Review

Pharmacogenetics of ATP-binding Cassette Transporters in Cancer and Chemotherapy

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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 ABC3 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 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 limi-
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 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 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 cancer 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.

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

**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 Mr 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.
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 descent 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, 40, 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 shortened 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, 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 MSD1, six transmembrane segments in MSD2, and four or six transmembrane segments in MSD3 (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 1: Localization and role of the ABC transporters involved in cancer and chemotherapy**

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<tr>
<th>Symbol</th>
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<th>Tissue location</th>
<th>Role in cancer or chemotherapy</th>
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<td>9q34</td>
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<td>Estramustine resistance</td>
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<td>Adrenal, kidney, brain, intestine, liver</td>
<td>Multidrug resistance, tumor prognostic factor</td>
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**Table 2 Continued**

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With regard to the role of MRP1 as a drug transporter, the best-characterized substrate for MRP1 is LTC₄, 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 alkylating agents as well as etoposide and doxorubicin, and usually conjugated with glutathione, glucuronide, and sulfate.

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 LTC₄, 17β-estradiol 17β-(β-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 LTC₄ 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 NH₂ terminus. However, MRP2 and MRP1 differ in their tissue localization and their transport kinetic properties (90, 91).

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**Table 2 Continued**

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* ?, unknown effect on transport function; ←→, does not alter transport activity; ↓, decreased transport activity; φ, null function.


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 chemotherapeutics, 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 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 NH2-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).
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 (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 NH2-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 anticaner 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 ABCG2 (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 over-expressed 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 Mr 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 NH2 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
of the ABCG2 gene results in a 2.4-kb mRNA encoding a 655-amino acid, M, 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). 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.

References


74. Ito, S., Ieiri, I., Tanabe, M., Suzuki, A., Higuchi, S., and Otsubo, K. Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/
Maehara, Y., Sugimachi, K., and Kuwano, M. Increased expression of an
98. Hinoshita, E., Uchiumi, T., Taguchi, K., Kinukawa, N., Tsuneyoshi, M.,
icular multispecific organic anion transporter (cMOAT) is expressed in
human hepatic cancer cells. Cancer Res.,
Akiyama, S., Ono, M., and Kuwano, M. A canalicular multispecific organic
drug resistance genes MDR1 and cMOAT in human hepatocellular carci-
97. Ito, K., Oleschuk, C. J., Westlake, C., Vasa, M. Z., Deeley, R. G., and
96. Keppler, D., and Konig, J. Hepatic secretion of conjugated drugs and
95. Cui, Y., Konig, J., Buchholz, J. K., Spring, H., Leier, I., and, Keppler, D.
Drug resistance and ATP-dependent conjugate transport mediated by the
apical multidrug resistance protein, MRP2, permanently expressed in human
94. Hinoshita, E., Uchiumi, T., Haga, S., Nakamura, T., Toh, S., Furukawa, M.,
average expression in cisplatin-resistant cancer cells with decreased ATP-
93. Kool, M., de Haas, M., Scheffer, G. L., van der Valk, P., Borst, P., and
MRP3, an organic anion transporter (cMOAT) gene. Am. J. Physiol.
transport properties of cloned rat multidrug resistance-associated protein 3 (MRP3).
90. Hirohashi, T., Suzuki, H., and Sugiyama, Y. Characterization of the
89. Toh, S., Wada, M., Uchiumi, T., Inokuchi, A., Makino, Y., Horie, Y.,
and mutations in the ATP-binding-cassette region in Dubin-Johnson syn-
87. Paulusma, C. C., Bosma, P. J., Zaman, G. J., Bakker, C. T., Otter, M.,
86. Kobayashi, K., Sogame, Y., Hayashi, K., Nicotera, P., and Orrenius, S.
metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. Cancer Chemother. Pharma-
85. Conrad, S., Kauffmann, H. M., Ito, K., Leslie, E., Deely, R., Schrenk, D.,
and Cole, S. A naturally occurring mutation in MRP1 results in a selective
84. Conrad, S., Kauffmann, H. M., Ito, K., Leslie, E., Deely, R., Schrenk, D.,
and Cole, S. A naturally occurring mutation in MRP1 results in a selective
83. Nies, A. T., Konig, J., Pfannschmidt, M., Klar, E., Hofmann, W. J., and
82. Tsujii, H., Konig, J., Rost, D., Stockel, B., Leuschner, U., and, Kepp-
er, D. Exon-intron organization of the human multidrug-resistance pro-
tein 2 (MRP2) gene mutated in Dubin-Johnson syndrome. Gastroenterol-
81. 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. A
80. Keitel, V., Kartenbeck, J., Nies, A. T., Spring, H., Brom, M., and
79. Abe, H., and Okuda, K. Biliary excretion of conjugated sulfobromo-
phthalein (BSP) in constitutional conjugated hyperbilirubinemias. Di-
75. Keitel, V., Kartenbeck, J., Ni’inuma, K., Suzuki, H., and Sugiyama, Y. A naturally occurring mutation in MRP1 results in a selective
74. Paulusma, C. C., Bosma, P. J., Zaman, G. J., Bakker, C. T., Otter, M.,
and mutations in the ATP-binding-cassette region in Dubin-Johnson syn-
73. 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.,


Molecular Cancer Therapeutics

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A. Craig Lockhart, Rommel G. Tirona and Richard B. Kim


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