The role of reduced intracellular concentrations of active drugs in the lack of response to anticancer chemotherapy

Jose JG MARIN*, Maria J MONTE, Alba G BLAZQUEZ, Rocio IR MACIAS, Maria A SERRANO, Oscar BRIZ

Laboratory of Experimental Hepatology and Drug Targeting (HEVEFARM), Biomedical Research Institute of Salamanca (IBSAL), University of Salamanca, National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Salamanca, Spain

A major difficulty in the treatment of cancers is the poor response of many tumors to pharmaceutical regimens. This situation can be accounted for by the existence of a variety of complex mechanisms of chemoresistance (MOCs), leading to reduced intracellular concentrations of active agents, changes in the molecular targets of the drugs, enhanced repair of drug-induced modifications in macromolecules, stimulation of anti-apoptotic mechanisms, and inhibition of pro-apoptotic mechanisms. The present review focuses on alterations in the expression and appearance of the genetic variants that affect the genes involved in reducing the amount of active agents inside tumor cells. These alterations can occur through two mechanisms: either by lowering uptake or enhancing efflux (so-called MOC-1a and MOC-1b, respectively), or by decreasing the activation of prodrugs or enhancing inactivation of active agents through their biotransformation (MOC-2). The development of chemosensitizers that are useful in implementing the pharmacological manipulation of these processes constitutes a challenge to modern pharmacology. Nevertheless, the important physiological roles of the most relevant genes involved in MOC-1a, MOC-1b, and MOC-2 make it difficult to prevent the side effects of chemosensitizers. A more attainable goal in this area of pharmaceutical enquiry is the identification of proteomic profiles that will permit oncologists to accurately predict a lack of response to a given regimen, which would be useful for adapting treatment to the personal situation of each patient.

Keywords: cancer; anticancer drug; chemoresistance; ATP-binding cassette protein; drug refractoriness; drug metabolism; cytochrome P450-related enzyme; prodrug; plasma membrane transporter

Introduction

One of the main problems in the treatment of cancer is the poor response of many tumors to the pharmacological regimen imposed. This situation can be explained by the existence of a variety of complex mechanisms of chemoresistance (MOCs), which lead to reduced intracellular concentrations of active agents, changes in the molecular targets of the drugs, and enhanced repair of drug-induced modifications in macromolecules; additionally, anti-apoptotic mechanisms are stimulated, whereas pro-apoptotic mechanisms are inhibited. Here, we review the MOCs that involve changes in the expression level and appearance of the genetic variants that affect the genes involved in i) drug uptake or efflux (so-called MOC-1a and MOC-1b, respectively) of the genes encoding the proteins that belong to the “transportome”, which is a group of expressed transporters that govern the traffic across the plasma membrane of tumor cells, and ii) activation of prodrugs or inactivation of already active agents through their biotransformation (MOC-2) (Figure 1). Together, these events form an important group of MOCs, as the mechanism of action of many anticancer agents occurs inside cells, either by inhibiting the key processes required for tumor cell survival or interacting in non-tumor cells with the signaling pathways involved in cancer development, such as those responsible for angiogenesis.

MOC-1a

Reduced expression of genes involved in drug uptake

The uptake of most anticancer agents occurs via plasma membrane transporters belonging to the Solute Carrier Family (SLC). The natural substrates of these transporters include a large variety of endogenous or xenobiotic compounds. Many drugs use these transporters to enter cells because they have structural characteristics that are similar to those of the
endogenous substrates\(^3\). Down-regulation or the expression of less functional variants of SLC proteins in tumor cells may impair drug uptake, reduce the intracellular concentration of the active agent, and, as a consequence, hinder the efficacy of chemotherapy (Table 1).

Organic anion-transporting polypeptides (OATPs) are plasma membrane transporters encoded by SLCO genes and are able to transport a large variety of endogenous compounds and drugs\(^4\). OATP1B1 and OATP1B3, encoded by the SLCO1B1 and SLCO1B3 genes, respectively, are able to transport such anticancer drugs as flavopiridol, methotrexate, paclitaxel, irinotecan, tyrosine kinase inhibitors\(^5\), and cisplatin-conjugated bile acid derivatives\(^6\). A reduction in OATP1B1 and OATP1B3 expression or their functionality appears to result in a lower accumulation of antitumor drugs in liver cancer\(^7\). Indeed, a decrease in the expression of these proteins in hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCG) has been described\(^8\). The expression of another mem-

Table 1. Important genes involved in mechanisms of chemoresistance type 1 and 2 to antitumor drugs.

| Gene       | Protein | Function           | Gene       | Protein | Function           | Gene       | Protein | Function           |
|------------|---------|--------------------|------------|---------|--------------------|------------|---------|--------------------|
| SLCO1B1    | OATP1B1 | Organic anion uptake | ABCB1      | MDR1    | Export pump        | TYMP       | TYMP    | Nucleotide metabolism |
| SLCO1B3    | OATP1B3 | Organic anion uptake | ABCB1      | MRP1    | Export pump        | UPP1/2     | UPP1/2 | Nucleotide metabolism |
| SLCO1A2    | OATP1A2 | Organic anion uptake | ABCB2      | MRP2    | Export pump        | UPRT       | UPRT    | Nucleotide metabolism |
| SLCO22A1   | OCT1    | Organic cation uptake | ABCB3      | MRP3    | Export pump        | DPDY       | DPDY    | Nucleotide catabolism |
| SLCO28     | CNTs    | Nucleoside uptake   | ABCB4      | MRP4    | Export pump        | CYP2A6     | CYP2A6 | Phase I enzyme     |
| SLC29      | ENTs    | Nucleoside uptake   | ABCG2      | BCRP    | Export pump        | CYP2C19    | CYP2C19 | Phase I enzyme     |
| SLC31A1    | CTR1    | Copper uptake       | ATP7A      | Menkes  | Copper efflux      | CYPD6      | CYPD6   | Phase I enzyme     |
| SLC19A1    | RFC     | Reduced folate carrier | ATP7B      | Wilson  | Copper efflux      | NQO1       | NQO1    | Phase I enzyme     |
|            |         |                    | LRP/MVP    | LRP/MVP | Nucleo-cytoplasmic transport |

BCRP, breast cancer resistance protein; CESs, carboxylesterases; CYPs, cytochrome P-450; DPD, dihydropyrimidine dehydrogenase; FPGS, folicpolyglutamate synthase; GSTs, glutathione S-transferases; LRP/MVP, lung resistance-related protein or major vault protein; MDR, multidrug resistance; MOC, mechanism of chemoresistance; MRPs, multidrug resistance-associated proteins; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; OPRT, orotidine phosphoribosyl transferase; SLC, solute carriers; SULTs, sulfotransferases; TYMP, thymidine phosphorylase; UGT, uridine disphosphate glucuronosyl transferase; UMPS, uridine monophosphate synthetase; UPP, uridine phosphoribases.

Figure 1. Schematic representation of the genes involved in chemoresistance due to a reduction in the intracellular concentrations of anticancer drugs via reduced uptake or enhanced efflux and by a lower activation of prodrugs and enhanced inactivation of anticancer agents.
ber of the OATP family, OATP1A2 (SLCO1A2), is markedly reduced in both polyps of the large intestine and colon cancer compared to a healthy colon[8]. Furthermore, alterations in the expression level or functionality of OATP1A2 have been associated with the development of resistance to methotrexate[10] and imatinib[11].

The human SLC22A family includes organic cation transporters (OCTs), Na+ -zwitterion/cation cotransporters (OCTNs), and a heterogeneous group of transporters that can transport organic anions (OATs)[13]. The organic cation transporter-1 (OCT1, the SLC22A1 gene) is one of the transporters involved in the uptake of tyrosine kinase inhibitors, such as sorafenib[13], and other important cationic antitumor drugs, such as platinum derivatives and anthracyclines[14]. Thus, tumor response to these drugs depends in part on OCT1 expression. We have recently described that primary liver cancer, ie, HCC, CGC, and hepatoblastoma, share decreased expression of OCT1 as a common feature[8]; OCT1 expression is also reduced in colon cancer[9]. In HCC[15] and CGC patients[16], low OCT1 expression has been associated with advanced tumor stages and poorer overall survival rates, and hypermethylation of the SLC22A1 promoter has been proposed as a mechanism for the down-regulation of OCT1 in HCC[15]. Moreover, imatinib uptake in chronic myeloid leukemia (CML) primary cells and cell lines has been shown to be dependent on OCT1 expression[17], and the degree of OCT1 expression has been suggested to be a useful biomarker for predicting the success of imatinib-based therapy in leukemia patients[18].

The SLC28 and SLC29 families include high-affinity concentrative nucleoside transporters (CNTs) and low-affinity equilibrative nucleoside transporters (ENTs), which are involved in the uptake of nucleoside-derived anticancer drugs, such as gemcitabine, flouxuridine, and catarabine[19]. Changes in the expression of these transporters account for the differential uptake of nucleoside derivatives by tumors, and this may significantly modulate intracellular drug bioavailability and hence responsiveness to therapy[19, 20].

Copper is an essential micronutrient, and uptake by human cells is mainly performed by the high-affinity copper transporter CTR1, which is encoded by the SLC31A1 gene. The expression of this transporter is regulated by the transcription factor Sp1, which acts as a copper sensor[21]. Recent research has revealed that CTR1 can also transport platinum drugs, underscoring the important role of CTR1 in platinum-drug sensitivity in cancer chemotherapy. Elevated CTR1 expression has consistently been associated with favorable treatment outcomes in cisplatin-based cancer chemotherapy, although down-regulation of CTR1 has been observed when tumor cells are exposed to this agent[22]. Because CTR1 expression can be induced through the depletion of cellular copper levels, which results in enhanced cisplatin uptake, a phase I clinical trial using a combination of trientine (a copper chelator) and carboplatin has been conducted, with encouraging results (for a review, see[21]).

Less functional variants of proteins involved in drug uptake
In addition to differences in the expression pattern of SLC proteins in tumors, the presence of of SLC protein variants is responsible for the considerable inter-individual variation in the efficiency of antitumor drugs[23] and also contributes to MOC-1b[24]. This section offers an overview of the transporter variants that are clinically relevant in the pharmacological treatment of cancer.

The reduced folate carrier (RFC, SLC19A1), which is ubiquitously expressed in human tissues, is involved in the uptake of endogenous folate and antifolate drugs, such as methotrexate and pemetrexed. SLC19A1 genetic variants have been associated with a variable response to methotrexate in several types of cancer[25], and the common missense variant His27Arg (c.80A>G) has been investigated for its effect on methotrexate response and toxicity. In children with acute lymphoblastic leukemia (ALL) treated with methotrexate, the presence of the wild-type variant was associated with a 50% better chance of staying in remission than the GG or GA variant[25]. Polymorphisms in the SLC1A91 gene can also be predictive of survival differences in advanced non-small cell lung carcinoma (NSCLC) patients treated with pemetrexed-based chemotherapy[26].

Resistance is a major cause of imatinib treatment failure in CML patients. Because imatinib uptake is mainly performed by OCT1, the SLC22A1 genotype of CML patients can be used to predict the outcome of imatinib treatment. Thus, patients harboring the wild-type GG genotype of the common variant Leu160Phe (c.480G>C) have a higher rate of treatment failure to imatinib therapy than patients with the mutation[27]. Moreover, the Met420del variant (c.1258_1260delATG) may alter imatinib pharmacodynamics, resulting in a reduction in drug efficacy in the targeted leukemia cells[28].

Similar results have been found for sorafenib in the treatment of liver cancer. The results of a recent study performed by our group suggest that liver carcinogenesis is accompanied by the appearance of aberrant variants of OCT1 that may dramatically affect the ability of HCC and CGC to take up and hence respond to sorafenib[13]. In addition, the existence of inactivating SNPs in the SLC22A1 gene in HCC and CGC may affect the response of these tumors to treatment with sorafenib[13]. The expression of non-functional truncated OCT1 isoforms originating through alternative splicing mechanisms, such as exon skipping and intron retention, has also been found in tumor cells[13, 29].

MOC-1b
Increased expression of genes involved in drug efflux
Most genes involved in MOC-1b encode export pumps belonging to the ABC (ATP-binding cassette) superfamily. The over-expression of some members of this superfamily in tumor cells, particularly those of the ABCB, ABCC, and ABCG families, is one of the major problems in chemotherapy resistance because of their role in the so-called multidrug resistance (MDR) phenotype[30]. These membrane transporters use energy derived directly from the hydrolysis of ATP to export a
large variety of compounds, including antitumor drugs, from inside the cell, thereby reducing their intracellular concentrations. The expression of ABC proteins can be increased as part of the cellular response to the chemical stress induced by compounds that are structurally very different. Even compounds that are not substrates of these transporters have been found to increase the levels of some ABC proteins in tumor cells[30].

The ABCB family includes the prototype of MDR proteins, i.e., MDR1 (gene symbol ABCB1), also known as P-glycoprotein. This pump is broadly expressed in epithelia and is able to transport a large variety of anticancer drugs[30]. MDR1 is also involved in conferring the MDR phenotype to many types of cancer cells that have developed resistance to anticancer drugs. For example, high ABCB1 expression has been associated with a poor response of HCC[31] and hepatoblastoma[32] to pharmacological treatments.

Several members of the ABCC family involved in chemoresistance are typically designated multidrug resistance-associated proteins (MRPs). Among them, the most important with regard to drug refractoriness and the development of chemoresistance in human tumors are MRP1 (ABCC1), MRP2 (ABCC2), MRP3 (ABCC3), and MRP4 (ABCC4)[6]. Indeed, a clear relationship between MRP1 and the resistance to many antineoplastic agents, such as doxorubicin and vincristine, has been established based on studies using tumor cell lines[33]. MRP2 plays an important role in the resistance to platinum derivatives, presumably because this protein is able to efficiently export glutathione conjugates of cisplatin, and a markedly increased expression of MRP2 has been found in colon cancer cells after exposure to cisplatin[34]. MRP2 is mainly detected in the membrane of HCC tumor cells, and this localization has been associated with the refractoriness of HCC to chemotherapy that is due to reduced drug accumulation in these cells[35]. Over-expression of MRP2 is considered to be one of the most important mechanisms accounting for the failure of cisplatin treatment in colon cancer[35], whereas MRP3 expression in CGC has been suggested to contribute to the intrinsic MDR phenotype characteristic of this type of cancer[36]. MRP4 expression has been found to be increased by more than 10-fold when cisplatin resistance was induced in cell lines derived from HCC[37].

The ABCG gene family includes an important transporter that is responsible for the failure of chemotherapy in many tumors: ABCG2, which is also known as breast cancer resistance protein (BCRP). ABCG2 expression may be a selective marker for the efficacy of chemotherapeutic regimens in treating some types of cancer, such as colorectal carcinoma[38], and esophageal squamous carcinoma[39]. ABCG2 over-expression in response to anticancer drug exposure has also been observed. ABCG2 was found to be markedly upregulated in a study in which the expression of a broad panel of both ABC and SLC transporters were measured in an ovarian cancer cell line compared to MDR sublines[40]. Similarly, increased ABCG2 expression has been found in colon cancer cells after exposure to cisplatin[34].

IMP3, a member of a family of insulin-like growth factor II (IGF-II) mRNA-binding proteins (IMPs), is preferentially expressed in triple-negative breast cancers, which are resistant to many chemotherapeutics. The IMP3 protein has also been reported to promote chemoresistance in breast cancer cells by regulating ABCG2 expression[41].

Owing to the clinical relevance of ABCG2 in the multidrug resistance phenomenon, ABCG2 constitutes an appealing therapeutic target to increase drug distribution. The development of ABCG2 inhibitors, which can be used in combination with antitumor drugs to block drug efflux from cancer cells, has recently emerged[42].

Non-ABC transporters are also involved in drug efflux and have therefore been included in MOC-1b. Thus, the ATP7A and ATP7B genes that encode the copper-transporting P-type ATPases Menkes and Wilson proteins, respectively, are involved in regulating the copper status of the body[43]. However, in cancer cells, these primary active transporters also account for a reduction in the intracellular concentrations of several antitumor agents, such as cisplatin. Accordingly, ATP7B has been found to be over-expressed in tumors with marked resistance to platinum-based chemotherapy[44].

Lung resistance-related protein (LRP) or major vault protein (MVP) is not a pump but plays a similar role in chemoresistance because it forms cytoplasmic nano-organelles, the vaults, which are able to encapsulate anticancer drugs and hence reduce their active intracellular concentrations. In tumors, an inverse relationship between MVP expression and the degree of response to anticancer agents has been reported[45, 46].

Genetic variants in genes involved in drug efflux

Most SNPs described in ABC genes do not contribute to MOC-1b because many of them affect the mRNA stability of the variant transcript, resulting in lower protein levels, or alter residues that are important for the structure of the protein, thereby impairing transport activity. Nevertheless, the presence of certain SNPs in the 5′ flanking region of ABC genes can increase their expression in tumor cells, accounting for enhanced resistance to antitumor drugs. Moreover, genetic variants in MOC-1b genes might result in an increased transport ability of the proteins and hence a reduced intracellular active drug concentration[34].

To date, more than 50 SNPs have been identified in ABCB1, and changes in protein (MDR1) structure and function that are due to altered protein folding have been suggested as a possible consequence of these mutations[47]. Some SNPs have been reported to affect either the substrate specificity of MDR1 and/or increase its transport activity, which in turn enhances drug refractoriness in tumor cells expressing these variants. The identification of SNPs in ABCB1 has been proposed as a valuable marker for the outcome of the chemotherapeutic treatment of several types of tumors[48–50]. The MDR1 variant Ser400Asn (c.1199G>A) in patients with ovarian cancer has been associated with a higher likelihood of failure of treatment with paclitaxel plus carboplatin[51]. Although c.1236C>T in ABCB1 is a synonymous SNP (p.Gly412Gly), it may result in higher MDR1 expression levels, and its presence in CML patients has been associated with a higher resistance to ima-
tinib\(^[62]\). Other ABCB1 genetic variants involved in the drug refractoriness of tumor cells are \(c.2677G>T/A\) (p.Ala893Ser/Thr) and \(c.3435C>T\) (p.Ile1145Ile). The presence of these variants has been related to increased resistance to irinotecan\(^{[53]}\) and platinum-derived compounds\(^{[54]}\) in advanced NSCLC, to imatinib in CML\(^{[55]}\), and to 5-fluorouracil plus leucovorin in colorectal cancer\(^{[59]}\). Additionally, the 1236T/2677G/3435C haplotype has been found more frequently in patients with resistance to imatinib\(^{[56]}\).

High MRPI expression in cancer is characteristic of the MDR phenotype\(^{[27]}\). The \(c.-1666G>A\) SNP in ABCC1 impairs promoter activity, and HCC patients bearing the wild-type \(-1666 GG\) genotype have a poor outcome and survival rate\(^{[58]}\). The most frequent SNPs in ABCC2 are \(c.-24C>T\), \(c.1249G>A\) (p.Val417Ile), and \(c.3972C>T\) (p.Ile1324Ile). The presence of \(c.-24C>T\) has been related to an increased platinum-based chemotherapy response in NSCLC\(^{[28]}\); furthermore, this SNP has also been related to higher methotrexate plasma concentrations in childhood ALL due to a lower MRPI activity\(^{[160]}\). An association between the \(c.3972C>T\) variant and the risk of CGC has also been found\(^{[22]}\). Moreover, the haplotype consisting of both \(c.-24C>T\) and \(c.3972C>T\) SNPs has been related to an enhanced sensitivity to irinotecan therapy in advanced NSCLC\(^{[23]}\).

Enhanced MRPI3 expression has been associated with a lower remission rate and decreased survival in leukemias\(^{[62,63]}\). Because the \(c.-211C>T\) SNP in the ABCC3 gene reduces MRPI expression levels\(^{[64]}\), the wild-type CC genotype has been associated with a poor prognosis in daunorubicin-treated ALL patients\(^{[66]}\). However, in this respect, controversial results have been found because the presence of the \(c.-211T\) mutant allele has been associated with significantly reduced progression-free survival in lung cancer patients treated with etoposide\(^{[169]}\). The \(c.-189A>T\) SNP has also been proposed as a marker of clinical outcome in childhood ALL patients, whereas it has been reported that methotrexate-treated patients bearing the wild-type AA allele, which results in enhanced ABCC3 promoter activity, have reduced survival outcomes\(^{[62]}\).

The prognostic usefulness of ABCC4 SNPs in childhood ALL has also been reported. Because the \(c.-1393T>C\) SNP results in higher ABCC4 promoter activity, the methotrexate and 6-mercaptopurine plasma levels in ALL patients are lower than in those bearing the wild-type genotype\(^{[67]}\).

More than 80 ABCG2 genetic variants have been described. Among them, Gln141Lys (c.421C>A) is one of the most extensively studied and results in reduced protein expression and increased sensitivity to many anticancer drugs\(^{[68]}\). For example, the presence of this polymorphism confers higher sensitivity to tyrosine kinase inhibitors\(^{[69]}\) and camptothecins\(^{[70]}\). In contrast, this ABCG2 variant has recently been associated with a poor prognosis in acute myeloid leukemia (AML) patients treated with idarubicin\(^{[71]}\).

The Arg482Gly (c.1444A>G) and Arg482Thr (c.1445G>C) variants result in higher sensitivity to topotecan but enhanced resistance to anthracyclines and rhodamine-123. These variants have been found during the course of drug-induced selec-

tion and have been shown to be responsible for differential drug efflux and sensitivity\(^{[72]}\).

**MOC-2**

**Reduced intracellular activation of prodrugs**

The detoxification processes occurring in both tumor and healthy cells include oxidation/reduction reactions by phase I enzymes, such as cytochrome P450 (CYP)-related enzymes, and conjugation with polyatomic groups by phase II enzymes, such as those involved in glucuronic acid, sulfate, and glutathione conjugation. Changes in the expression or function of these enzymes that are due to the appearance of variants in the genes involved in drug biotransformation in cancer cells may dramatically affect the final success of pharmacological treatment\(^{[72]}\). Thus, on the one hand, the enhanced activity of phase I and phase II enzymes may result in the inactivation of drugs and enhanced chemoresistance; on the other hand, several anticancer agents are administered as inactive prodrugs and hence require metabolic activation by phase I proteins. Accordingly, reduced sensitivity to these drugs may be due to the reduced expression or function of the enzymes. In summary, alterations in drug metabolism that are due either to an enhanced production of inactive metabolites or a reduced activation of prodrugs (MOC-2) may decrease the intracellular concentrations of the active agent, reducing the efficacy of chemotherapy\(^{[69]}\).

For instance, 5-fluorouracil activation involves three enzymatic activities: thymidine phosphorylase (TYMP), uridine phosphorylase (UPP), and orotate phosphoribosyl transferase (OPRT). The reduced expression or impaired activity of these enzymes in tumors has been associated with a lower sensitivity to 5-fluorouracil\(^{[74]}\). Similarly, irinotecan must be converted by carboxylesterases (CESs) into SN-38, which is an active inhibitor of topoisomerase I. The mRNA levels of these enzymes are consistently reduced in irinotecan-resistant colorectal carcinomas\(^{[75]}\). One of the mechanisms accounting for the resistance to methotrexate derivatives is a reduction in folypolyglutamate synthase (FPGS) activity, the role of which is to enhance the affinity of the drug for its molecular target\(^{[76]}\).

CYP belongs to a large group of more than 60 enzymes that are responsible for the activation or inactivation of most clinically important drugs, including those used against cancer. The human CYP genes are highly polymorphic, yet to date only certain genetic variants have been demonstrated to be clinically relevant.

CYP2A6 activates tegafur to generate 5-fluorouracil, and one study reported that the poor response to tegafur by a patient with gastric cancer was caused by a poor metabolic phenotype due to mutations in the CYP2A6 gene. The presence of the Ser224Pro variant (c.670T>C, CYP2A6*11), which has a very low metabolizing capacity, along with the deletion of one of the alleles of the CYP2A6 gene (also known as the CYP2A6*4C allele) were found in this patient\(^{[77]}\).

CYP2C19 is involved in thalidomide and cyclophospha-
drine activation. Two variants resulting in the synthesis of non-functional truncated proteins, Ile215_Pro227del fs*20 or
CYP2C19*2 (c.681G>A, which results in an aberrant splicing of exon 5) and Trp212X or CYP2C19*3 (c.636G>A), have been associated with a lack of response to thalidomide in patients with multiple myeloma\cite{78,79}. The CYP2C19*2 variant has also been associated with lower drug efficacy in cyclophosphamide-treated patients as a result of inefficient activation of the prodrug\cite{80}.

CYP2D6 metabolizes tamoxifen to produce active 4-hydroxytamoxifen and endoxifen. More than 80 genetic variants encoding non-functional CYP2D6 or an enzyme with reduced activity have been described, and tamoxifen-treated breast cancer patients harboring these variants are at a greater risk of cancer relapse\cite{81}. The most common variants in the Caucasian population are CYP2D6*3 (c.2549delA in exon 5), CYP2D6*4 (1846G>A in intron 3), CYP2D6*5 (deleted gene), and CYP2D6*6 (c.1707delT in exon 3)\cite{82}.

Enhanced drug inactivation
Dihydropyrimidine dehydrogenase (DPYD) inactivates fluoropyrimidines, such as 5-fluorouracil; therefore, changes in DPYD activity affect the overall exposure of tumor cells to these drugs. Among the 39 genetic variants identified in the human DPYD gene, the most frequent is an intronic mutation that leads to exon-14 skipping (c.IVS14+1G>A). This alteration is associated with decreased DPYD activity and hence an increased risk of developing severe or fatal side effects during treatment with 5-fluorouracil or its prodrugs capecitabine and tegafur\cite{83}.

NAD(P)H quinone oxidoreductase 1 (NQO1) catalyzes the reduction of quinines and nitro derivatives using NADP or NADPH as a cofactor. A lower survival of women with breast carcinoma harboring the Pro187Ser variant (c.558C>T; NQO1*2) after treatment with anthracycline-based adjuvant chemotherapy with epirubicin has been reported\cite{84}.

Glutathione S-transferases (GSTs) form a superfamily of enzymes that play an important role in the detoxification of a broad variety of hydrophobic and electrophilic compounds through conjugation with reduced glutathione, a modification that inactivates the drugs and/or increases their water solubility, thus favoring their elimination from the body\cite{85}. Accordingly, an enhanced conjugating activity due to GST can determine the degree of resistance to platinum compounds\cite{86}, and the cellular content of glutathione is a key factor in this mechanism of resistance. Indeed, the amount of intracellular glutathione and the expression of the enzymes responsible for the synthesis of glutathione determine sensitivity to cisplatin\cite{87,88}.

Functionally different glutathione S-transferase pi (GSTP1) variant proteins are encoded by a polymorphic gene. These enzymes are able to recognize several anticancer drugs as substrates, such as etoposide, doxorubicin, thioredoxin, oxaliplatin, and carboptatin. The Ile105Val variant, or GSTP1*B, is a non-synonymous SNP (c.313A>G) of special pharmacogenetic relevance and has been associated with anticancer drug responses and toxicity. Indeed, an increase in overall survival was observed in patients with metastatic colorectal cancer bearing the GSTP1*B polymorphism who had been treated with 5-fluorouracil plus oxaliplatin\cite{89}. It was also reported that children with ALL treated with etoposide displayed different drug clearances, depending on their genotype\cite{90}.

Uridine disphosphate glucuronosyl transferases (UGTs) catalyze the glucuronidation of xenobiotics and endogenous compounds to form more water-soluble molecules that can be more readily excreted. UGT1A1 is involved in the metabolism of bilirubin and other endogenous compounds. UGT1A1 also mediates the glucuronidation of SN-38, which is secreted into bile\cite{91}, and also participates in the metabolism of other drugs, such as etoposide, epirubicin, and tyrosine kinase inhibitors. In addition, an increase in UGT1A1 activity may contribute to chemoresistance to irinotecan in colon cancer\cite{92}.

A TA insertion in the (TA)6TAA promoter region (UGT1A1*28) is the most common genetic variant in the Caucasian population, whereas the Gly71Arg variant, or UGT1A1*6 (c.211G>A), and Pro229Gln, or UGT1A1*27 (c.686C>A), are more frequent in Asian populations. All of these variants are associated with enhanced exposure to SN-38 and higher drug-induced toxicity due to a reduction in SN-38 glucuronidation\cite{92,93}.

Sulfotransferase 1A1 (SULT1A1) catalyzes the sulfation of the active metabolite of tamoxifen. The most common variant is Arg213His, or SULT1A1*2 (c.638G>A), which has been associated with reduced activity and a three-fold enhanced risk of death in patients with breast cancer treated with tamoxifen\cite{94}.

The clinical relevance of genetic variants in other drug-metabolizing enzymes, such as carboxylesterase, epoxide hydrolase, cytidine deaminase, deoxyxycytidine kinase, N-acetylation, thiopurine methyltransferase, and metallothionein, to chemoresistance is considered minor\cite{24}.

Conclusions and future perspectives
To produce a higher likelihood of success, a better understanding of the molecular bases of MOC is required to tackle one of the major hurdles in the treatment of cancer, ie, the lack of response to available drugs. Indeed, the development of chemosensitizers that are useful for the pharmacological manipulation of MOC is a challenge to modern pharmacology. Nevertheless, the important physiological role of many of the most relevant genes involved in MOC-1a, MOC-1b, and MOC-2, creates difficulty in avoiding the side effects of chemosensitizers. Another goal in this area of pharmacological research that has a higher chance of success in the near future is the identification of proteomic profiles. Such data may allow oncologists to accurately predict the lack of response to a given pharmacological regimen, which would be useful for adapting treatments to the personal situation of each patient.

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Abbreviations
AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; CES, carboxylesterase; CML, chronic myeloid leukemia; HCC, hepatocellular carcinoma; CGC, cholangiocarcinoma; CYP, cytochrome P-450; DPD, dihydropyrimidine dehydrogenase; GST, glutathione S-transferase; LRP/MVP, lung resistance-related protein or major vault protein; MDR, multidrug resistance; MOC, mechanism of chemoresistance; MRP, multidrug resistance-associated protein; NSCLC, non-small cell lung carcinoma; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; OPRT, orotate phosphoribosyl transferase; SLC, solute carrier; SNP, single-nucleotide polymorphism; TYMP, thymidine phosphorylase; UGT, uridine disphosphate glucuronosyl transferase; UMPS, uridine monophosphate synthetase; UPP, uridine phosphorylase.

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