THE ROLE OF UDP-GLUCURONOSYLTANSFERASES AND DRUG TRANSPORTERS IN BREAST CANCER DRUG RESISTANCE

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One of the major limitations of chemotherapy is that often, over time, tumor cells become either inherently resistant or develop multidrug resistance to the treatment. Another limitation of chemotherapy is toxicity to normal tissues and adverse side effects. The reasons for the failure of some cancers to respond to chemotherapeutic drugs are not clear but have been attributed to alterations in many molecular pathways, which include drug metabolizing enzymes and drug transporter genes. Alterations in the energy-dependent ATP-binding cassette (ABC) transporter genes have been suggested to confer a drug-resistant phenotype by decreasing the intracellular accumulation of chemotherapeutic drugs via efflux mechanisms [10]. In addition, polymorphisms in UDP-glucuronosyltransferases (UGTs) have been reported to correlate with clinical outcome and drug resistance. In this review, we provide an overview of known polymorphisms within UGTs and ABC transporter genes that have been reported to have altered expression and/or activity in breast cancer. Those polymorphic variants that affect the clinical efficacy and confer drug resistance of chemotherapeutic agents, including hormonal therapies, taxanes, anthracyclines, and alkylating agents, in breast cancer. 

Key Words: UDP-glucuronosyltransferase, ATP-binding cassette transporters, multi-drug resistance.

Breast cancer is a major health problem that will affect approximately 1.3 million women worldwide this year alone [1]. Although breast cancer continues to be one of the leading causes of cancer death among women, there has been a statistically significant decline in breast cancer incidence from 2002–2009, which has been attributed to early detection, advances in prevention, and the development of targeted therapies and chemotherapeutic agents that eradicate cancer cells [2]. However, one of the major limitations of chemotherapy is that often, over time, tumor cells become either inherently resistant or develop multidrug resistance (MDR) to a variety of chemotherapy drugs over time. Drug resistance to chemotherapeutic agents accounts for treatment failure in more than 90% of patients with metastatic cancer [3]. This phenomenon is evident in many patients with advanced stage of disease who do not respond to the drug treatment, experience drug relapse, and/or subsequently die from the disease. In comparison, patients who are given chemotherapy with early stage breast disease tend to have an increased chance of survival due to better response to chemotherapeutic drugs [3]. Another limitation of chemotherapy is toxicity to normal tissues and adverse side effects. Some of these adverse effects include nausea, loss of appetite, hair loss, fatigue, and increased susceptibility to infections due to low white blood cell counts. In addition, some chemotherapeutic agents, for example tamoxifen, a chemotherapeutic agent used for the treatment and prevention of estrogen receptor (ER)-positive or progesterone receptor (PR)-positive breast cancer, have been shown to increase the risk of endometrial cancer and venous thromboembolism [4]. Table 1 lists the major chemotherapeutic drugs used in the treatment of breast cancer and their reported adverse side effects. The reasons for the failure of some cancers to respond to chemotherapeutic drugs are not clear but have been attributed to alterations in many molecular pathways, which include the decreased uptake of water-soluble drugs, increased repair of DNA damage, reduced apoptosis, and increased energy-dependent efflux of hydrophobic drugs. Among the most studied genes and gene products that confer drug resistance are the ATP-binding cassette (ABC) transporters, primarily P-glycoprotein (ABCB1) [5, 6], multidrug resistance protein 1 (ABCC1) [7], and breast cancer resistance protein [8]. The ABC transporters are responsible for the cellular uptake and efflux of drugs, which are critical for drug absorption, distribution, and excretion [9]. Drug transporter genes have been suggested to confer a drug-resistant phenotype by decreasing the intracellular accumulation of chemotherapeutic drugs via efflux mechanisms [10].

In addition to drug transporters, alterations in drug metabolizing enzymes, in particular UGTs, have been linked to cancer drug resistance and drug toxicity. For example, UGTs have been demonstrated to be an intrinsic mechanism of resistance to the DNA topoisomerase I inhibitors 7-ethyl-10-hydroxycamptothecin and NU/ICRF 505 in human colon cancer cells [11]. Furthermore, large interindividual variability in UGT expression and activity has been observed in the clinical efficacy of chemotherapeutic agents used to treat breast cancer, which can lead to increased risk of...
cellular toxicity [12]. These interindividual differences in UGT expression within the population may be due to alterations in UGT genes that undergo genetic and/or epigenetic changes that may result in chemoresistance. Several studies have provided evidence that single nucleotide polymorphisms (SNPs) in both UGTs and ABC transporters can affect the pharmacokinetics and pharmacodynamics of various chemotherapeutic agents [13, 14]. This is of great importance since many of the glucuronide conjugates of chemotherapeutic agents are substrates of ABC transporters. Therefore, alterations in UGTs and ABC transporter genes may impact drug response and disease susceptibility.

Table 1. Major adverse effects associated with chemotherapeutic drugs used for breast cancer treatment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Serious Adverse Effects</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoxifen</td>
<td>Joint and chest pain, depression, increased risk of blood clots, vaginal bleeding, hypertension, hypercholesterolemia, endometrial cancer</td>
<td>Competitive inhibitor of ER</td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>Depression, anxiety, increased risk of blood clots</td>
<td></td>
</tr>
<tr>
<td>Raloxifene</td>
<td>Increased risk of blood clots, gall stones, uterine bleeding and stroke, depression</td>
<td></td>
</tr>
<tr>
<td>Aromatase Inhibitors</td>
<td>Osteoporosis, joint pain, hypercholesterolemia, cardiovascular risk</td>
<td>Inhibit the enzyme aromatase</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Ankylostomiasis, bloody stool, hyper/hypotension, diarrhea, infections, severe mouth sores, fluid retention</td>
<td>Enhance the stability of microtubules</td>
</tr>
<tr>
<td>Docetaxel</td>
<td>Bloody stool, diarrhea, infections, severe mouth sores, fluid retention</td>
<td></td>
</tr>
<tr>
<td>Vinca alkaloids</td>
<td>Congestive heart failure, arrhythmia, bloody stool, seizures, diarrhea, infections, severe mouth sores</td>
<td>Inhibit the incorporation of tubulin into microtubules DNA double strand breaks</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Congestive heart failure, arrhythmia, bloody stool, seizures, diarrhea, infections, severe mouth sores</td>
<td></td>
</tr>
<tr>
<td>Epirubicin</td>
<td>Congestive heart failure, arrhythmia, bloody stool, diarrhea, infections, severe mouth sores</td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>Thrombocytopenia, seizures, hyperuricemia, kidney failure, anemia, hearing loss/tinnitus, liver/viscera problems</td>
<td>Cross-link subunits of DNA</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>Hearing/viscera problems, mouth/throat sores, bleeding, infections, hypocalcemia/hypokalemia</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td>Hearing/viscera problems, mouth/throat sores, infections, hypocalcemia/hypokalemia</td>
<td></td>
</tr>
</tbody>
</table>

UGTs are classified into two families, UGT1 and UGT2, based on the similarity of their primary amino acid sequences [18]. The human UGT1 and UGT2 gene families encode 19 RNA transcripts that have been identified in several human tissues [19]. The UGT1 isoforms in humans are encoded by a single gene locus on chromosome 2-q37 [20]. The UGT1A isoforms share more than 50% sequence homology with each other but less than 50% identity with members of the 2B family. UGT1A isoforms are generated by alternative splicing of a unique exon 1 to common exons 2–5. The UGT1A locus encodes nine functional first exon cassettes (UGT1A1 and UGT1A3–10) and three non-functional exon 1 sequences (UGT1A2, UGT1A11, and UGT1A12) that encode the unique N-terminal domains and approximately two-thirds of the luminal domains of the UGT1A proteins. Common exons 2–5 encode the UDP-glucuronic acid (UDP-GA) binding site and the remainder of the luminal domain, which is identical in all UGT1A family members [21].

The human UGT2 gene family is divided into two subfamilies, UGT2A and UGT2B. In contrast to the UGT1 family, most of the UGT2 genes are compromised of six exons that are not shared between the UGT2 family members. UGT2 genes are clustered on chromosome 4-q13. UGT2B isoforms are the most abundant of the UGT2 isoforms and are, therefore, the most studied. Furthermore, polymorphic variations within the coding region of UGT genes have been associated with altered UGT expression and activity that may affect glucuronidation of various drugs, including breast cancer drugs (Table 2), environmental pollutants, and endogenous compounds.

The pharmacokinetics of UGT enzymes toward estrogens and their metabolites (catechol estrogens) have also been studied extensively [22–25]. In our previous study, several UGT isoforms, including UGT1A10 and UGT2B7, displayed high catalytic activity and affinity for estrogens and their metabolites and therefore were further characterized in the breast tissue [26]. The results demonstrated that in African-American women and Caucasian women, UGT1A10 and UGT2B7 was down-regulated in malignant breast tissues compared to the corresponding normal breast tissue [26]. Furthermore, glucuronidation activity towards 17β-estradiol, the most physiologically active form of estrogen, was decreased in the majority of breast cancers compared to normal breast tissues. In the same study, glucuronidation activity towards 4-hydroxyestrone, the 4-hydroxy metabolite of estrone, which has been implicated in breast carcinogenesis, was significantly lower in all breast tissues compared to normal breast tissues [26]. In another study, Gestl and coauthors [27] demonstrated that the rates of glucuronidation of 4-hydroxyestrone were significantly lower in neoplastic tissues compared to normal tissues. In the same study, UGT2B7 protein in the epithelium lining the mammary gland ducts was shown to be highly variable among individuals. Collectively, these data suggest that UGT1A10 and

**GENERAL OVERVIEW**

**UDP-Glucuronosyltransferases.** UGTs are a group of Phase II drug-metabolizing enzymes that have been extensively studied for many years due to their broad substrate specificity and ability to conjugate a variety of endogenous and exogenous substrates with diverse chemical structures and physiochemical properties [15]. UGTs biotransform a broad range of substrates by catalyzing the transfer of glucuronic acid from UDP-glucuronic acid (UDP-GA) to hydrophobic endogenous and exogenous substrates in a process referred to as glucuronidation. The glucuronide end product is generally an inactive, hydrophilic compound that can be readily excreted from the body via the urine or bile [16]; however, UGTs can also generate bioactive or even toxic products [17].
UGT2B7 might play a role in protecting the breast from carcinogenic estrogens.

### Table 2. Summary of polymorphic variants within the coding region of UGTS and ABC transporter genes and their influence on breast cancer drug response

<table>
<thead>
<tr>
<th>Genes</th>
<th>Polymorphism</th>
<th>Drug</th>
<th>Gene Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>UGT1A1*12Glu to Val</td>
<td>Tamoxifen</td>
<td>Increased activity of tamoxifen and 4-hydroxytamoxifen [54]</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>UGT2B7*1T to C</td>
<td>Doxorubicin</td>
<td>Decreased expression of doxorubicin and endoxifen [54]</td>
</tr>
<tr>
<td>UGT2B15</td>
<td>UGT2B15*2</td>
<td>Doxorubicin</td>
<td>Reduced 5-year survival rate [56]</td>
</tr>
<tr>
<td>ABCC2</td>
<td>ABCC2C2</td>
<td>Fulvestrant</td>
<td>Reduced recurrence-free survival [47]</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>UGT1A1*28</td>
<td>Fulvestrant</td>
<td>Increased activity and hip bone density [77]</td>
</tr>
<tr>
<td>ABCB1</td>
<td>ABCB1*8</td>
<td>Docetaxel</td>
<td>Dominant clearance of doxorubicin and lower plasma concentration of doxorubicin in patients with CC-GG-CC compared to CG-GT-CT haplotype [97]</td>
</tr>
<tr>
<td>ABCB1</td>
<td>ABCB1*8</td>
<td>Anthracyclines</td>
<td>Better clinical response to docetaxel and lower clearance of doxorubicin and endoxifen [54]</td>
</tr>
<tr>
<td>ABCB1</td>
<td>CC-GG-CC</td>
<td>Doxorubicin</td>
<td>Increased clearance of doxorubicin in patients with CC-GG-CC compared to CT-GT-CT haplotype [93]</td>
</tr>
</tbody>
</table>

Interestingly, polymorphic variants that lead to alterations in UGT enzymes that conjugate estrogens and/or their metabolites have been associated with increased breast cancer risk in African-American women. In a study consisting of 200 African-American women with invasive breast cancer and 200 matched controls, who were genotyped for the presence of the UGT1A1*28 genetic variant, premenopausal African-American women carrying the UGT1A1*28 genetic variant were shown to be at slightly increased risk for developing ER-negative breast cancers [28]. In addition, several population-based studies of breast cancer patients have demonstrated an association between genetic variability in UGT1A1, which conjugates estrogens, and breast cancer risk [29, 30].

### ATP-binding cassette transporters

ATP-binding cassette transporters (ABC-transporter) are transmembrane proteins that make up one of the largest and oldest superfamilies that transport a wide variety of substrates across extracellular and intracellular membranes. Most eukaryotic ABC transporters are localized in the plasma membrane and are responsible for exporting substrates including metabolic products, lipids and sterols, toxins, and drugs out of the cell [31]. ABC transporters possess a broad tissue distribution and have also been shown to protect the body against xenobiotics and their metabolites. ABC transporters utilize the energy of adenosine triphosphate (ATP) hydrolysis to translocate various substrates across membranes, and they have also been demonstrated to play a role in translation of RNA and DNA repair [9].

Currently, 48 human ABC transporter genes have been identified and sequenced [32]. ABC transporters are classified into seven subfamilies, A to G, based on the arrangement of their nucleotide-binding domains and transmembrane domains [32].

The transmembrane domain has a variable sequence and consists of alpha helices that recognize a variety of substrates. The alpha helices undergo conformational changes to transport substrates across the membrane. The nucleotide-binding domain is located in the cytoplasm, has a highly conserved sequence, and is the site for ATP binding [33]. Proteins are classified as ABC transporters based on their characteristic peptide sequence and organization of their ABC domain. ABC transporters that extrude substances out of the cell contain an intracellular domain that joins the membrane-spanning helices and the ABC domain. The intracellular domain is believed to be responsible for communication between the transmembrane domain and nucleotide-binding domain [33]. Most exporters are made up of a homodimer consisting of two half transporters or monomers of a transmembrane domain fused to a nucleotide-binding domain. Dimer formation of the ABC domains of transporters requires the binding of ATP [34].

It is well accepted that alterations in ABC transporters can result in MDR. MDR develops as a result of over-expression of certain ABC transporters, which, in turn, increases the efflux of the drug out of the cell. There are several ABC transporters that confer drug resistance including, ABCB1 [35], ABCG2 [36], and ABCC1 (MRP1) [7]. The ABCB1 gene, which encodes P-glycoprotein, is the most highly studied ABC transporter and is associated with MDR [35]. ABCB1 consists of a functional monomer with two transmembrane domains and two nucleotide-binding domains. ABCB1 transports large hydrophobic, mainly cationic or electrically neutral, substrates. ABCB1 is expressed in cancerous as well as noncancerous tissues, such as the brush-border membrane of the intestine, liver, and kidney and the blood-brain barrier [37]. The most-studied member in the ABCG family is ABCG2, which encodes the breast cancer resistance protein. The breast cancer resistance protein was first discovered in 1998 in doxorubicin-resistant breast cancer cells [36] and later in several other tissues, including the placenta, which suggests that it serves to protect the fetus [38]. Over-expression of breast cancer resistance protein is associated with a poor response to cancer chemotherapy and causes resistance to chemotherapeutic agents [39], including the DNA topoisoamerase I inhibitor topotecan and the antifolate agent methotrexate [40]. The ABCC family member MRP1 has also been demonstrated to participate in MDR of tumors [7]. Multidrug resistance proteins are essential for transporting glutathione and glucuronide conjugates, as in the case of SN-38 glucuronide, the active metabolite of irinotecan [41].

In this review, we will summarize various factors that have been reported to alter the expression and/or activity of UG Ts and ABC transporter genes in breast cancer, including known polymorphisms that may confer drug resistance of the most common chemotherapeutic agents used to treat breast cancer and thus effect of these drugs.
GLUCURONIDATION AND TRANSPORT MECHANISMS OF CHEMOTHERAPEUTIC AGENTS FOR BREAST CANCER

Antiestrogen Therapies

The majority of breast cancer cases depend on estrogen for tumor growth and differentiation. Furthermore, it is well accepted that one of the major risk factors for developing breast cancer in women is a cumulative exposure to estrogen. Approximately 75% of breast tumors in postmenopausal women are ER-positive and/or PR-positive and these patients are therefore candidates for endocrine treatment [42]. Antiestrogen therapies or selective estrogen receptor modulators act to bind competitively to and block the action of estrogen at the ER.Outlined below are some of the most common antiestrogens used for the treatment and prevention of breast cancer.

Tamoxifen (Nolvadex). Tamoxifen, a selective estrogen receptor modulator, is generally prescribed within the United States, usually for 5 years, as a component of adjuvant endocrine therapy to prevent endocrine receptor-positive breast cancer recurrence, to treat metastatic breast cancer, and to prevent disease in high-risk populations and in women with ductal carcinoma in situ [43]. Since the approval of tamoxifen by the U.S. Food and Drug Administration in 1977, tamoxifen has demonstrated significant beneficial effects by reducing breast cancer incidence and increasing overall survival rates in patients who had been taking tamoxifen for several years, including patients with ER-positive breast cancer and women at risk of breast cancer [44]. Furthermore, tamoxifen has been shown to reduce the risk of ischemic heart disease and osteoporosis [45]. Although tamoxifen use has led to significant reductions in breast cancer incidence, studies have shown that some women on tamoxifen may be at increased risk of experiencing adverse side effects, including nausea, hot flashes, vaginal bleeding, fatigue, venous thromboembolism [4], endometrial cancer, and uterine sarcoma [43]. In addition, 30–50% of patients with adjuvant tamoxifen therapy experience relapse and subsequently die of the disease [12].

Tamoxifen undergoes hydroxylation mediated by cytochrome P450 (CYP) enzymes. Both CYP2D6 and CYP3A4/5 play a role in metabolizing tamoxifen to several primary and secondary metabolites. 4-Hydroxytamoxifen and endoxifen are the therapeutically active forms of tamoxifen and have a high affinity for ER [46]. Variants of the CYP2D6 gene, which result in a protein with reduced or absent enzyme activity and lower plasma levels of active tamoxifen metabolites, have been observed in approximately 5% to 10% of women diagnosed with breast cancer [47]. In a retrospective study, there was an association between women with the presence of a nonfunctional or reduced-function CYP2D6 (*10, *41) alleles or absent (*3, *4, *5) and worse clinical outcome and worse event-free survival. Furthermore, the recurrence rates were highest among poor metabolizers at 29.0% compared to 14.9% for extensive metabolizers and 20.9% for heterozygous extensive/intermediate metabolizers and all-cause mortality rates were 22.8%, 18.0%, and 16.7%, respectively [48].

Recently, several studies using human recombinant UGT isozymes have provided evidence for the specific UGTs responsible for conjugating tamoxifen and its metabolites [49–52]. Of the UGTs involved, including UGT1A8, UGT1A10, and UGT2B7, UGT1A4 was identified as the major UGT isoform involved in the glucuronidation of tamoxifen and its metabolites, while UGT2B7 displayed the highest affinity and activity against trans-4-hydroxytamoxifen [53]. In addition, polymorphisms in UGT isoforms, including UGT2B7*28[54], UGT1A10*9[55], and UGT1A8*7[56] have been studied and compared to their wild-type counterparts to determine their impact on the glucuronidation of tamoxifen and/or its metabolites. A study by Lazarus et al. [55], showed that a variant in UGT1A8, 173Ala/277Tyr, exhibited no detectable glucuronidation activity against the trans isomers of either 4-hydroxytamoxifen or endoxifen, while glucuronidation activity towards tamoxifen was not affected for the UGT1A8*7[57], UGT1A8*10[58], and UGT1A10*9[59] variants compared to their wild-type counterparts. However, the UGT2B7*28[60] variant exhibited significantly decreased activity against the trans isomers of 4-hydroxytamoxifen and endoxifen compared with wild-type UGT2B7*28[61] [54]. Furthermore, there was no significant effect on the glucuronidation rates of the UGT1A1*24Thr/48Leu variant against trans-4-hydroxytamoxifen; however, higher N-glucuronidation activities were observed for UGT1A4*24Thr/48Leu-overexpressing cell microsomes as compared to wild-type UGT1A4*24Thr/48Leu-overexpressing cell microsomes. On the other hand, wild-type UGT2B7*28[62] showed significantly higher glucuronidation activity against trans-4-hydroxytamoxifen and trans-endoxifen compared to the UGT2B7*28[63] variant [53]. A retrospective study by Nowell and coauthors identified a nonsynonymous polymorphism, UGT2B15*2, in a putative substrate binding domain of UGT2B15 that significantly reduced the 5-year recurrence rate and survival rate in 162 breast cancer patients treated with tamoxifen compared to 175 patients who did not receive tamoxifen therapy and recurrence rate [55]. However, in a study that consisted of 677 tamoxifen-treated postmenopausal patients with breast cancer, of whom 238 were randomized to either 2 or 5 years of tamoxifen, there was no association between UGT2B15 genotype and disease-free survival rates was observed [56]. Collectively, these findings suggest that polymorphisms in UGTs responsible for tamoxifen conjugation and metabolism may alter the pharmacological response to tamoxifen therapy.

Studies from the literature also provide evidence of inter-individual variability in ABC transporters and patients receiving tamoxifen therapy. In a study of 282 patients with ER-positive, invasive breast cancer receiving tamoxifen monotherapy, the effects of allelic variants of haplotype-tagging single nucleotide polymorphisms of ABCB1, ABCCC2, and ABCG2 on recurrence-free survival were assessed. In that study, Kiyotani et al. [57] demonstrated that five single nucleotide poly-
morphisms in ABCC2 was significantly associated with shorter recurrence-free survival (P=0.001; HR = 10.64; 95% CI, 1.44 to 78.88) in patients with AA versus GG genotypes. ABCN1 (Multidrug resistance protein 8), an ABC transporter that confer [58] resistance to fluoropyrimidines and to efflux methotrexate, has been positively correlated with ER-alpha expression in breast cells and tumors from postmenopausal patients. In addition, expression of ABCN1 was up-regulated in MCF7 cells exposed to tamoxifen for 72 h, and was over-expressed in tamoxifen-resistant cell lines [59].

Several studies have correlated levels of P-glycoprotein, a transporter encoded by the MDR1 gene, before tamoxifen therapy to survival and response rates in breast cancer patients [60, 61]. Keen et al. [61] found that breast cancer patients who did not express P-glycoprotein were more likely to respond to tamoxifen. Subsequently, Linn et al. [62] showed that early breast cancer patients who did not express P-glycoprotein had a 3-year survival rate of 85% compared to 15% in those who expressed P-glycoprotein [62].

Studies from the literature also provide evidence of activation of ABC transporter genes through steroid receptors. A study by Koibuchi et al. [63] revealed that 4-hydroxytamoxifen activated the expression of CYP3A4 and MDR1 mRNA through steroid receptor, SXR, in MCF-7 cells. In another study, a significant increase in resistance toward tamoxifen was also observed in MCF-7 through the human pregnane X receptor (hPXR). Activation of the pregnane X receptor led to increased expression of CYP3A4 and MDR1 [64]. A study by Choi et al. [65] demonstrated that tamoxifen-resistant MCF-7 cells expressed higher levels of MRP2 than control MCF-7 cells also through the pregnane X receptor. These studies suggest that the induction of CYPs and ABC transporters by tamoxifen may affect tamoxifen metabolic pathways in estrogen responsive breast cells.

**Fulvestrant (Faslodex).** Fulvestrant is another antiestrogen that has been used for the treatment of ER-positive metastatic breast cancer in postmenopausal women with disease progression after previous antiestrogen therapy. Fulvestrant is a pure ER antagonist that, unlike tamoxifen, has not been reported to present estrogen agonistic effects in other tissues [66]. Since fulvestrant is an ER antagonist it acts to bind, block, and increase degradation of ER protein, leading to an inhibition of estrogen signaling through the ER [42]. Although fulvestrant has been suggested to be an effective and well-tolerated treatment in postmenopausal women with advanced breast cancer progressing prior tamoxifen therapy [42], fulvestrant has been shown to cause adverse side effects. In a multinational double-blind, randomized trial that compared the efficacy and tolerability of fulvestrant with tamoxifen as the initial hormonal treatment of advanced breast cancer in postmenopausal women, adverse effects were experienced by 269 of 310 patients who received fulvestrant (86.8%) and by 239 of 271 patients who received tamoxifen (88.2%). The most frequent side effects from fulvestrant, tamoxifen, or both included nausea, asthenia, vasodilatation, pain, and bone pain. In a phase III clinical trial, reported side effects from fulvestrant use included hot flashes, gastrointestinal problems, vaginitis, and weight gain [67].

Fulvestrant is primarily metabolized by CYPs, sulfotransferases and UGTs [68]. Fulvestrant has been shown to be glucuronidated by human recombinant UGT1A1, UGT1A3 [69], UGT1A4, and UGT1A8 enzymes. Kinetic analysis revealed that UGT1A4 displayed the highest affinity for fulvestrant with a Km, of 0.51μM and UGT1A3 and UGT1A4 displayed the highest catalytic efficiency than UGT1A1 and UGT1A8 for fulvestrant glucuronidation. In the same study, fulvestrant glucuronide was identified by LC-MS/MS. Another study demonstrated the expression of UGT1A3, UGT1A4, and UGT1A8 mRNA in mammary gland tissue, suggesting that fulvestrant may be inactivated by glucuronidation in mammary gland [70]. These findings indicate that alterations in UGT1A3 and UGT1A4 may significantly affect the glucuronidation rates of fulvestrant. Interestingly, seven regulatory and 10 exonic polymorphisms with six leading to amino-acid changes have been identified in UGT1A3. Reduced transcriptional activity towards estrone was also associated with all six variant promoters (two-fold; P <0.001) [71]. As previously described, missense polymorphisms in UGT1A4, namely SNPs at codon 24 and 48, may also alter the efficiency and activity towards tamoxifen. Thus, it would be important to determine whether UGT1, UGTA3 and UGT1A4 genetic variant may cause differential activity towards fulvestrant.

**Raloxifene (Evista).** Raloxifene is a selective estrogen receptor modulator used for the prevention and treatment of osteoporosis. In 2007, the FDA approved raloxifene for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women at high risk for invasive breast cancer. Raloxifene has shown promising results compared to tamoxifen in reducing breast cancer incidence [72]; however, raloxifene has been shown to produce significantly more strokes and blood clots compared to placebo [73].

Significant interindividual variability in raloxifene pharmacokinetics has been observed. Raloxifene shows high interindividual variability with linear pharmacokinetics [74]. Raloxifene has also been shown to be rapidly absorbed after oral administration and have a low absolute bioavailability of only 2% and long half-life [74]. It is primarily metabolized by UGT1A1, UGT1A8, and UGT1A10 to form raloxifene-4'-glucuronide or raloxifene-6'-glucuronide [75, 76]. Glucuronidation of raloxifene has been suggested to affect the low oral bioavailability of raloxifene in humans [77]. Recently, in vitro metabolic systems in human liver microsomes have been established to study raloxifene glucuronides [78]. In 2009, a study was conducted with serum samples from postmenopausal osteoporotic patients treated with raloxifene to compare the levels raloxifene glucuronides with genotypes for common UGT1A1 gen-
netic variants [79]. Patients who were homozygous for the UGT1A1*28 allele showed significantly higher raloxifene glucuronide concentrations and an increase in hip bone mineral density compared to wild-type allele.

The identification of raloxifene as a substrate of ABC1 has led to the development of several raloxifene-based multidrug resistance protein 1 inhibitors. Two raloxifene analogs that inhibit ABC1, namely LY117018 and LY329146, have been shown to reverse doxorubicin resistance in the MRP1-expressing HL60/ADR cell line. P-glycoprotein has also been observed to cause bioactivation of raloxifene in cryopreserved and freshly isolated human hepatocytes [80].

Other Chemotherapeutic Drugs

**Taxanes.** Taxanes, (i.e., paclitaxel and docetaxel) and the vinca alkaloids are the most active chemotherapeutic drugs used to treat breast cancer. The major limitation of taxane drugs is that only 30–50% of previously untreated patients respond to these agents [81]. Several studies have investigated the role of ABC polymorphisms, including ABCB1, BCRP, and MRP2, on the pharmacokinetics of these drugs; however, results have been conflicting. In one study that consisted of 92 patients, those individuals who were homozygous for the C1236T polymorphism in the ABCB1 gene (ABCB1*8) were significantly correlated with a decreased docetaxel clearance (-25%; P = 0.0039). Furthermore, there was no association between haplotypes of CYP3A and ABCB1 and pharmacokinetics [58]. By contrast, another study that consisted of 93 patients with high-risk primary or stage IV breast cancer, who received dose-intense paclitaxel, did not find an association between ABCB1 and ABCG2 genotypes and paclitaxel clearance [82]. Additional large population studies should be conducted to determine the pharmacokinetics and pharmacodynamics of ABC transporters on taxane drugs.

**Anthracyclines.** Anthracyclines (doxorubicin and epirubicin), derived from *Streptomyces* are effective against more types of cancer than any other class of chemotherapy agents. Adverse effects associated with anthracyclines are heart damage and vomiting. There also exists wide variability in response to anthracyclines. Epirubicin has been shown to undergo glucuronidation, catalyzed by UGT2B7, to form epirubicin glucuronide conjugates that are excreted [83]. A study in 2006, showed that epirubicin glucuronidation activity in an adult population was significantly higher compared with all pediatric age groups (P < 0.01). In that study, UGT2B7 expression was also significantly higher in infants compared with children below 11 years of age [84].

Doxorubicin and epirubicin are inactivated by cytochrome P450s and transported out of cells by ABC transporters, namely ABCB1, ABC1, ABC2, and ABCG2. Several investigations of the possible influence of variations in ABC transporter genes relevant to anthracyclines have been conducted. A polymorphism in the MDR-1 gene, C3435T, was demonstrated to correlate with a better clinical response in 68 patients who received preoperative chemotherapy to anthracyclines alone or combined with taxanes for locally advanced breast cancer [85]. A study conducted in Pakistani women (n = 68) with breast cancer using fluorouracil, doxorubicin and cyclophosphamide for treatment of breast cancer showed that a higher frequency of variant ABCB1 alleles was more prevalent in Pakistani women with breast cancer when compared to Caucasian, Chinese, Japanese, Hispanic, and African women [86].

**Platinum drugs.** Platinum drugs (cisplatin, carboplatin and oxaliplatin) induce damage to tumors via the induction of apoptosis. Apoptosis is responsible for the toxic effects associated with platinum use. The major limitation in the clinical applications of cisplatin has been the development of cisplatin resistance by tumors [87]. Several mechanisms have been implicated in platinum resistance, including DNA detoxification, mediated by glutathione transferases, and transport mechanisms mediated via ABC2 and ABCG2 [82]. However, only recently has there been a positive association between polymorphisms in ABC transporter genes and clinical outcomes [88]. In a recently reported study, 973 lung cancer patients were genotyped for the ABCC4 SNP. The results demonstrated an association of that ABCC4 polymorphism with survival for lung cancer patients, and ABCC4 over-expression significantly decreased cisplatin sensitivity in lung cancer and HEK293T cell lines [89]. In another study, Ekblad and coauthors showed that ABCB1 was highly over-expressed in the three most oxaliplatin-resistant sublines, but was significantly under-expressed in two of the more cisplatin-resistant cell lines [90].

**FUTURE DIRECTIONS**

In summary, interindividual differences in expression and activity have been observed to affect the clinical pharmacokinetics and pharmacodynamics, and in turn, response to chemotherapeutic agents and targeted therapies used for the treatment of breast cancer. Extensive studies from the literature have concentrated on the role that direct genetic alterations, such as insertions, deletions, and mutations, have on DNA and gene expression. However, it is now recognized that epigenetic mechanisms also play a key role in carcinogenesis. One of the major epigenetic mechanisms is DNA methylation. DNA methylation is a form of chemical modification of DNA that involves the addition of a methyl group to DNA and can be inherited without changing the DNA sequence. DNA hypermethylation of CpG rich regions (also referred to as CpG islands) located in the promoter region of many genes is a critical epigenetic pathway resulting in the silencing of genes involved in carcinogenesis. Studies are now being performed to determine if DNA methylation may be used to predict therapeutic efficacy of anticancer drugs. To our knowledge the only study that has provided evidence of UGT regulation via DNA methylation was published by Gagnon et al. [91]. In that study, DNA methylation repressed UGT1A1 expression in colon cancer and this repression was attributed to the tumoral inactivation of
the anticancer agent irinotecan, SN-38, used to treat colorectal cancer. This study was the first to suggest that not only is UGT1A1 regulated by genetic mechanisms, as in the case of the UGT1A1*28 low activity allele, but also through DNA methylation. Since isoforms of the UGT1A family share similar sequence homology, other UGT isoforms, like UGT1A1, might also be regulated by DNA methylation, in addition to genetic polymorphisms. Collectively, the continued study of genetic, and inclusion of epigenetic, pathways that may regulate UGTs and transporter genes, will continue to provide novel breakthroughs in the cause of breast cancer drug resistance. Furthermore, the incorporation of conjugating and drug transport pathways in large population-based studies would prove beneficial by providing insight as to how alterations in these pathways might impact the clinical response and clearance of chemotherapeutic drugs in women with breast cancer.

**Note:** The views expressed in this paper do not necessarily represent those of the U.S. Food and Drug Administration.

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