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Editorial

Role of ABC transporters in cancer chemotherapy

Yue-Li Sun[1,2,3], Atish Patel[1], Priyank Kumar[1] and Zhe-Sheng Chen[1]

Abstract

Multidrug resistance (MDR) in cancer cells can significantly attenuate the response to chemotherapy and increase the likelihood of mortality. The major mechanism involved in conferring MDR is the overexpression of ATP-binding cassette (ABC) transporters, which can increase efflux of drugs from cancer cells, thereby decreasing intracellular drug concentration. Modulators of ABC transporters have the potential to augment the efficacy of anticancer drugs. This editorial highlights some major findings related to ABC transporters and current strategies to overcome MDR.

Key words ABC transporter, multidrug resistance (MDR), ABCB1, multidrug resistance protein (MRP), ABCG2

Cancer cells develop resistance to structurally and mechanistically unrelated drugs over a period of time. This phenomenon of resistance is known as multidrug resistance (MDR). MDR studies suggested that drug transport is a carefully controlled process, and this process was later found to be regulated by members of the ATP-binding cassette (ABC) transporter family of proteins. P-glycoprotein (P-gp)/ABCB1 was the first identified ABC transporter, an accomplishment of Ling *et al.*[1] in 1976, and this protein has since been found to play a role in many cellular functions. Currently, 49 different ABC transporters have been identified in humans, though there are more in bacteria and parasites[2]. The overexpression of specific ABC transporters in cancer cell lines and tumors produces MDR, which is the major factor contributing to the failure of chemotherapy. The ABC transporter superfamily includes membrane proteins that extrude a wide variety of substrates across cellular membranes. An increasing number of chemotherapeutic drugs transported by ABC transporters have been recognized since the discovery of P-gp.

The ABC transporter family has been divided into seven subfamilies based on the sequence similarity as well as structural organization[3]. Three ABC transporters appear to account for most of the reported MDR in humans: P-gp, multidrug resistance protein 1 (MRP1)/ABCC1, and breast cancer resistance protein (BCRP)/ABCG2/MXR/ABCP[3]. These proteins can recognize and transport a large number of structurally diverse compounds. Numerous studies have reported that inhibiting these aforementioned ABC transporters can reverse MDR. This editorial briefly discusses these three ABC transporters as well as strategies to surmount MDR in cancer chemotherapy.

P-gp/ABCB1

The specific association between cell membrane transporters and a drug resistant phenotype was first found in the Chinese hamster ovary cell line in the 1970s. When this cell line was selected for resistance to colchicine, it also exhibited resistance to daunomycin and puromycin[1]. The protein associated with this phenomenon, a 170-kDa glycoprotein, was purified in 1979 and later known as P-gp[4]. Additional evidence in support of its role in drug resistance came in 1982 when it was shown to confer an MDR phenotype to drug-sensitive cells after DNA-mediated gene transfer of DNA obtained from colchicine-resistant mutants[5]. In 1985, *MDR1*, the gene encoding P-gp, was cloned[6]. It is located on chromosome 7 at q21 and contains 28 exons encoding a protein of 1280 amino acids[7].

P-gp is composed of 12 hydrophobic transmembrane domains and 2 nucleotide-binding domains. One nucleotide-binding domain connects two transmembrane domains with a hydrophilic nucleotide-binding domain

Authors' Affiliations: [1]Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professionals, St. John's University, Jamaica, NY 11439, USA; [2]State Key Laboratory of Oncology in South China; [3]Department of Medical Oncology, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 51006, P. R. China.

Corresponding Author: Zhe-Sheng Chen, Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professionals, St. John´s University, Jamaica, NY 11439, USA. Tel: +1-718-990-1432; Email: chenz@stjohns.edu.

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loop. The transmembrane domains are responsible for determining the characteristics of the substrates, whereas nucleotide-binding domains are responsible for ATP binding and hydrolysis, which drives substrate transport[8].

Using the energy provided by ATP hydrolysis, P-gp can pump out a wide spectrum of compounds including vinca alkaloids, anthracylines, epipodophyllotoxins, and taxanes[9]. P-gp is expressed in normal tissues, such as the epithelium of the kidneys, liver, intestine, pancreas, placenta and adrenal gland[10], where it functions to excrete toxic metabolites. P-gp is expressed in a variety of blood and solid cancer cells that have developed drug resistance, leading to failure of chemotherapy. A study by Peng *et al*.[11] published in this issue shows that P-gp mRNA and protein levels were increased in imatinib-resistant chronic myelogenous leukemia (CML) K562 cells, and the drug accumulation was decreased. These data suggest that overexpression of P-gp could be at least one of the factors that mediate resistance to imatinib in CML cells.

P-gp phenotype can be detected in clinical specimens through methods such as reverse transcription-polymerase chain reaction, immunodetection, and functional assays employing fluorophore substrates. Furthermore, its presence is negatively correlated with clinical efficacy in response to therapy in cancer patients[12]. Consequently, P-gp has been the target of intense academic, clinical, and pharmaceutical researches aimed at inhibiting its activity. Verapamil is a first-generation modulator demonstrated to reverse drug resistance in P-gp-expressing cell lines[13]. A series of P-gp modulators, such as cyclosporine A, quinine, monoclonal antibodies and others, have been subsequently identified through *in vitro* studies[14]. However, due to variability in absorption, excessive binding to plasma proteins or unacceptable toxicity at effective plasma concentration, most of these modulators were deemed inadequate for clinical use. The requirement for more selective, potent and high binding affinity compounds as P-gp modulators led to the development of the second-generation of modulators including PSC833 (valspodar), GF120918, LY335979, and XR9051[15]. However, when these modulators were used in patients, serious drug-drug interactions occurred because these modulators were inhibitors of cytochrome P450[16]. Subsequently, third-generation modulators, such as tariquidar (XR9576), elacridar (GF120918), zosuquidar (LY335979), and laniquidar (R1010933)[17], were developed and were found to possess high efficacy, low toxicity, and increased selectivity. Initial clinical trials indicated that the majority of these compounds had favorable efficacy profiles. However, later trials indicated that these compounds were unacceptable due to various toxicities and low patient survival advantages[9]. Currently, studies for the development of efficacious, non-toxic modulators that are not CYP450 inhibitors are ongoing.

MRP1/ABCC1

Subsequent to the discovery of P-gp, further research indicated that cancer cells had an MDR phenotype not associated with P-gp expression. This led to the discovery of MRP1 (ABCC1), the first member of the C subfamily[18]. In 1992, Cole *et al*.[18] cloned a cDNA encoding a 190-kDa protein that was named MRP (renamed MRP1), which was overexpressed in a doxorubicin-resistant cell line. Sequence analyses of *MRP1* revealed a similarity to *MDR1*, thereby indicating that its gene product was an ABC transporter. The C subfamily of ABC transporters is composed of 13 members (MRPs), and 9 are related to MDR[19]. MRPs 1–9 have two transmembrane domains and two cytoplasmic nucleotide-binding domains[20]. MRPs can be categorized according to the presence or absence of a third NH2-terminal membrane-spanning domain [trans-membrane domain 0 (TMD_0)] in their structures[21]. This topological feature is present in MRP1, MRP2, MRP3, MRP6, and MRP7 (long MRPs), but not in MRP4, MRP5, MRP8, and MRP9 (short MRPs)[22].

MRPs are lipophilic anion pumps that confer resistance to anticancer drugs. Studies about MRPs are discussed in detail in the review article of this issue by Sodani *et al*.[23]. MRP1 is localized in the plasma membrane of the intestine, liver and kidney, as well as the blood-brain and other vital biological barriers. MRP1 plays an important role in mediating the cytoplasmic concentration of endogenous and exogenous substances[23]. Thus, MRP1 can influence the pharmacokinetic profile of a variety of drugs.

MRP1 is overexpressed in many multidrug-resistant human cancer cell lines. Transfection experiments have shown that MRP1, like P-gp, confers resistance to a wide range of antitumor drugs[9]. The substrate selectivity of MRP1 is quite broad, allowing MRP1 to confer resistance to anthracyclines, epipodophyllotoxins, vinca alkaloids, and camptothecins[21]. A variety of inhibitors of MRP1 have been identified, but their specificity remains to be determined. Probenecid, sulphnpyrazone, and indomethacin, general inhibitors of organic anion transport, are able to inhibit MRP1[24]. Verapamil, quercetin, genistein, and cyclosporine, inhibitors of P-gp, can also suppress the function of MRP1[25]. Other P-gp and MRP1 dual inhibitors include PAK-104P, agosterol A, steroid analogues, and imidazothiazole derivatives[25]. The discovery and development of MRP1 inhibitors with high efficacy and appreciable safety have been much more difficult than those of P-gp, mostly because MRP1 is an anionic transporter. The development of probenecid

as a clinical MRP1 modulator was limited by its clinical toxicity at the requested dose[26]. Sulindac was identified as a competitive substrate for MRP1, but the clinical benefit in patients with acute or chronic leukemia is yet to be explored[27]. Studies aimed at determining the potency of compounds for MRP1 modulation are still needed.

BCRP/ABCG2

A pattern of MDR similar to P-gp or MRP1 overexpression was found in a breast cancer cell line selected for resistance to mitoxantrone, MCF-7/MX, and a subline that was made resistant to doxorubicin in presence of P-gp inhibitor verapamil, MCF-7/AdrVp[28]. In 1998, Doyle *et al.*[28] isolated a new ABC transporter from the MCF-7/AdrVp subline. This transporter was named breast cancer resistance protein (BCRP) because it was isolated from multidrug-resistant human breast cancer cells[28]. Studies on BCRP were discussed in detail by Nakanishi *et al.*[29].

BCRP, a 72-kDa protein with 655 amino acids, is encoded by a gene located on chromosome 4q22. BCRP belongs to a small group of half transporters because it contains six transmembrane helices and one ATP-binding site. Xu *et al.*[30] reported that the function of BCRP transporters is regulated by homodimerization or homotetramerization.

BCRP is present mainly in the plasma membrane of cells that comprise the placenta, small intestine, prostate, brain, liver, and ovaries[31]. As an efflux transporter, BCRP pumps out a variety of xenobiotic and toxic compounds, including anticancer drugs. Mitoxantrone-resistant colon cancer cells, S1-M1-80, were found to overexpress BCRP and hence, BCRP is also considered a mitoxantrone-resistance protein (MXR)[32]. Cellular resistance to mitoxantrone is attributable to BCRP-mediated drug efflux, which reduces the intracellular accumulation of mitoxantrone[33]. Mitoxantrone-mediated BCRP overexpression can occur not only in colon cancer cells but also in a wide variety of cancer cell lines, including breast cancer, gastric carcinoma, fibrosarcoma, non−small cell lung cancer, glioblastoma, and myeloma[34-36]. Thus, BCRP is a constituent of the cellular defense mechanism in response to mitoxantrone selection[29]. Several reports show that BCRP also induces resistance to irinotecan-based therapy[37]. BCRP overexpression was found in the topotecan-selected human ovarian cancer cell line IGROV1/T8 as well as in the breast cancer cell line MCF-7/TPT3000, and this resulted in resistance to SN-38, an active metabolite of irinotecan[37,38]. *BCRP* mRNA expression was higher in hepatic metastases in patients treated with irinotecan-based chemotherapy than in patients with irinotecan-naïve metastases, suggesting that BCRP is also involved in irinotecan resistance *in vivo*.

Mutations of *BCRP* gene produce varied substrate profiles for mutants as well as wild-type variants. Mutations at codon 482 in *BCRP* mRNA involving the replacement of arginine with threonine or glycine (R482T or R482G) caused higher resistance to anthracyclines[35,39].

Data from several studies have shown that tumors contain a small population of cells called cancer stem cells or side population cells that can recapitulate the original tumors[40,41]. Zhou *et al.*[42] reported that the expression of *Bcrp1*, the gene encoding the BCRP transporter protein, is not only highly conserved in various primitive stem cells but also serves as a marker for the side population phenotype. Moreover, a study conducted by Scharenberg *et al.*[43] reported similar expression of BCRP in the stem cell population of human hematopoetic cells. A recent report by Hirsch *et al.*[44] showed that metformin combined doxorubicin kills more cancer stem cells as well as non-stem cancer cells *in vitro* than doxorubicin alone. Furthermore, this combination treatment significantly reduced tumor mass and relapse compared to doxorubicin alone in a tumor xenograft model[44].

Fumitremorgin C (FTC) is a specific inhibitor of BCRP transporter. It is only used for research because it is a neurotoxic mycotoxin[45]. Other BCRP inhibitors include elacridar (GF120918), 17β-estradiol, flavonoids quercetin, biochanin A, and genistein[45]. Further studies are needed for detemining whether any of these inhibitors can be used for enhancing therapeutic effect on BCRP overexpression tumors in clinics.

Strategies to Overcome MDR

Correlation between tyrosine kinase inhibitors and ABC transporters

Tyrosine kinases are a family of enzymes responsible for phosphorylation of different proteins. They play an integral part in the human signaling pathway by regulating various physiological processes including cell growth, differentiation, adhesion, motility, and apoptosis[46]. However, these tyrosine kinases, when abnormally activated, drive uncontrolled cell proliferation and growth, induction of anti-apoptotic effects, and promotion of oncogenesis and metastasis. All tyrosine kinases share common structural features: an extracellular domain, a transmembrane domain, and a tyrosine kinase domain. Ligand-mediated dimerization of tyrosine kinases results in activation of downstream signaling pathways. Thus, blockade of these functional pathways is a promising approach for killing cancer cells.

Drugs that block the activity of tyrosine kinases are known as tyrosine kinase inhibitors and are widely used either alone or in combination to treat various cancers. They are divided into three main categories according to their specific targets: epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), and multi-targeted tyrosine kinase inhibitors. The first tyrosine kinase inhibitor to be used clinically was imatinib for the treatment of CML and gastrointestinal stromal tumors[47].

Recent studies have shown that tyrosine kinase inhibitors inhibit ABC transporters by binding to the substrate-binding sites within the transmembrane domain at clinically achievable concentrations[45,48]. Imatinib was reported to inhibit the transport of substrates of P-gp, BCRP, and MRP1[49,50]. Nilotinib, a second-generation tyrosine kinase inhibitor of breakpoint cluster region-Abelson (BCR-ABL), is used to treat CML patients who are resistant or intolerant to imatinib. In addition, nilotinib was reported to be an inhibitor of P-gp, BCRP and MRP7[51,52]. Sunitinib, another tyrosine kinase inhibitor used to treat imatinib-resistant gastrointestinal stromal tumors, is an inhibitor of P-gp and BCRP[53,54]. Similarly, lapatinib, which is used to treat Her-2 positive advanced or metastatic breast cancer, is an inhibitor of P-gp, BCRP, and MRP7[55-57]. A review article about tyrosine kinase inhibitors as modulators of ABC transporter-mediated MDR will be published in the following issue of this journal.

Personalized medicine

Pharmacogenomics is a branch of pharmacology involving the study of the influence of genetic factors on the pharmacodynamic and pharmacokinetic profile of a drug. Numerous reports indicate that genetic differences in individuals play an important role in the efficacy and adverse effects produced by drugs[58]. Intense knowledge of pharmacogenomics and its application to the field of ABC transporters would provide valuable information related to the use of personalized medicine[59]. Considerable evidence indicates that ABC transporters protect the human body against endogenous and exogenous substrates[60]. The first report of a single nucleotide polymorphism (SNP) in the *MDR1* gene was by Mickley *et al*.[61], who in 1998 found SNPs in exons 21 (2677G>T) and 24 (2995G>A) in the *MDR1* gene. Subsequently, over 50 different SNPs and insertion/deletion polymorphisms have been reported for *MDR1*. The most well-characterized polymorphisms include 1236C>T (rs1128503, exon 12), 2677G>T/A (rs2032582, exon 21), and 3435C>T (rs1045642, exon 26). These SNPs have a wide array of effects on the expression pattern[62], toxicity, response, and survival of patients when anticancer drugs such as taxanes are used to treat breast, ovarian, and lung cancers[63,64]. However, the results among these studies have been highly inconsistent, thereby preventing any conclusions[65]. BCRP, another widely expressed ABC transporter, regulates intestinal absorption, biliary secretion of substrates, and protection of the fetus and brain from toxic xenobiotics. The two most frequent polymorphisms of *BCRP* among over 50 others are 34G>A and 421C>A[66,67]. The 421C>A SNP has been associated with a lower protein expression of BCRP[67], which led to increased bioavailability of substrate drugs such as topotecan[68] and sulfasalazine[69]. Overall, there are accumulating data indicating that individual genotyping could be useful for clinical decision making, especially for the selection of medications.

Use of nanotechnology to overcome MDR

Introduction of nanotechnology into the world of medicine is bound to change the foundations of how a disease is diagnosed and treated and how it can be prevented. The National Institute of Health (Bethesda, MD) believes that nanomedicines have the potential to turn nanomolecular techniques into benefits for the patients. The unique way nanoparticles carry anticancer drugs to the sites of solid tumors after systemic administration provides new opportunities for drug delivery[70,71]. Their small size and increased surface area allows improved solubility and stability of anticancer drugs, resulting in a corresponding improvement in prevention, detection, and treatment of drug-resistant cancer cells with minimal toxicity towards normal cells[72]. Nanoparticles range in size from 10 to 100 nm and penetrate the tumor vasculature via a phenomenon called enhanced permeability and retention (EPR)[73,74], hence prolonging the exposure time of chemotherapeutic drugs. The advantages of nanotherapy include the ability to target the site of action and to bypass resistance mechanisms such as transporters. Indeed, a diverse array of therapeutic agents can be incorporated into nanoparticles and administered at the site of action, thereby increasing the bioavailability of the therapeutic agent. Furthermore, a drug incorporated in a nanoparticle can also be protected from physiological and biological degradation. Controlled release and prolonged circulation are a few more advantages that come with the use of nanoparticles in the field of medicine[75,76]. The future of nanotherapy is promising against tumor resistance. This will be briefly discussed in the current issue by Xue *et al*.[72].

Network systems for targeted therapy

Reducing the toxicity produced by anticancer drugs is something that scientists across the world are working

to achieve. One way to do this is to devise an approach that targets cancer cells without affecting the normal homeostasis of the body. Network systems are used to find connections between components of biological pathways to better understand the whole pathways involved. In a network system analysis, different biological systems are laid onto a graph, and nodes and edges are used to connect each individual component on different levels. Network types include protein-protein interaction networks[77], transcriptional regulatory networks, and signal transduction networks [78-82]. Using a network systems approach, Pujana *et al.*[83] successfully identified that hyaluronan-mediated motility receptor (HMMR), which is encoded by a gene *HMMR* previously unknown, has associations with the breast cancer-associated gene *BRCA1*. This finding is of exceptional importance because it provides not only a new target for cancer treatment but also a roadmap for other scientists to follow. The network systems approach, which will be reviewed in the following issue of *CJC*, will provide new insight into the critical molecular players and events underlying cancer and could lead to a new way of treating cancer.

Conclusions

The ABC transporters play an important role in effluxing endogenous toxicants and xenobiotics such as antineoplastic drugs from cells. Therefore, to design an effective chemotherapeutic regimen, thorough knowledge of the mechanisms of MDR caused by ABC transporters is required. Although structural and functional insights about ABC transporters have been useful in elucidating the mechanisms of MDR, co-administration of ABC transporter modulators with antineoplastics may produce unacceptable clinical toxicities. Consequently, ABC transporter modulators cannot be used clinically. However, knowledge of ABC transporters as cancer stem cell markers can be exploited to determine the plausible prognosis for various types of cancers. In addition, newer approaches to target MDR, such as use of tyrosine kinase inhibitors, nanomedicines or pharmacogenomics, can be beneficial in combating the MDR.

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Review

Multidrug resistance associated proteins in multidrug resistance

Kamlesh Sodani, Atish Patel, Rishil J. Kathawala and Zhe-Sheng Chen

Abstract

Multidrug resistance proteins (MRPs) are members of the C family of a group of proteins named ATP-binding cassette (ABC) transporters. These ABC transporters together form the largest branch of proteins within the human body. The MRP family comprises of 13 members, of which MRP1 to MRP9 are the major transporters indicated to cause multidrug resistance in tumor cells by extruding anticancer drugs out of the cell. They are mainly lipophilic anionic transporters and are reported to transport free or conjugates of glutathione (GSH), glucuronate, or sulphate. In addition, MRP1 to MRP3 can transport neutral organic drugs in free form in the presence of free GSH. Collectively, MRPs can transport drugs that differ structurally and mechanistically, including natural anticancer drugs, nucleoside analogs, antimetabolites, and tyrosine kinase inhibitors. Many of these MRPs transport physiologically important anions such as leukotriene C4, bilirubin glucuronide, and cyclic nucleotides. This review focuses mainly on the physiological functions, cellular resistance characteristics, and probable *in vivo* role of MRP1 to MRP9.

Key words Multidrug resistance protein (MRP), multidrug resistance (MDR), ABC transporter, chemotherapy

Chemotherapy is one of the major treatment modalities available for cancer patients. Unfortunately, during the course of treatment, cancer cells develop resistance to functionally and structurally different anticancer drugs by either acquired (due to host factors) or intrinsic (due to genetic or epigenetic) mechanisms[1,2]. This phenomenon of resistance to different classes of anticancer drugs by cancer cells is termed multidrug resistance (MDR). This pervasive and insidious clinical problem eventually leads to cancer relapse and death among patients. The mechanisms of MDR have been intensively studied, although not all mechanisms producing MDR have been elucidated. The detailed mechanisms that cancer cells utilize or develop to evade chemotherapy are complex and have been described in detail in several recent reviews[3-5]. One of the most important mechanisms underlying MDR is overexpression of adenosine triphosphate (ATP)–binding cassette (ABC) transporters, which efflux a wide spectrum of anticancer drugs against the concentration gradient using ATP-driven energy.

The ABC transporter family, representing the largest family of transmembrane proteins, comprises 49 transporters that are further subdivided into seven subfamilies, ABC-A to -G, based on sequence similarities[6]. Of them the major ABC transporters involved in MDR development are ABC subfamily B member 1 [(ABCB1/P-glycoprotein (P-gp)], ABC subfamily G member 2 [ABCG2, also known as breast cancer resistance protein (BCRP)/mitoxantrone resistance protein (MXR)/placenta-specific ABC protein (ABCP)], and ABC subfamily C member 1 (ABCC1/MRP1)[6,7]. This review will provide in-depth details about the MRPs involved in conferring MDR in cancer cells.

The MRP subfamily, the C subset of the ABC transporter superfamily, is composed of thirteen members, and nine of these are primarily involved in MDR (Table 1)[8]. Based on functional characterization, localization, and cloning studies, these nine MRPs have been established as ATP-dependent efflux transporters for endogenous substances and xenobiotics. The other three members of the MRP subfamily, namely ABCC7/cystic fibrosis transmembrane conductance regulator (CFTR), ABCC8/sufonylurea receptor 1

Authors' Affiliation: Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Queens, NY 11439, USA.

Corresponding Author: Zhe-Sheng Chen, Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Queens, NY 11439, USA. Tel: +1-718-990-1432; Fax: +1-718-990-1877; Email: chenz@stjohns.edu.

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