ROLE OF THE ABC TRANSPORTER BREAST CANCER RESISTANCE PROTEIN (BCRP, ABCG2) IN DRUG DISPOSITION

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Among the many biomolecular mechanisms known to affect drug disposition in mammalian systems, the Breast Cancer Resistance Protein (BCRP) is a relative newcomer. Originally identified as transporter capable of causing multidrug resistance in cancer cells, BCRP is a 655 amino acid member of the G subfamily of the ABC transporter superfamily, and is formally designated ABCG2 (1, 2). The BCRP gene is located on chromosome 4q22. Like all ABC subfamily members, BCRP is a half-transporter, possessing only 6-transmembrane domains, and one ATP-binding domain (Figure 1). For functional reasons, ABC transporters require at least two ATP-binding domains; hence to be functionally active, BCRP must dimerize or multimerize with another half-transporter. Current evidence suggests that BCRP is a homodimer or homomultimer. BCRP localizes to the plasma membrane of cells and results in low intracellular accumulation of its substrates by effluxing these molecules out of the cell. Because of its location in tissues critical to drug clearance, the ability of BCRP to transport certain drugs, xenobiotics and naturally occurring small molecules bestows this transporter with the potential for having a major impact on the disposition of pharmaceutical agents.

In polarized cells such as placental syncytiotrophioblast, hepatocytes (bile canaliculi), and intestinal mucosal cells, BCRP is expressed on the apical membranes, where it serves to efflux substances out of the fetal circulation, or into the lumen of the bile duct or gut, respectively. Hence, BCRP has demonstrated a substantial effect on drug clearance, which is of considerable interest regarding potential interactions of BCRP substrate drugs or natural compounds, many of which can also function as BCRP inhibitors. The rat homologue of BCRP is expressed on the luminal surface of rat brain capillary endothelial cells, where it may function as a component of the blood brain barrier.

Based on the above, it is likely that a major physiologic function of BCRP/ABCG2 is to protect critical tissues such as brain and fetus (via the placenta) from potentially toxic xenobiotics, and to assist the clearance of xenobiotics from the organism. Xenobiotics currently known to be substrates for BCRP include many currently approved cancer chemotherapeutic agents such as mitoxantrone, camptothecin-derived topoisomerase inhibitors (eg., topotecan and SN-38), methotrexate, methotrexate polyglutamates, and anthracyclines (daunorubicin and doxorubicin), as well as anticancer drugs undergoing clinical trials such as the HER-tyrosine kinase inhibitor CI-1033, and the cyclin-dependent kinase inhibitor flavopiridol (2). The ability of BCRP to efflux anthracyclines is greatly enhanced by the presence of a mutation at codon 482 (R482T or R482G), which has been observed in cells that overexpress BCRP following drug selection (3, 4). Such mutations lose the ability to efflux methotrexate (4). Recently, the Bcr-Abl tyrosine kinase inhibitor imatinib (Gleevec, STI-517) was found to be a substrate for BCRP (5). Imatinib is currently the most effective drug known for the treatment of the chronic phase of CML.

A number of natural or physiological substrates for BCRP have been identified. BCRP transports folic acid, and may play a role in cellular folate homeostasis (6). In mice, BCRP transports pheophorbide a, a breakdown product of chlorophyll found in mouse chow; BCRP knockout mice suffered photosensitivity as the result of tissue accumulation of pheophorbide a (7). This work led to the discovery that BCRP transports protoporphyrin IX, which is structurally related to pheophorbide a. BCRP binds to hemin, and may transport products of the heme biosynthetic or catabolic pathway. Conjugates of estrogens (estrone 3 sulfate and estradiol glucuronide) are also substrates, but free estrogens are not.

Potent and specific inhibitors of BCRP/ABCG2 are currently available, with some of the inhibitors in clinical trials or available for clinical use. Fumitremorgin C (FTC), a mycotoxin isolated from Aspergillus fumagatis, is highly specific for BCRP, and active at micromolar concentrations. Ko143, a derivative of FTC, is considerably more potent than the parent compound. Currently these specific BCRP inhibitors are useful for functional assays of BCRP transporter activity. GF120918 inhibits both P-gp and BCRP, and is currently undergoing clinical trials as an oral agent. One of these trials demonstrated that co-administration of GF120918 increased the oral bioavailability of topotecan, a BCRP-substrate drug, from 30% to 90% (8). Other BCRP inhibitors include tressa, flavopiridol, reserpine, certain taxane derivatives (orataxel and tRA96023), flavopiridol, flavonoids, reserpine, estrogens and antiestrogens, and certain HIV protease inhibitors. Hence, many natural products and currently approved pharmaceuticals are substrates and/or
inhibitors of BCRP, which raises the possibility that these agents could, when administered simultaneously, alter the clearance and disposition of other BCRP substrate molecules.

### Table 1. Selected Genetic Variations in Human BCRP/ABCG2 Coding Region

<table>
<thead>
<tr>
<th>Variation</th>
<th>Domain of BCRP</th>
<th>Population/freq of at least one allele</th>
<th>Effect/comments</th>
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<tbody>
<tr>
<td>G34A/V12 M</td>
<td>N terminal</td>
<td>Swedish, 2%; African-Americans, 8%, Mexican Indians, 100%, Japanese 30%, Chinese 40%</td>
<td>Decreased transporter activity</td>
</tr>
<tr>
<td>C421A/Q141K</td>
<td>ABC signature</td>
<td>Japanese, 46%; Swedish, 10%; North American White, ~25%; African Amer., 0%; Chinese 60%</td>
<td>Decreased activity; impaired clearance of diflomotecan</td>
</tr>
<tr>
<td>A616C/I206L</td>
<td>Walker B</td>
<td>Very low</td>
<td></td>
</tr>
<tr>
<td>A1768T/N590Y</td>
<td>Extracellular domain</td>
<td>Very low</td>
<td></td>
</tr>
<tr>
<td>A1444G/R482G</td>
<td>TM3</td>
<td>Drug selected cell lines only</td>
<td>Gain of anthracycline transport, and loss of methotrexate transport</td>
</tr>
<tr>
<td>G1445C/R482T</td>
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**Pharmacogenomics:** Partial sequencing of BCRP exons from multiple genomic DNAs demonstrates that the genetic sequence is highly conserved, although single nucleotide polymorphisms (SNPs) are found. Allelic variation as a result of SNPs results in alterations of the BCRP protein at amino acids 12 (V12M), 141 (Q141K), 206 (I206L), and 590 (N590Y), with the most frequent polymorphisms being the exon 2 SNP (G34A) and the exon 5 SNP (C421A), which produce changes in amino acids 12 and 141 (9-11). Additionally, a splice variant was found that resulted in deletions of A315 and T316 (10). The effect of most of these polymorphisms in BCRP function remain to be determined, although one study suggested that the C421A/Q141K variant may result in very low levels of BCRP protein expression (10). These investigators found that in a normal Japanese population, 39% were heterozygous and 7% were homozygous for the variant C412A allele. In a North American white population, patients heterozygous for the C421A allele had 3-fold higher plasma levels of diflomotecan following IV administration, compared to patients with the wild-type allele (12). Hence, the potential for inter-individual variation in drug disposition resulting from BCRP polymorphisms is quite high.

**Selected References:**