, no transport function has been ascribed to MRP8, and the role that MRP8 plays in breast cancer development or treatment has not yet been determined.

ABCC12 (MRP9). Bera *et al.* (140) and Tammur *et al.* (141) also identified MRP9 using the same computer-based screening approach to generate ESTs. Interestingly, the *MRP9* gene appears to encode two different mRNA transcripts that are differentially expressed in different tissues (142, 143). The larger 4.5-kb transcript is highly expressed in breast cancer, with lesser expression in normal breast tissue and testis, whereas the smaller 1.3-kb transcript is expressed in brain, skeletal muscle, and ovarian tissues. Structurally, the larger MRP9 transcript is predicted to encode one NBD and two transmembrane domains, each containing four membrane-spanning regions (143). The function of MRP9 has not been determined.

ABC Subfamily G: ABCG2 (BCRP)

Mitoxantrone is an antitumor antibiotic used most commonly in the treatment of hormone-refractory metastatic prostate cancer. Exposure to mitoxantrone can select for tumor cells exhibiting a MDR phenotype not associated with an overexpression of the ABC transporters commonly implicated in this phenotype or reversed by the usual MDR modulators (144–148).

Almost simultaneously, three research groups described a novel ABC half-transporter that conferred resistance to mitoxantrone. This protein, termed BCRP, MXR, or ABCP, had a single ATP-binding domain at the NH_2 terminus and a single COOH-terminal set of transmembrane segments. The gene has been mapped to chromosome 4q22, and expression of the encoded protein conferred resistance to mitoxantrone, doxorubicin, and daunorubicin and reduced daunorubicin accumulation and retention (149–151). Transcription

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of the *ABCG2* gene results in a 2.4-kb mRNA encoding a 655-amino acid, M_r 72,600 polypeptide localized to the plasma membrane (152, 153). ABCG2 may require dimerization for transport activity (154, 155).

ABCG2 mRNA expression analyses of normal tissues indicate highest expression in the placenta, heart, ovary, and kidney, and lower levels in the liver, colon, small intestine, prostate, and brain (149, 150). In tumor cell lines, ABCG2 expression is seen in breast, colon, stomach, myeloma, and fibrosarcoma cell lines and appears to mediate cross-resistance not only to mitoxantrone but also to anthracyclines, topotecan, and SN-38. However, sensitivity to cisplatin, paclitaxel, and *Vinca* alkaloids appeared to be retained (154, 156).

The normal role and the physiological substrates of ABCG2 are unknown, and its clinical relevance to cancer and chemotherapy has not been clarified. Nevertheless ABCG2 appears to play an important role in normal physiological functions because Bcrp1(−/−) mice developed protoporphyria and diet-dependent phototoxicity when exposed to normal food constituents (157). One known substrate for ABCG2, Hoechst 33342 dye, identifies a "side population" of stem cells with considerable plasticity that are present in a number of tissues (158). Expression of the *ABCG2* gene appears to determine the side population phenotype, and expression decreases with cellular differentiation (159). Of note, *ABCG2* mRNA has been detected in a subset of blast cells from patients with drug-resistant acute leukemia and is potentially associated with chemotherapy resistance in patients with AML (160, 161). Experiments in Bcrp1-null mice appear to confirm the role of ABCG2 in chemotherapy resistance because hematopoietic stem cells from these mice were more sensitive to treatment with mitoxantrone (162). Conversely, ABCG2 expression was low or undetectable in a panel of human tumors, including primary tumors as well as drug-treated breast cancer and acute myeloid leukemia samples (152).

A number of polymorphisms in *ABCG2* have been discovered, and two polymorphisms in particular, C421A and C376T, have been demonstrated in a cohort of Japanese subjects and may have functional consequences. These polymorphisms may result in hypersensitivity of certain individuals to ABCG2 substrates because *in vitro* assays indicate that both polymorphisms result in reduced protein expression (163). The clinical relevance of these polymorphisms and the functional status of other known polymorphisms in ABCG2 have not been assessed (Table 2). In cell lines, mutations leading to an amino acid change at arginine 482, predicted to be located at the start of the third MSD, appears to play an important role in determining the drug resistance phenotype, depending on the amino acid substitution; mutations in this hot spot have not been demonstrated in humans (164, 165).

Conclusion

Cancer chemotherapy treatment is complicated by interindividual variations in responses and toxicities and the narrow therapeutic index of the available chemotherapy agents. Current data strongly implicate certain drug transporters, especially multidrug transporters of the ABC superfamily, as a key determinant of tumor drug resistance as well as altered drug disposition or responsiveness. More recently, functional genetic polymorphisms in these transporters have been identified. Future studies that integrate the role of genetic heterogeneity in ABC transporters may allow for targeted and individualized chemotherapy that minimizes toxicity while maximizing efficacy.

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