Molecular Pharmacology of ABCG2 and Its Role in Chemoresistance

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Received July 23, 2013; accepted August 28, 2013

ABSTRACT

The ATP-binding cassette, subfamily G, isoform 2 protein (ABCG2) is an important member of the ABC transporter superfamily, which has been suggested to be involved in multidrug resistance (MDR) in cancer. Its diverse range of substrates includes many common chemotherapeutics such as imatinib, doxorubicin, and mitoxantrone. Physiologically, ABCG2 is highly expressed in areas such as the blood-brain barrier and gastrointestinal tract, where it is thought to play a role in protection against xenobiotic exposure. High ABCG2 expression has also been found in a variety of solid tumors and in hematologic malignancies and has been correlated with poorer clinical outcomes. Furthermore, ABCG2 expression is a characteristic feature of cancer stem cells, which are able to self-renew and differentiate. These cancer stem cells have been postulated to play an important role in MDR, where their inherent ABCG2 expression may allow them to survive chemotherapy and repopulate the tumor after exposure to chemotherapeutics. This observation raises the exciting possibility that by inhibiting ABCG2, cancer stem cells and other cancers may be targeted and eradicated, at which point conventional chemotherapeutics would be sufficient to eliminate the remaining tumor cells. Inhibitors of ABCG2, such as tyrosine kinase inhibitors, phosphodiesterase-5 inhibitors, and the fumitremorgin-type indolyl diketopiperazine, Ko143 [(3S,6S,12aS)-1,2,3,4,6,7,12,12a-octahydro-9-methoxy-6-(2-methylpropyl)-1,4-dioxopyrazino[1',2':1,6]pyrido[3,4-b]indole-3-propanoic acid 1,1-dimethyl-ethyl ester], could potentially be used for this purpose. However, these agents are still awaiting comprehensive clinical assessment.

Introduction: The ATP-Binding Cassette Transporters

The successful treatment of cancer can be impeded by the concurrent development of resistance to multiple chemotherapeutics that are both structurally and mechanistically unrelated (Robey et al., 2009). This multidrug resistance (MDR) phenotype can be associated with an increase in the expression of ATP-binding cassette (ABC) transporters, which efflux substrates from the cell in an energy-dependent manner, driven by ATP hydrolysis (Robey et al., 2009). Of the 48 ABC transporters currently recognized, three are most commonly associated with MDR: P-glycoprotein (P-gp; also known as MDR1 or ABCB1), MDR-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP) or ABC subfamily G, isoform 2 protein (ABCG2) (Robey et al., 2009).

It is well known that P-gp can transport a range of hydrophobic chemotherapeutics such as anthracyclines, taxanes, etoposide, and mitoxantrone (Juliano and Ling, 1976; Gottesman et al., 2002) and was originally thought to be solely responsible for the MDR phenotype (Juliano and Ling, 1976; Gottesman et al., 2002). However, it soon became clear that other transporters could also confer resistance to chemotherapeutics. The gene encoding MRP1 (also known as ABCC1) was cloned by Cole et al. in 1992, and, similarly to P-gp, its overexpression conferred resistance to a range of chemotherapeutics (Cole et al., 1992). The discovery that P-gp and MRP1 alone could not account for the efflux of chemotherapeutic drugs from a subset of leukemic cells subsequently led to the identification of BCRP from the breast cancer cell line, MCF-7.
AdrVp (Doyle et al., 1998). The Human Genome Organization Nomenclature Committee has since formally designated BCRP as ABCG2, as it belongs to the ABC transporter superfamily and is the second member of the G subfamily.

Although their substrate specificities differ, there is considerable overlap between ABCG2, P-gp, and MRP1 in terms of their ability to transport many structural classes of agents (Fig. 1). This raises the question of the physiologic rationale for this apparent redundancy. All three transporters appear to be involved in protecting the body from toxicity associated with xenobiotic exposure, and the answer may lie in where the transporter is expressed in a cell or organ. In the case of the gastrointestinal tract, P-gp and ABCG2 are apically localized (Thiebaut et al., 1987; Maliepaard et al., 2001a), whereas MRP1 is found at the basolateral surface of crypt cells (Peng et al., 1999). A potential explanation for this differential expression is that in the gut, P-gp/ABCG2 may function to modulate the absorption of xenobiotics, whereas MRP1 may protect these highly proliferating cells from xenobiotic damage (Leslie et al., 2005).

Chromosomal Localization and Regulation of Expression

The human ABCG2 gene is located on band 4q22, between the markers D4S2462 and D4S1557 (Knutsen et al., 2000). To date, no other ABC transporter has been assigned to chromosome 4. Cytogenetic studies using the ABCG2 overexpressing cell lines, MCF-7/AdrVp 3000 and MCF-7/MX, showed gene amplification exclusively between 4q21 and 4q22, suggesting that ABCG2 homodimerizes to create an active transporter (Knutsen et al., 2000). The ABCG2 gene consists of 16 exons and 15 introns, spanning 66 kb (Bailey-Dell et al., 2001). The 5′-untranslated region is found mainly in exon 1, whereas the translational start site is found in the second exon (Bailey-Dell et al., 2001). The Walker A and B motifs, which are crucial for nucleotide binding, have been identified in exon 3 and 6, respectively, whereas the ABC motif (ALSGGQ) is found in exon 6 (Bailey-Dell et al., 2001).

Like other ABC transporters, the promoter region of ABCG2 (Fig. 2) is located downstream of a putative cytosine-phosphate-guanine island, is TATA-less, and contains several putative specificity protein 1, activator protein 1, and activator protein 2 binding sites, a common feature of promoters lacking a TATA box (Bailey-Dell et al., 2001). Additionally, several transcription factors and their cis and trans response elements have been shown to regulate ABCG2 expression (Ee et al., 2004; Krishnamurthy et al., 2004; Wang et al., 2008; Pradhan et al., 2010). The presence of a hypoxia response element may contribute to MDR in some tumors, as hypoxia-inducible factor 1α is highly active within the hypoxic interior of solid tumors and thus can increase the expression of ABCG2 via the hypoxia response element ( Krishnamurthy et al., 2004). Likewise, the hormones progesterone and estrogen have response elements located within the ABCG2 promoter and have been shown to increase ABCG2 expression (Ee et al., 2004; Wang et al., 2008; Pradhan et al., 2010). The estrogen response element is located adjacent to a nuclear factor light chain enhancer of activated B cells response element, and binding of the estrogen receptor and p65 (the active nuclear factor κB subunit) at these response elements leads to a pronounced increase in ABCG2 mRNA expression (Pradhan et al., 2010). The ability of hormones to regulate ABCG2 expression may be important during pregnancy, as high ABCG2 expression is found in the placenta, where ABCG2 protects the fetus from exposure to harmful xenotoxins (Jonker et al., 2000).

Single-Nucleotide Polymorphisms

Screening for single-nucleotide polymorphisms (SNPs) in ethnically diverse subjects has identified more than 80 synonymous and nonsynonymous SNPs in the ABCG2 gene to date (Sharom, 2008). The two most frequent polymorphisms identified were the G34A (resulting in V12M) and C421A (resulting in a Q141K substitution) transitions (Fig. 3), found in 18 and 35.5% of the studied population, respectively (Kobayashi et al., 2005).

The functional impact of these SNPs on drug transport remains contentious because the results are not consistent. No significant change in irinotecan pharmacokinetics was observed in relation to the ABCG2 C421A genotype in American

![Fig. 1. There is considerable substrate overlap between the ABC transporters P-gp, MRP1, and ABCG2. *Substrates transported by ABCG2 mutant R482G.](image-url)
Caucasians, African Americans, Africans, Han Chinese, and European Caucasian patients treated with irinotecan (de Jong et al., 2004). This was not corroborated by Morisaki et al. (2005), who found that cells with the Q141K variant of ABCG2 had IC50 values 1.2- to 5-fold lower than cells transfected with wild-type (Arg482) ABCG2, suggesting that the Q141K SNP affects drug transport (Morisaki et al., 2005). Recently, studies aimed at evaluating the clinical relevance of the Q141K SNP in patients undergoing chemotherapy have also been reported. In these studies, elevated plasma concentrations of gefitinib (Li et al., 2007) and diflomotecan (Sparreboom et al., 2004) and increased bioavailability of oral topotecan (Sparreboom et al., 2005) were found. The Q141 mutation has also been associated with increased adverse effects in response to gefitinib treatment (Mizuarai et al., 2004). Additionally, the Q141K mutation has been demonstrated to decrease ATPase activity by 1.3-fold compared with wild-type ABCG2 (Mizuarai et al., 2004). Furthermore, kinetic analysis of ATPase activity showed that the Km value in Q141K cells was 1.4-fold higher than that of wild-type ABCG2 (Mizuarai et al., 2005). This was not corroborated by Morisaki et al. (2005), who found that cells with the Q141K variant of ABCG2 had IC50 values 1.2- to 5-fold lower than cells transfected with wild-type (Arg482) ABCG2, suggesting that the Q141K SNP affects drug transport (Morisaki et al., 2005). Recently, studies aimed at evaluating the clinical relevance of the Q141K SNP in patients undergoing chemotherapy have also been reported. In these studies, elevated plasma concentrations of gefitinib (Li et al., 2007) and diflomotecan (Sparreboom et al., 2004) and increased bioavailability of oral topotecan (Sparreboom et al., 2005) were found. The Q141 mutation has also been associated with increased adverse effects in response to gefitinib treatment (Mizuarai et al., 2004). Additionally, the Q141K mutation has been demonstrated to decrease ATPase activity by 1.3-fold compared with wild-type ABCG2 (Mizuarai et al., 2004). Furthermore, kinetic analysis of ATPase activity showed that the Km value in Q141K cells was 1.4-fold higher than that of wild-type ABCG2 (Mizuarai et al., 2004). These results suggest that this SNP may result in altered transport functions of ABCG2 transporter, which has important implications for the pharmacokinetics and drug-resistance profiles of chemotherapeutics.

**Structure**

The 655-amino-acid ABCG2 protein is in a reversed configuration to most other ABC transporters, as the ATP-binding domain is at the N terminus, and the six putative transmembrane domains are at the C terminus (Polgar et al., 2008) (Fig. 3). The large extracellular loop between transmembrane domains 5 and 6 contains the only proven N-linked glycosylation site, Asp596 (Diop and Hrycyna, 2005). Additionally, ABCG2 is unusual among ABC transporters in that it is a “half transporter” (Doye et al., 1998). In fact, although most ABC transporters contain two repeated halves, each containing a membrane-spanning domain and a nucleotide-binding domain, ABCG2 contains one only membrane-spanning domain and one nucleotide-binding domain (Allikmets et al., 1998). As it is widely accepted that functional ABC transporters require two transmembrane domains and two nucleotide-binding domains to form a central substrate translocation pathway, it is believed that ABCG2 must at least dimerize to become functional (Ni et al., 2010a). Similar to other related members of the ABC family, which are known heterodimers (Hilbrand et al., 2007), ABCG2 probably dimerizes in the endoplasmic reticulum (ER) (Graf et al., 2003). Then ABCG2 may exit through the ER to the Golgi apparatus for post-translational processing before it is trafficked to the apical membrane of the cell (Graf et al., 2003).

**Homodimer or Higher-Order Homo-Oligomer?** Under conditions of reducing SDS-PAGE, ABCG2 migrates as a 70-kDa band, but in the absence of reducing agents, it migrates as a 140-kDa complex, which implies that it is a homodimer linked by intermolecular disulfide bridges (Kage et al., 2002). This is supported by studies using chemical cross-linking agents, which theoretically link the monomers and cause a shift in molecular mass from 72 to 180 kDa (Litman et al., 2002). Although it is apparent that ABCG2 could be capable of forming homodimers, several studies have shown that it can also form higher oligomeric states, including tetramers to even a dodecamer (Xu et al., 2007; Xie et al., 2008). Additionally, electron microscopy has shown an octameric complex organized as a tetramer of dimers in the mutant R482G isoform of ABCG2 (McDevitt et al., 2006). This same isoform was demonstrated to form higher-order oligomers of 150 and 290 kDa (Velamakanni et al., 2008). Recently, human recombinant ABCG2 purified from *Pichia pastoris* led to structural analysis at 5 Å resolution, and this has shown ABCG2 assembled as a tetramer (Rosenberg et al., 2010). The conflicting results found between laboratories are likely due to several factors, such as the type of detergents used and the extraction conditions. Hence, whether ABCG2 forms a dimer or a higher-order oligomer under physiologic conditions remains unclear.

**Sequences Involved in Dimerization.** Mutational analysis has indicated that two regions of ABCG2 may be involved in dimerization (Wakabayashi et al., 2006; Polgar et al., 2006, 2010). The GXXXG sequence has been linked to dimerization in other proteins such as glycoporphin a (Langosch et al., 1996), and this motif is also located in transmembrane domain 1 of ABCG2 (Fig. 3) (Polgar et al., 2010). Mutation of the glycine residues to leucines resulted in impaired drug transport of several ABCG2 substrates, suggesting that this motif plays a role in dimerization, although it also may be important in the formation of higher-order complexes (Polgar et al., 2004).

Mutational analysis of Thr402, which is located near the GXXXG motif (TXXGXXXG), in combination with mutations of the GXXXG motif (T402L or T402R and G406L or G410L; Fig. 3) resulted in a reduction in protein expression and drug efflux, alterations in glycosylation, and retention of ABCG2 in the ER (Polgar et al., 2010). Although the mutants could still

**Structure**

The ABCG2 promoter has multiple cis regulatory elements—Sp1, AP1, and AP2 sites—the position of which is depicted in relation to the transcription start site of the first ABCG2 exon. Ap, activator protein; CpG, cytosine-phosphate-guanine; HRE, hypoxia response element; ERE, estrogen response element; AhRE, aryl hydrocarbon response element; NFkBRE, response element; SP, specificity protein.

**Fig. 2.** The ABCG2 promoter has multiple cis regulatory elements—Sp1, AP1, and AP2 sites—the position of which is depicted in relation to the transcription start site of the first ABCG2 exon. Ap, activator protein; CpG, cytosine-phosphate-guanine; HRE, hypoxia response element; ERE, estrogen response element; AhRE, aryl hydrocarbon response element; NFkBRE, response element; SP, specificity protein.

**Fig. 3.** Schematic illustration of the membrane topology of ABCG2 and highlights of residues or mutations that affect the functioning of the transporter. Val12M and Q141K are SNPs thought to affect the pharmacokinetics of ABCG2 substrates. Notably, T402, G406, and G410 are near or part of the GXXXG motif and are thought to play a role in dimerization, as do Gly553 Cys592, 603, and 608. The R482G isoform of ABCG2 appears in drug-selected cell lines and displays altered substrate specificity to the wild-type transporter. Asp596 is the sole N-linked glycosylation site.
be chemically cross-linked, this may be simply an indication of their close proximity within the ER (they must be physically close to dimerize) (Polgar et al., 2010). It does not necessarily reflect their ability to form a fully functional transporter since functions such as drug efflux were shown to be affected by the mutations (Polgar et al., 2010).

Additionally, the results of the mutation of Gly553 found in transmembrane domain 5 to Leu553 mimicked those found for the TXGXXG motif (Fig. 3) (Polgar et al., 2006). Although the monomers of the mutant forms of ABCG2 can be cross-linked and form disulfide bonds, the fact that they display impaired glycosylation and trafficking is consistent with, but does not prove, the involvement of the TXGXXG motif and Gly553 in homodimerization and formation of a fully functional ABCG2 transporter (Polgar et al., 2006).

Mutagenesis studies targeting cysteine residues (Cys592, Cys603, and Cys608) suggest that they are likely to be involved in the formation of intramolecular disulfide bonds (Fig. 3) (Wakabayashi et al., 2007). Although most evidence demonstrates that Cys603, which is highly conserved among ABCG2 orthologs, plays a key role in the formation of disulfide bonds, the disulfide bonds themselves do not appear to be essential for protein function (Shigeta et al., 2010). This observation suggests that noncovalent protein-protein interactions could also contribute to dimerization (Henriksen et al., 2005; Ni et al., 2010b). Notably, Cys592 and Cys608 also appear to be important residues, as substituting these residues for Gly results in reduced expression and aberrant localization to the intracellular compartments as opposed to the plasma membrane (Wakabayashi et al., 2006). Taken together, these results suggest that correct disulfide bond formation involves these residues and impacts on intracellular sorting and function of the protein (Wakabayashi et al., 2007).

**Homology Modeling.** To elucidate more fully the structure of ABCG2, homology models have been generated. Notably, models based only on computer predictions have been found to be inconsistent with experimental topology structures (Ni et al., 2010a). Because of this problem, three homology models of ABCG2 have been deduced that are based on experimental topology structures and represent different conformational states. The templates used were MsbA (an *Eschericia coli* ABC transporter) for the substrate-unbound, nucleotide-free, inward-facing apo (open) conformation (Ward et al., 2007); parts of murine P-gp to model the substrate-bound, nucleotide-free, inward-facing holo (closed) conformation (Aller et al., 2009); and Sav1866 (a *Staphylococcus aureus* ABC transporter) to model the nucleotide-bound, outward-facing conformation (Dawson and Locher, 2006). The nucleotide-binding domains were modeled using the nucleotide-binding domains of the *E. coli* maltose transporter MalK (Chen et al., 2003a). The results for homology modeling of MsbA suggest that in the presence of a substrate, ABCG2 displays a more closed conformation (Ward et al., 2007; Rosenberg et al., 2010).

The models suggest that the large extracellular loop that connects transmembrane domain 1 and transmembrane domain 2 may form contacts with the extracellular loop connecting transmembrane domain 5 and transmembrane domain 6, and this could act to stabilize the dimeric structure (Figs. 3 and 4) (Rosenberg et al., 2010). The large extracellular loop, which connects transmembrane domain 5 and transmembrane domain 6, might also play a critical role in modulating substrate binding (Özvegy-Laczka et al., 2008). This is corroborated by evidence from Xu et al. (2007), who demonstrated that the transmembrane domain 5-loop-transmembrane domain 6 fragment plays a critical role in ABCG2 oligomerization.

Interestingly, the coupling helix 1 that is involved in the interactions between the nucleotide-binding domain and the transmembrane domain was not predicted by the homology models for ABCG2, although it is present in the templates and in other ABC efflux transporter structures (Rosenberg et al., 2010). However, there is a putative linker region that connects the nucleotide-binding domain to the transmembrane domain (Fig. 4), and this is much longer in ABCG2 than in other ABC transporters (Ni et al., 2010a). This potential linker region may perform the same function as coupling helix 1, although it could not be modeled because of the lack of appropriate templates (Rosenberg et al., 2010). A second coupling helix is also present in the model and associates with the nucleotide-binding domain of the opposite monomer (Rosenberg et al., 2010). This motif has been found in the intracellular loop that connects transmembrane domains 4 and 5 (Fig. 4) and may enable the flexibility of the transporter, which in turn aids substrate interactions (Rosenberg et al., 2010). On substrate binding, the homology models predict dramatic conformational changes. In fact, the intracellular entry of ABCG2 closes, which alters the shape of the substrate-binding cavity to form the “ATP sandwich” necessary for ATP
hydrolysis to occur (Rosenberg et al., 2010). A large V-shaped gap then forms on the extracellular side of the transporter and may release the substrates after ATP hydrolysis (Ward et al., 2007; Rosenberg et al., 2010).

These homology models are in agreement with existing data. For example, Arg482 has been found in the homology models to be located in the central cavity, with the side chain pointing toward the drug translocation pathway (Fig. 4) (Cai et al., 2010). This residue has been the subject of many site-directed mutagenesis studies that have pointed to its importance in determining substrate specificity and transport activity (Miwa et al., 2003; Robey et al., 2003; Örzály-Laczka et al., 2005). This observation could explain why resistance to methotrexate (which interacts with Arg482) is decreased by the R482G and R482T mutations (Chen et al., 2003b), whereas efflux of prazosin (which binds to a separate and distinct binding site) was not affected (Giri et al., 2009). It would therefore appear that homology models of ABCG2 do provide relatively accurate and useful information and may be valuable tools in the development of ABCG2 substrates and inhibitors.

**ABCG2 Transport**

**Substrates.** ABCG2 transports a structurally diverse array of substrates (Fig. 5), the list of which is constantly expanding (Polgar et al., 2008). Initially, many of the ABCG2 substrates were reported to be chemotherapeutics, such as mitoxantrone (Doyle et al., 1998), which can be used to select for increased ABCG2 expression (Ross et al., 1999). Substrates of the wild-type ABCG2 transporter include tyrosine kinase inhibitors, such as imatinib and gefitinib (Elkind et al., 2005), flavopiridol (Robey et al., 2001; Allen et al., 2002a; Elkind et al., 2005), and the camptothecins: topotecan, irinotecan, and its active metabolite, SN-38 [7-ethyl-10-hydroxycamptothecin] (Yang et al., 1995; Ma et al., 1998; Maliepaard et al., 2001b). Some drug-selected cell lines that express mutant forms of ABCG2, R482G, and R482T (discussed in “Homology Modeling”) are considered to be gain-of-function mutants, as their altered substrate specificity increases resistance to anthracyclines (doxorubicin, daunorubicin) and rhodamine 123 (Fig. 6) (Chen et al., 1990; Honjo et al., 2001; Allen et al., 2002a). The residue at this position therefore

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**Fig. 5.** The structural diversity of selected ABCG2 substrates and inhibitors.
appears to play a key role in substrate recognition (Chen et al., 2003b).

Other drugs also act as substrates of ABCG2 and include cimetidine (Pavek et al., 2005), prazosin (Litman et al., 2000), statins (Hirano et al., 2005; Huang et al., 2006), and zidovudine (Wang et al., 2004). Additionally, ABCG2 can transport biologic substrates like estrone, 17β-estradiol, porphyrins such as heme (Krishnamurthy et al., 2004), protoporphyrin IX (Robey et al., 2004), and the dietary carcinogen, 2-amino-1-methyl-6-phenylimidazo(4,5-b) pyridine (Pavek et al., 2005). It should also be noted that only sulfated steroids have been found to be transported by ABCG2 in mammalian cells (Imai et al., 2003; Suzuki et al., 2003), whereas unconjugated estradiol can be transported by ABCG2 identified in Lactococcus lactis (Janvilisri et al., 2003).

For those studying ABCG2, the discovery of fluorescent substrates has been extremely useful. Fluorescent substrates include rhodamine 123 (Litman et al., 2000) and phaeophor- bide a (Robey et al., 2004). The ABCG2-specific substrate, phaeophor- bide a, is a dietary chlorophyll breakdown product that was discovered serendipitously in ABCG2-/- mice, which were extremely sensitive to the compound and developed lethal phototoxic lesions on light-exposed skin (Jonker et al., 2002). Lysotracker, prazosin-BODIPY, and Hoechst 33342 have also been reported to be ABCG2 substrates (Litman et al., 2000).

Although there is considerable substrate overlap between ABCG2, P-gp, and MRP1 (Fig. 1), some characteristics of this transporter set ABCG2 apart (Leslie et al., 2005). ABCG2 is able to transport hydrophilic conjugated anions, especially if they have been sulfated, in addition to hydrophobic substrates, which P-gp can also transport (Kodaira et al., 2010). This substrate overlap is thought to exert a synergistic effect to limit the penetration of drugs across the blood-brain barrier (BBB) and other areas (Kodaira et al., 2010) and may affect the pharmacokinetics of substrate drugs. It is still unknown what features determine whether a compound will be a substrate of ABCG2, as to date there has only been one group of compounds (camptothecin analogs) in which there is an example of a clear structure-activity relationship (SAR) (Yoshikawa et al., 2004). For instance, in the camptothecin analog, SN-38 (the active metabolite of irinotecan), the presence of hydroxyl or amino groups at carbons 10 and 11 increases polarity and facilitates its recognition by ABCG2 (Fig. 7) (Yoshikawa et al., 2004).

ABCG2 Inhibitors. The list of inhibitors of ABCG2 is similarly large and diverse (Fig. 5). The first inhibitor to be identified was fumitremorgin C, isolated from Aspergillus fumi- gates (Rabindran et al., 2000). Fumitremorgin C was reported to inhibit mitoxantrone resistance in the S1-M1-3.2 cell line, before ABCG2 had been cloned (Rabindran et al., 2000). Fumitremorgin C was reported to inhibit mitoxantrone resistance in the S1-M1-3.2 cell line, before ABCG2 had been cloned (Rabindran et al., 1998). Because of the neurotoxicity of fumitremorgin C, several analogs were produced, of which Ko143 [(3S,6S,12aS)-1,2,3,4,6,7,12,12a-

![Fig. 6](image_url)

**Fig. 6.** Drug-selected cell lines can express the mutant form of ABCG2-R428G. This isoform has altered substrate specificities as compared with wild-type ABCG2, possibly because some substrates interact with the amino acid at position 428. This figure was adapted from Sarkadi et al. (2004).

![Fig. 7](image_url)

**Fig. 7.** Substitution of polar groups at carbons in positions 10 and 11 (circled in red) of camptothecin analogs has been found to increase the substrate recognition by ABCG2, as demonstrated by the increase in IC<sub>50</sub> for SN-38 compared with SN-443 in the ABCG2-expressing cell line PC-6/SN2-H2. A planar structure due to double bonds (circled in red) has been found to increase the potency of both flavonoids (between carbons 2 and 3) and rotenoids (between carbons 6 and 12). This is illustrated by the lower EC<sub>50</sub> for increasing mitoxantrone accumulation in MCF-7 MX100 ABCG2-expressing cells for flavone compared with flavanone; and the increase in maximal mitoxantrone accumulation for boeravinone G compared with boeravinone C in human embryonic kidney (HEK)-293/ABCG2 cells.
octahydro-9-methoxy-6-(2-methylpropyl)-1,4-dioxopyrazino[1',2':1,6]pyrido[3,4-b]indole-3-propanoic acid 1,1-dimethylethyl ester) was found to be the most potent and selective inhibitor of ABCG2 (Allen et al., 2002b). Unlike fumitremorgin C and Ko143, many inhibitors of ABCG2 are not selective and inhibit other ABC transporters as well, such as elacridar (de Bruin et al., 1999), tariquidar (Robey et al., 2004), sildenafil [commercially marketed as Viagra (Pfizer, New York, NY)] (Shi et al., 2011), and cyclosporin A (Gupta et al., 2006). Also of note, some inhibitors of ABCG2 are also substrates of this molecule, suggesting that they are competitive inhibitors. For instance, the tyrosine kinase inhibitors imatinib and gefitinib fall into this category (Houghton et al., 2004; Özvegy-Laczka et al., 2004).

Several SAR and quantitative SAR studies have been carried out for ABCG2 inhibitors, allowing the prediction of some of the structural requirements for an inhibitor. Lipophilicity has been shown to be a predictor of inhibition in both flavonoids (Zhang et al., 2005) and fumitremorgin C analogs (van Loevezijn et al., 2001). However, this was not shown to be the case for either tariquidar analogs (Pick et al., 2008) or propafenone analogs (Cramer et al., 2007). Planar structure has also been suggested to increase the ABCG2-inhibitory properties of a drug, as evidenced by results from SARs of different structural series of compounds. For example, the two cyclin-dependent kinase inhibitors, purvalanol A and WHI-P180 (3-[(6,7-dimethoxyquinazolin-4-yl)amino]phenol), have a planar structure and are also the two strongest ABCG2 inhibitors of this class (An et al., 2009). A planar structure including a 2,3-double bond in ring C increases the inhibition potency for flavonoids (Zhang et al., 2005), and the same effect is seen for rotenoid derivatives (Fig. 7) (Ahmed-Belkacem et al., 2007). Although quantitative SAR analysis has provided some useful information for the development of ABCG2 inhibitors, their efficacy will remain limited until a high-resolution three-dimensional structure has been determined.

Localization and Physiologic Function

Many studies have investigated the tissue distribution of ABCG2 expression, providing clues as to the physiologic function of the transporter (Robey et al., 2009). High ABCG2 expression has been found in the placenta, brain, prostate, small intestine, testes, ovaries, liver, adrenal gland, uterus, and central nervous system (Doyle and Ross, 2003). Several physiologic roles have been posited for ABCG2, and in general it seems to protect cells and organs from harmful xenobiotics (Robey et al., 2007). As a result of this function, ABCG2 has been shown in many instances to affect the pharmacokinetics of its substrates, making its expression relevant in a clinical setting (Fig. 8).

Placenta. High ABCG2 expression has been found on the apical membrane of syncytiotrophoblasts of the chorionic villi, leading to the assertion that ABCG2 protects the fetus from exposure to toxins and also assists in removing toxins from the fetus (Fig. 8) (Litman et al., 2002). Importantly, ABCB1a/1b−/− (P-gp knockout) mice were often used to avoid the confounding effects of having P-gp present, as P-gp and ABCG2 have overlapping substrate specificities (Fig. 1). The administration of oral topotecan and elacridar to ABCB1a/1b−/− mice resulted in fetal plasma topotecan levels twice as high as the maternal plasma levels (Jonker et al., 2000). Other groups have also similarly demonstrated that i.v. administration of the antibiotic nitrofurantoin to pregnant mice leads to a 5-fold higher fetal concentration in ABCG2−/− mice versus wild-type mice (Zhang et al., 2007). In humans, an ex vivo study employing human placental vesicles demonstrated that fetal-to-maternal concentration ratios of the gestational diabetes drug glyburide were increased 2-fold by coadministration of the ABCG2 inhibitor novobiocin (Gedeon et al., 2008).

There is also evidence demonstrating the transport of toxins that are ABCG2 substrates from the fetal to the maternal space (Staud et al., 2006; Myllynen et al., 2008). In rats, the histamine 2 receptor antagonist cimetidine was shown to be transported against the concentration gradient from the fetal space to the maternal space (Staud et al., 2006), and analogous results have been obtained in perfused human placenta using the dietary carcinogen, 14C-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (Myllynen et al., 2008).

Blood-Brain Barrier. Generally, the influx and efflux of drugs through the BBB have been thought to depend, at least in part, on the physiochemical properties of the drug, such as lipophilicity and molecular weight (Pardridge, 2005). However, many drugs have lower BBB penetration than would be
predicted based on their physiochemical properties, and this is largely due to the expression of efflux transporters, such as the ABC proteins (Pardridge, 2005). This poor penetration of some drugs affects the treatment of diseases such as Alzheimer and Parkinson disease, as well as cancers such as gliomas because concentrations high enough to penetrate the BBB often cause systemic toxicity (Pardridge, 2005). The BBB also poses several problems for drug development as it places significant restrictions on the size, lipophilicity, and transporter affinity of drugs (Pardridge, 2005). Of the three ABC transporters in the BBB, ABCG2 is thought to be the most highly expressed, followed by P-gp and then MRP1, and all are localized to the luminal side of endothelial cells lining BBB capillaries (Fig. 8) (Eisenblätter et al., 2003).

The considerable substrate overlap between ABCG2 and P-gp (Fig. 1) makes it difficult to determine the pharmacological and potentially the physiologic roles of ABCG2 alone. This conclusion is exemplified by a study by Breedveld et al. (2005), who examined the uptake of imatinib in ABCG2⁻/⁻ and ABCB1a/1b⁻/⁻ (i.e., P-gp⁻/⁻) mice. In this investigation, ABCB1a/1b⁻/⁻ mice displayed a 3.6-fold higher brain penetration of imatinib compared with wild-type animals and ABCG2⁻/⁻ a 2.5-fold higher concentration, whereas inhibition with elacridar caused a 4.2-fold increase in imatinib penetration in wild-type mice (Breedveld et al., 2005). This suggests that although ABCG2 is highly expressed at the BBB, P-gp is perhaps more clinically relevant. This is corroborated by another study that found the blood-to-plasma ratios of imatinib to be increased by >10-fold in ABCB1a/1b/ABCG2⁻/⁻ mice, whereas in ABCB1a/1b⁻/⁻ mice, the increase was only approximately 2-fold, and no difference was observed for ABCG2⁻/⁻ mice (Oostendorp et al., 2009). Again, this observation suggests that although P-gp may play a more important role in limiting the penetration of substrates at the BBB, ABCG2 is also of importance and the combination of the two transporters may even be synergistic (Fig. 8).

**Mammary Gland.** ABCG2 has been found to be upregulated in the lactating breast of mice, cows, and humans, where it may function to concentrate vitamins and minerals into milk for breastfeeding young (Fig. 8) (Hogue et al., 1999). One such vitamin is riboflavin, which is necessary for fat metabolism and many redox reactions (Van Herwaarden et al., 2007). Unfortunately, ABCG2 may also play a role in the concentration in milk of toxins and drugs such as the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (Jonker et al., 2001a). These substrates include thera-
optics. Consistent with this, ABCG2 expression is found in the canalicular membrane of hepatocytes (Maliepaard et al., 2001a), in the bile ducts (Vander Borght et al., 2006), in the gallbladder (Aust et al., 2004), and in the apical membrane of proximal kidney tubule epithelial cells (Huls et al., 2008) (Fig. 8). Notably, ABCG2⁻/⁻ mice have also been shown to have impaired renal or hepatic excretion of several ABCG2 substrates (Mizuno et al., 2004).

**Subcellular Localization.** ABCG2 is located predominantly in the plasma membrane of drug-selected and ABCG2-transfected cell lines, as has been demonstrated by a number of immunohistochemical studies and confocal microscopy drug localization assays (Scheffer et al., 2000; Maliepaard et al., 2001a). This is somewhat unusual, as almost all other known half-transporters are localized to intracellular membranes such as those of the mitochondrion or the ER. Examples of such transporters include the ABC family members ABC7 and M-ABC1, which are localized in the mitochondria (Hogue et al., 1999), and ABCB2 and ABCB3 that are found in the ER (Kleijmeer et al., 1992). Only one other half transporter has been found to be at least partially localized to the plasma membrane, namely, the human homolog of the *Drosophila* white gene, ABCG1 (Vaughan and Oram, 2005). The localization of ABCG2 may be regulated in part by cell signaling pathways such as the phosphatidyl inositol 3-kinase (PI3K) /γ-Akt murine thymoma viral oncogene homolog (Akt) pathway. In fact, inhibition of both PI3K (Misra et al., 1998) and Akt (Chu et al., 2008) has been shown to cause ABCG2 translocation from the plasma membrane to an intracellular compartment. Moreover, ABCG2 may also be present in the membranes of acidic vesicles (e.g., lysosomes), as colocalization...
studies with Lysotracker demonstrated sequestration of mitoxantrone in acidic vesicles of both S1 cells and the S1-M1-80 subline that expresses high ABCG2 levels (Litman et al., 2000).

**Significance of ABCG2 in Cancer**

ABCG2 is overexpressed in several MDR cancer cell lines, which is indicative of its importance in the MDR phenotype of cancer cells (Allikmets et al., 1998; Doyle et al., 1998; Miyake et al., 1999). Given that a large number of commonly used chemotherapeutics are ABCG2 substrates, the clinical impact of ABCG2 on MDR in cancer is being actively investigated (Robey et al., 2007). To date, studies that have attempted to link ABCG2 expression to clinical outcomes have been inconclusive and often contradictory (Doyle and Ross, 2003). However, the possibility remains that modulation of ABCG2 may increase the oral bioavailability of chemotherapeutics or overcome MDR.

**Expression in Hematologic Malignancies.** ABCG2 has been found to be frequently expressed on malignant hematopoietic and lymphoid cells and has been most thoroughly investigated in acute myeloid leukemia (AML) 6. (Table 1; Sargent et al., 2001; Van der Heuvel-Eibrink et al., 2002; van der Kolk et al., 2002; Doyle and Ross, 2003; van der Pol et al., 2003; Galimberti et al., 2004). In AML, ABCG2 has shown potential as a prognostic indicator despite the fact that common first-line therapies for AML are not ABCG2 substrates (Natarajan et al., 2012). To date, all AML patients examined have possessed the wild-type ABCG2 sequence (Plasschaert et al., 2003; Suvannasankha et al., 2004), and these patients, therefore, should not be resistant to anthracyclines as they are not substrates of wild-type ABCG2 (Fig. 6) (Honjo et al., 2001). However, a correlation was found between ABCG2 expression and failure to achieve complete remission in AML patients after induction therapy with the cytotoxic agent cytarabine (Steinbach et al., 2002). This is likely due to P-gp expression, as high levels of P-gp have been found in 45% of AML cases when the patient is aged over age 55 (Leith et al., 1997), although this was not directly investigated. High ABCG2 levels in AML have also been correlated with a relapsed or refractory disease state, lower complete response rate, and shorter survival (Table 1) (Benderra et al., 2004, 2005).

These latter results are inconsistent with those found by other investigators who reported a correlation with survival in AML patients, but not the complete response rate (Ugga et al., 2005). Interestingly, ABCG2 expression has been observed in small subpopulations of AML cells (Abbott et al., 2002; Suvannasankha et al., 2004) and seems to be most highly expressed on CD34+/CD38− “cancer stem cells” (see “Cancer Stem Cells and MDR”), which have an immature phenotype (Raaijmakers et al., 2005; de Figueiredo Pontes et al., 2008). It should be noted that the detection of ABCG2 in AML cells has been affected by methodological issues. These problems have potentially contributed to the discordance observed between studies correlating ABCG2 expression to prognostic outcomes (Table 1).

The role of ABCG2 in acute lymphoblastic leukemia (ALL) drug resistance has also been investigated (Table 1). As in AML, high ABCG2 expression has been correlated with resistance to the cytotoxic agent cytarabine, a non-ABCG2 substrate (Stam et al., 2004). Many common drugs used to treat ALL are not ABCG2 substrates, but this latter observation suggests that in both AML and ALL, ABCG2 expression may be a marker of resistance, as opposed to a mechanism that is

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>Results</th>
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<tr>
<td><strong>AML</strong></td>
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<tr>
<td>Sargent et al., 2001</td>
<td>20 Blast samples</td>
<td>Twenty-seven percent (27%) of samples were ABCG2 positive. Low ABCG2 expression, no increase in relapse samples.</td>
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<td>van der Kolk et al., 2002</td>
<td>20 Paired (pretreatment and refractory/relapse) samples</td>
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<td>Abbott et al., 2002</td>
<td>40 De novo samples</td>
<td>Seventy-eight percent (78%) of samples had higher ABCG2 expression than normal blood and bone marrow; 7% had levels within the range of drug-resistant cell lines. Relapsed samples had higher ABCG2 expression, ABCG2 expression associated with P-gp expression.</td>
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<tr>
<td>van Den Heuvel-Eibrink et al., 2002</td>
<td>20 Paired (de novo and refractory/relapse) samples</td>
<td>No ABCG2 detected. ABCG2 expression detected in 56% of samples, intermediate levels in 48.2%. ABCG2 expression associated with P-gp expression.</td>
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<tr>
<td>van der Pol et al., 2003</td>
<td>45 Paired samples</td>
<td>No correlation between function, protein, or gene expression. Median ABCG more than 10× higher in patients who did not achieve remission compared with responders. ABCG2 expression higher at relapse than diagnosis. High ABCG2 linked to worse prognosis.</td>
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<tr>
<td>Galimberti et al., 2004</td>
<td>51 AML samples</td>
<td>ABCG2 expression and ABCG2 expression associated with P-gp function were correlated with poorer treatment outcomes: achievement of complete remission, 4-year disease-free survival and 4-year overall survival.</td>
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<tr>
<td>Suvannasankha et al., 2004</td>
<td>31 Pretreatment blast samples</td>
<td>Patients with higher ABCG2 expression who respond to therapy had shorter overall survival. No difference in expression between responders and nonresponders.</td>
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<td>Steinbach et al., 2002</td>
<td>59 Untreated AML, 9 relapse samples</td>
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<td>Benderra et al., 2005</td>
<td>149 De novo samples</td>
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<td>Ugga et al., 2005</td>
<td>40 AML samples</td>
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<td><strong>ALL</strong></td>
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<td>Sauerbrey et al., 2002</td>
<td>47 De novo, 20 relapsed samples</td>
<td>No correlation between expression and response, ABCG2 expression lower in T-cell lineages. EXP-34 positivity in B-cell lineage 2.4-fold higher than T-cell lineage. ABCG2 expression 2.4-fold less in infant samples. Expression correlated with Ara-C resistance.</td>
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<tr>
<td>Plaschaert et al., 2003</td>
<td>46 De novo samples</td>
<td>43% of cases BXP-21 positive. BXP-21 staining predictive of shorter disease-free survival.</td>
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<tr>
<td>Stam et al., 2004</td>
<td>13 Noninfant and 13 infant samples</td>
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<tr>
<td>Suvannasankha et al., 2004</td>
<td>30 Pretreatment samples</td>
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directly involved in this process (Natarajan et al., 2012). Results linking ABCG2 expression to prognostic outcomes in ALL have been inconsistent, particularly between adults and children. For example, ABCG2 expression in adults has been correlated with poorer disease-free survival (Suvannasankha et al., 2004), whereas in children, ABCG2 expression has shown not to be related to relapse-free survival time (Table 1) (Sauerbrey et al., 2002). In contrast, in children, low expression of ABCG2 is correlated to a higher incidence of death resulting from drug toxicity (Cortez et al., 2009). This observation may be due simply to the fact that children have better clinical outcomes than adults in ALL.

**Expression in Solid Tumors.** The expression of ABCG2 in solid tumors was initially not detected by immunohistochemical studies (Scheffer et al., 2000), although with the use of a different monoclonal antibody (BXP-21 as opposed to BXP-34), ABCG2 was subsequently identified by the same group of researchers in more than 40% of tumors examined (Diestra et al., 2002). These tumors represented more than 21 different types of untreated cancer, such as colon, esophageal, endometrial, breast, pancreatic, and lung cancers (Diestra et al., 2002).

Like hematologic malignancies, ABCG2 expression in some types of solid tumors has been correlated with poorer outcomes (Mo and Zhang, 2012). In small cell lung cancer, high ABCG2 expression has been associated with poor clinical response and progression-free survival to platinum drug (carboplatin and cisplatin)-containing regimens (Kim et al., 2009) and lower overall survival in patients with non–small cell lung cancer (Ota et al., 2009). Unfortunately, the results of studies examining this latter tumor-type and ABCG2 expression lack consistency (Kanzaki et al., 2001; Faneyte et al., 2002; Burger et al., 2003), as do those for breast cancer (Kanzaki et al., 2001; Burger et al., 2003), and so it is impossible to draw firm conclusions about whether there is a link between ABCG2 and clinical outcomes in solid tumors (Kanzaki et al., 2001; Faneyte et al., 2002; Burger et al., 2003). Most recent studies have moved away from attempting to assess this link and have instead focused on ABCG2 and its role in the cancer stem cell phenotype (see the following).

**Cancer Stem Cells and MDR.** Although the idea of cancer stem cells is still controversial, the theory has gained increasing traction in recent years (Visvader and Lindeman, 2008). Cancer stem cells are a subset of tumor cells so named to reflect their shared properties with normal stem cells as they retain the ability to self-renew and differentiate, although to a limited extent (Visvader and Lindeman, 2008). The first evidence of cancer stem cells was found in immune-deficient mice, where it was ascertained that a small subset (0.1–1%) of AML cells transplanted into these mice could induce leukemia (Lapidot et al., 1994). Cancer stem cells have also been separated from solid tumors and usually express specific markers, which have been useful in their isolation (Hirschmann-Jax et al., 2004). These markers include CD24 (Al-Hajj et al., 2003), CD44 (Al-Hajj et al., 2003), and CD133 (Singh et al., 2004). Notably, ABCG2 is also a marker of cancer stem cells, as the efflux of the ABCG2 substrate, Hoechst 33342, is exploited to isolate them (Zhou et al., 2001). Cancer stem cells are often referred to as a “side population” because they display low Hoechst fluorescence on flow cytometry and appear as negatively-stained cells to one side of the main population (Zhou et al., 2001). Although Hoechst 33342 is also a P-gp substrate, it has been established that the side population phenotype is due to ABCG2 expression since the side population is still present in ABCB1a/1b−/− (P-gp knockout) cells (Zhou et al., 2001).

Cancer stem cells offer an alternative to the conventional model of acquired MDR, in which MDR occurs by cells gaining mutations which confer drug resistance, giving them an advantage over other cells in the tumor population by allowing them to survive chemotherapy (Fig. 9A) (Dean et al., 2005). The cancer stem cell model of MDR differs in that the resistant cancer stem cells are inherently present in the tumor cell population, and after chemotherapy, these cells survive (possibly owing to the presence of ABCG2 or P-gp) and ultimately repopulate the tumor with both cancer stem cells and more differentiated cells (Fig. 9B) (An and Ongkeko, 2009). In an additional model of cancer stem cell MDR, some cancer stem cells acquire new mutations, which further increase

![Fig. 9. Different models of tumor MDR](molpharm.aspetjournals.org)
Pharmacological Targeting of ABCG2 in Cancer. The expression of ABCG2 by cancer stem cells raises the possibility of selectively targeting cancer stem cells in chemoresistant tumors. As yet, this remains an elusive ideal. ABCG2 is expressed on normal hematopoietic stem cells, as well as in the BBB, so any drug would need to be carefully titrated to ensure that toxicity is minimized. Serious adverse events related to altered pharmacokinetics of coadministered cytotoxics are one of the many reasons why clinical trials of P-gp inhibitors have been disbanded (Nobili et al., 2006). The first ABCG2 inhibitor, fumitremorgin C, is not used in clinical settings because of its severe neurotoxic effects, and this has led to the development of a series of structural analogs, of which Ko143 is the most potent and does not cause neurotoxicity in mice (Allen et al., 2002b). These inhibitors of ABCG2 have not been tested in clinical trials to date, but they remain a promising possibility if cell-type specificity were to be achieved.

As ABCG2 inhibitors, tyrosine kinase inhibitors are an interesting option for pharmacological targeting of ABCG2. Clearly, these agents have already been in clinical trials, and thus, it may be theoretically easier to gain approval for their use, making them a more tenable possibility than untested inhibitors such as Ko143. In cell-based assays, tyrosine kinase inhibitors have been shown to be substrates of ABCG2 (possibly binding at the prazosin-binding site (Özvegy-Laczka et al., 2004; Yang et al., 2005). Erolitinib, gefitinib, and imatinib have all been shown to increase the accumulation and, therefore, the cytotoxicity, of ABCG2 substrates such as mitoxantrone and methotrexate in vitro (Misra et al., 1998; Özvegy-Laczka et al., 2004; Yang et al., 2005).

In addition to inhibiting ABCG2, tyrosine kinase inhibitors may downregulate the expression of ABCG2 via the P13K-Akt pathway (Nakanishi et al., 2006). These results suggest that combination therapies involving tyrosine kinase inhibitors and conventional chemotherapeutics may prevent MDR and enhance the efficacy of both treatments. This intervention has yet to be trialed, although coadministration of imatinib and the photodynamic therapy, 2-(1-hexylloxyethyl)-2-devinyl pyropheophorbide a, has been tested in vivo (Liu et al., 2007). Many photosensitizers are ABCG2 substrates, meaning that their intracellular accumulation is decreased in tumors that express the transporter (Robey et al., 2005). The combination of imatinib and 2-(1-hexylloxyethyl)-2-devinyl pyropheophorbide a significantly enhanced the efficacy of the photosensitizer, halving the rate of tumor growth (Liu et al., 2007). These promising results warrant further investigation in clinical trials.

The potential of phosphodiesterase-5 inhibitors as potential chemosensitizers has also been investigated (Black et al., 2008; Das et al., 2010). In fact, sildenafil has been shown to inhibit ABCG2 and P-gp at clinically achievable concentrations and reverse the efflux of mitoxantrone, paclitaxel, and methotrexate (Shi et al., 2011). Currently, no clinical trial results have been published on examining the effect of phosphodiesterase-5 inhibitors on ABC transporters. However, studies in animal models have demonstrated that the use of sildenafil in combination with doxorubicin results in improved transport of doxorubicin across the BBB (Black et al., 2008) and improved efficacy of doxorubicin in prostate cancer (Das et al., 2010). It is possible that the increased activity of doxorubicin is due at least in part to the inhibition of ABCG2 and P-gp by sildenafil, and this suggests that phosphodiesterase-5 inhibitors may prove to be useful chemoadjuvants in the future (Tiwari and Chen, 2013).

Conclusion

Although a plethora of research has been conducted examining ABCG2 biochemistry and pharmacology with respect to substrates, inhibitors, protein structure and function, and gene regulation, vast gaps in our knowledge remain. For example, it is still not known what form higher-order oligomers of ABCG2 take, as a high-resolution crystal structure has yet to be solved. Hence, this shortcoming limits the ability to predict structural requirements for developing new substrates and inhibitors. Such information would be invaluable in exploiting ABCG2 as a therapeutic target, although this is another area that has not been thoroughly investigated. Probably the major reason for this failure of P-gp inhibitors in clinical trials is due principally to errors in methods, such as not selecting patients based on tumor expression of P-gp (Robey et al., 2009). This failure has led to an unwillingness to invest in targeted treatments for MDR in cancer, and to date, the importance of ABCG2 in cancer biology and treatment remains unclear.

Recent research suggests that combination therapies involving tyrosine kinase inhibitors and phosphodiesterase-5 inhibitors with conventional chemotherapeutics have potential to treat MDR tumors, and this is currently the best prospect for exploiting ABCG2 in the clinic, provided lessons are learned from past errors. Until this and further translational research has been completed, it will remain difficult to establish the impact of ABCG2 on chemotherapy outcomes.

Acknowledgments

The authors thank Dr. Darius Lane, Dr. Vera Richardson, and Nicole Seebacher of the Molecular Pharmacology and Pathology Program (University of Sydney) for critical appraisal of the manuscript before submission.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Stacy, Jansson, Richardson.

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