Role of membrane-embedded drug efflux ABC transporters in the cancer chemotherapy

Sonu Kumar Gupta, Priyanka Singh, Villayat Ali, Malkhey Verma

Department of Biochemistry, School of Basic & Applied Sciences, Central University of Punjab, Punjab, India

Abstract

One of the major problems being faced by researchers and clinicians in leukemic treatment is the development of multidrug resistance (MDR) which restrict the action of several tyrosine kinase inhibitors (TKIs). MDR is a major obstacle to the success of cancer chemotherapy. The mechanism of MDR involves active drug efflux transport of ABC superfamily of proteins such as P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) that weaken the effectiveness of chemotherapeutics and negative impact on the future of anticancer therapy. In this review, the authors aim to provide an overview of various multidrug resistance (MDR) mechanisms observed in cancer cells as well as the various strategies developed to overcome these MDR. Extensive studies have been carried out since last several years to enhance the efficacy of chemotherapy by defeating these MDR mechanisms with the use of novel anticancer drugs that could escape from the efflux reaction, MDR modulators or chemosensitizers, multifunctional nanotechnology, and RNA interference (RNAi) therapy.

Correspondence: Malkhey Verma, Department of Biochemistry, School of Basic & Applied Sciences, Central University of Punjab, Mansa Road, Bathinda-151001, Punjab, India.
Tel.: +91.7589489833 - Fax: +91.164.2864106.
E-mail: malkhey.verma@cup.edu.in; malkhey@yahoo.com

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Introduction

Chronic myeloid leukemia (CML) is characterized by the increased proliferation of pluripotent hematopoietic stem cells.[1-3] It is genetically distinguished by the presence of the t (9;22), (q34; q11) reciprocal chromosomal translocation. The Abelson proto-oncogene (ABL) on chromosome 9 is translocated to the Breakpoint Cluster Region (BCR) on chromosome 22, forming a fusion gene in which ABL-related tyrosine kinase activity is constitutively activated.[2,4-6] Imatinib, also called imatinib mesylate or signal transduction inhibitor 571 (STI571) or commercially known as Gleevec[7,8] is a BCR-ABL tyrosine kinase inhibitor (TKI), which has revolutionized the treatment of chronic myeloid leukemia. Imatinib is the first approved medicine that targets the ATP binding domain of BCR- ABL tyrosine kinase by reversing its effects.[9,10] While 5-years overall survival rate of CML patients without imatinib was recorded as 24%, it increased gradually to 39% (1966-1998) and 56.8% (1999-2006) and the overall survival was estimated to 85% with the application of imatinib.[2,11,12] Simultaneously during treatment, the development of resistance in many leukemia patients in the chronic phase[8,13,14] and accelerated/blast crisis phase[8] limits the use of tyrosine kinase inhibitors. The phenomenon of resistance against the chemotherapeutic agents called chemoresistance or multidrug resistance (MDR).[15] It was described by Keld Dano as the active outward transport of Vinca alkaloids and anthracyclines from murine Ehrlich ascites tumor cells.[16]

MDR is a phenomenon in which the cancer cell's resistance to a drug is accompanied by resistance to a pharmacologically and structurally different class of drugs.[17] Although, the mechanisms of anticancer drug resistances are a complex process and commonly categorized into drug dependent, target-dependent, and drug/target-independent. Drug-dependent MDR is caused by overexpression of efflux drug transporters and detoxifying enzymes which lowers the uptake or enhance the efflux of drugs in cancerous cells. Target-dependent MDR is primarily attributed to the factors influencing drug targets such as translocation, deletion, mutation, and amplification. Drug/target-independent MDR occur either genetically or epigenetically due to the inactivation of drug targeting by changing cell signaling cascades.[18-21] Interestingly, one of the most significant mechanism causing MDR is the overexpression of adenosine triphosphate (ATP)-binding cassette (ABC) superfamily of transporters, which efflux both cytotoxic

agents and targeted anticancer drugs using ATP driven energy.[22-24] Therefore, this review aims to discuss the role of those three mammalian ABC transporters that mediate MDR in cancerous cells and subsequent development of strategies to overcome MDR in cancer chemotherapy.

Properties of ABC transporters in human physiology

The human ABC transporters, one of the largest groups of membrane protein complexes consist of 49 members that has been divided into seven subfamilies (ABC-A through to ABC-G) (Table 1) based on the sequence similarities of nucleotide-binding domain (NBD) as well as structural organization,[25-27] though there are more in bacteria and parasites.[28]

Transporters are membrane-bound proteins that facilitate the translocation of substrate molecules across the biological membranes. Those mammalian ABC transporters which are localized to the plasma membrane throughout the body such as the liver, intestine, kidney, and organs with barrier functions, such as the brain, testes and placenta, as well as to membranes that compose various subcellular organelles, significantly decrease the intracellular concentration of various drugs, drug conjugates and metabolites by export.[27,29] Structurally, all ABC transporters contain two transmembrane domains (TMDs) and two nucleotides (ATP)-binding domains (NBDs).[30] Generally, they exhibit a common structural fold which is made up of a core of six TM helices per TMD. The hydrophobic TMDs are structurally different, which alternately identify and translocate various substrates upon conformational changes. So, the TMDs which lengthen the membrane and form channels may determine the transport features of substrates.[31,32] The energy required for translocation or efflux of physiological and xenobiotic substances from the cytosol to the extracellular space is provided by ATP hydrolysis via ATPase.[30] Till date, at least 15 human ABC superfamily transporter proteins such as P-glycoprotein (P-gp/ABCB1), multidrug resistance-associated protein 2 (MRP2/ABCC2) and breast cancer resistance protein (BCRP/ABCG2) deal with MDR as drug efflux pumps. However, various recent studies have confirmed that ABC transporter proteins efficacy enhance their role through some other mechanisms in addition to drug efflux (Figure 1).[33-36]

P-glycoprotein (ABCB1)

P-glycoprotein is the first member of ABC transporter family that linked with the overexpression of the ATP binding cassette transporter which is encoded by the ABCB1 gene.[37] It is the highly studied ABC drug efflux transporter to date[38,39] and its role in resistance against anticancer drugs has been known for more than thirty years.[40,41] The most outstanding property of P-gp is the difference in the structure of substrates transported, including a large number of drugs useful in therapeutic applications. These substrates include anticancer drugs, analgesics, antibiotics, antiarrhythmics, antihistamines, calcium-channel blockers, chemotherapeutic drugs, fluorophores HIV-protease inhibitors, immunosuppressive agents, natural products, neuroleptics, pesticides and many others (Table 2).[42-53] P-glycoprotein is highly expressed on the liver, placenta, lower gastrointestinal tract (jejunum, ileum, and colon), proximal tubules epithelia of kidney and luminal blood-brain barrier.[41] P-gp seems to be an essential factor of pharmacokinetics due to its wide range of substrate localization, and a good negotiator of transporter-mediated drug-drug interactions. Some of the current research findings also suggest that transport of cytotoxic substrates for P-glycoprotein can be suppressed by a large variety of chemical compounds known as *reversal agents* or P-gp blockers.[38] Not all but some of the identified inhibitors act as competitive inhibitors, for example, calcium channel inhibitor verapamil or an immunosuppressor cyclosporin A. Also, the other inhibitors probably work through some different mechanisms.

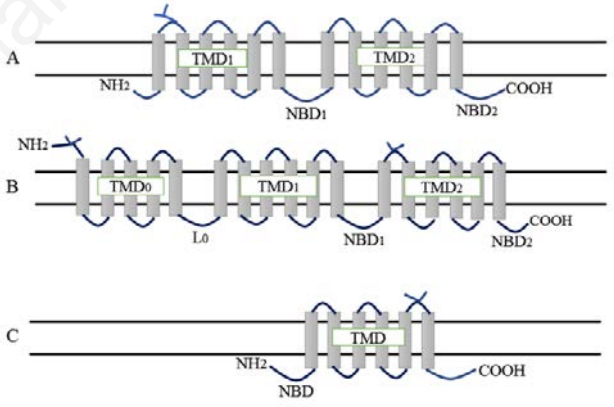

Figure 1. Model of the secondary structure of efflux membrane transporters of the ABC family. A) P-gp/ABCB1; B) MRP2/ABCC2; C) BCRP/ABCG2. TMD, transmembrane domain; NBD, nucleotide-binding domain; L0, loop 0.

Table 1. Human ABC transporter gene family.

Subfamily names	Assumed name	Number of genes	Number of pseudogenes
ABCA	ABC1	12	5
ABCB	MDR	11	4
ABCC	MRP	13	2
ABCD	ALD	4	4
ABCE	OABP	1	2
ABCF	GGN20	3	2
ABCG	White	5	2

Multidrug resistance protein 2 (MRP2/ABCC2)

The ABCC gene family has been divided into 13 subfamily members (ABCC1 through to ABCC13). MRP2 is the second member of the MRP subfamily of ABC transporter encoded by ABCC2 gene.[54,55] MRP2 was initially cloned from rat liver using the same approaches that took benefit of its structural similarity to human MRP1.[56,57] MRP2 has entirely different expression pattern in the apical plasma membrane of hepatocytes, the brush-border membrane of renal proximal tubules and small intestine, where it is situated to play an essential role in the elimination and oral bioavailability of a wide variety of drugs including endogenous glucuronides, sulphates and GSH conjugates from the cells. Furthermore, MRP2 involves in biliary elimination of LTC4 and bilirubin conjugates.[58,59] Also, MRP2 mRNA has been detected in the gallbladder, peripheral nerves, placental trophoblasts, and $CD4^+$ lymphocytes.[60-63] A new report on cancer chemotherapy suggest that effectiveness of cisplatin based treatment in patients with hepatocellular carcinoma depends upon MRP2 mRNA expression level.[64] Similar to MRP1, MRP2 can transport many different chemotherapeutic substrates which are listed in Table 2.

The transport of various substrates by MRP2 is based on different approaches, such as the analysis of distinct compounds transported into the bile of normal rats, but none of Mrp2 mutant rats; irregular uptake of substrates into vesicles of bile canalicular membrane obtained from normal and Mrp2 mutant rats; transduction or transfection of human MRP2 or rat/rabbit Mrp2 cDNA into cell lines followed by drug resistance analysis, accumulation of compounds into cells, and transepithelial transfer of compounds.[42,65]

Same as P-glycoprotein inhibitors some MRP2 inhibitors have been verified to work to a greater or lesser extent in entire cells which comprise of cyclosporin A, benzbromarone, probenecid, sulfinpyrazone, PSC 833, PAK-104P and MK571.[66-68] The compounds recognized so far have significant activity against MRP1 and P-gp but, hard to obtain against MRP2.

Table 2. Some clinically selected cytotoxic substrates associated with multidrug resistance transporters.

MDR1
Anticancer drugs: Actinomycin D, Anthracenes (bisantrene, mitoxantrone), Anthracyclines (daunorubicin, doxorubicin, epirubicin), chlorambucil, cisplatin, colchicine, cytarabine, Epipodophyllotoxins [etoposide (VP-16), Teniposide (VM-26)], 5-fluorouracil, gefitinib, hydroxyurea, irinotecan (CPT-11), methotrexate, mitomycin C, Taxanes (docetaxel, paclitaxel), tamoxifen, topotecan, Vinca alkaloids (vinblastine, vincristine, Vinorelbine Vindesine)
Analgesics: asimadoline, fentanyl, morphine, pentazocine
Antibiotics: cefoperazone, ceftriaxone, clarithromycin, doxycycline, erythromycin, gramicidin A, gramicidin D, grepafloxacin, itraconazole, ketoconazole, levofloxacin, rifampicin, sparfloxacin, tetracycline, valinomycin
Antiarrhythmics: amiodarone, digoxin, lidocaine, propafenone, quinidine, verapamil
Antihistamines: cimetidine, fexofenadine, ranitidine, terfenadine
Antilipidemic: lovastatin, simvastatin
Calcium channel blockers: azidopine, bepridil, diltiazem, felodipine, nifedipine, nisoldipine, nitrendipine, tiapamil, verapamil
Fluorophores: Hoechst 33342/33258, rhodamine 123, calcein AM (calcein acetoxymethyl ester), Fluo-3 AM, Fura-2 AM
HIV protease inhibitors: saquinavir, ritonavir, nelfinavir, lopinavir, indinavir, amprenavir
Immunosuppressive agents: cyclosporin A, cyclosporin H, FK506, sirolimus, tacrolimus, valspodar (PSC-833)
Natural products: curcuminoids, flavonoids
Neuroleptics: chlorpromazine, phenothiazine
Others: Amitriptyline (antidepressant), Dipyridamole (anticoagulant), BCECF-AM, bepridil, diltiazem, endosulfan, leupeptin, methyl parathion, paraquat, pepstatin A, trifluoperazine, trans-flupentixol
MRP2
Anticancer drugs: cisplatin, doxorubicin, epirubicin, etoposide, irinotecan, mitoxantrone, methotrexate, SN-38, Vinca alkaloids (vinblastine, vincristine)
Antibiotics: ampicillin, azithromycin, cefodizime, ceftriaxone, grepafloxacine, irinotecan
Antihypertensives: olmesartan, temocaprilate
HIV drugs: adevovir, cidofovir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir
Others: ethinylestradiol-3-O-glucuronide, genistein-7-glucoside, p-Aminohippurate, phloridzin, quercetin 4 -β-glucoside
BCRP
Anticancer drugs: Anthracenes (mitoxantrone), Anthracyclines (daunorubicin, doxorubicin, epirubicin), Epipodophyllotoxins (etoposide, teniposide), gefitinib, imatinib (TKI), irinotecan, methotrexate, SN-38, topotecan,
Antibiotics: ciprofloxacin, norfloxacin, ofloxacin
Antihypertensives: reserpine
Antivirals: delavirdine, lamivudine, lopinavir, nelfinavir, zidovudine
Calcium channel blockers: nicardipine
Lipid lowering drugs: cerivastatin, pravastatin, rosuvastatin
Others: azidothymidine, cyclosporin A, chrysin, lamivudine, ortataxel, quercetin

Data were collected from References.[42-53]

Breast cancer resistance protein (BCRP/ABCG2)

Although we have discussed earlier two transporters, MDR1 P-gp and MRP2 which are involved in MDR development similar to that, the third subfamily of transporter known as breast cancer resistance protein have an enormous impact in the physiology, pathophysiology, pharmacokinetics, and toxicokinetics.[69] Based on analogy with other ABC transporters BCRP is the second member of the G subfamily (ABCG2) which is thought to function as homo or heterodimers.[24,27,70] BCRP is the first clone based on its higher expression in doxorubicin-resistant breast cancer cell line (MCF-7).[69-73] This gene was isolated from the breast cancer cell line, that's why called breast cancer resistance protein (BCRP). This transporter is extensively distributed in the endothelial cells of brain capillaries, liver, intestine, proximal tubule cells of the kidney, and contributing to the absorption, distribution, and elimination of the drugs and endogenous compounds in addition to protection of tissues against deadly xenobiotic exposures.[22,74] Just like MDR1/P-gp and MRP2, BCRP transports structurally and functionally diverse kind of substrates such as analgesics, antibiotics, anticancer and antivirals drugs, *etc*. (Table 2). Furthermore, the transport through above transporters can be inhibited by small molecules listed in Table 3.[32,49,68,75-82]

Strategic development to overcome MDR in cancer chemotherapy

Several strategies have been made to overcome MDR in cancer cells through the synthesis of new molecules applying biochemical and physical approaches as well as nanotechnologies.[83-85] One such strategy is the development of novel anticancer drugs that are not P-gp substrates, but many different chemosensitizers identified were substrates for P-gp, therefore, worked by contesting with cytotoxic compounds for efflux by the P-gp pump. Verapamil and cyclosporine A are the first-generation P-gp modulators which block the transport function of the transporter.[86,87] Other examples of modulators are trifluoperazine, quinidine, and progesterone.[82] The second-generation P-gp modulators such as dexverapamil, dexniguldipine, valspodar (PSC 833), and biricodar (VX-710) are more effective and less toxic than their predecessors.[88] Among them, the best-studied agent is valspodar, a non-immunosuppressive derivative of cyclosporin D that inhibits P-gp by 10-20 folds higher than cyclosporin A.[89,90] Many cytotoxic agents that are substrates for P-gp are also substrates for the cytochrome P450 isoenzyme 3A4, and they create toxic pharmacokinetic interactions due to competition between them.[91] Therefore, different anticancer drugs are being developed that cannot be identified by P-gp and/or ABC transporters. For example, BMS-184476,[92] Ortataxel[93] and Taxane analogues DJ-927.[94] Similarly, Chinese traditional medicine is known as *indirubin* usually do not carried out by P-gp, but prevent the efflux of doxorubicin and vincristine by P-gp.[95]

Still, the problem with MDR continues which motivated the scientists to develop third-generation P-gp modulators such as Tariquidar (XR9576),[96] Zosuquidar (LY335979),[97] Laniquidar (R101933),[98] Elacridar (F12091).[99] While Tariquidar inhibits ATPase activity of P-gp even at a very low concentration (25-80 nM),[100] Zosuquidar (an oral P-gp inhibitor) stimulate the intake of daunorubicin, idarubicin, mitoxantrone, and mylotarg in acute myeloid leukemia.[101]

The other strategies involve the use of microRNAs (miRNAs). They are small, highly conserved non-coding RNA molecules that bind to the 3' UTR of mRNA and suppress the protein expression throughout the translation.[102,103] Generally, miRNAs get modified within cancer cells that may lead to the development of MDR.[104] These are some miRNAs (miR-27a, miR-296, miR-298, miR-451, miR-1253) which have been recognized as an inhibitor of P-glycoprotein, and their therapeutic index was evaluated in breast cancer cells lines (MCF-7) and esophageal squamous carcinoma cells.[105-107] The complete understanding of the mechanism of miRNAs regulation may contribute to the development of a drug against MDR.[108] siRNAs can also reverse the MDR through inhibition of MDR genes, for example, ABCB1 (MDR1), ABCB4 (MDR3), ABCG2 (BCRP).[109-112]

The monoclonal antibodies also play a crucial role in reversing drug resistance mediated by P-gp, such as MRK- 16 and MRK-17 were developed to reverse the drug resistance effect both *in vivo* and *in vitro* during the 1980s.[113-116] While MRK-16 act as an effective blocker against actinomycin D and vincristine efflux, MRK-17 has an active role in the inhibition of MDR cell proliferation. UIC2 is a newly designed mouse monoclonal antibody which binds to a cell surface epitope of P-gp in a specific manner and suppresses the drug efflux and boost cell cytotoxicity.[117] The conjugates of monoclonal antibodies with P-gp-reversing agents may increase anticancerous properties.

Currently, nanotechnology-based approaches are being used as a more efficient strategy to overcome MDR. Different types of nanoparticles such as metals, polymers, dendrimers, liposomes, solid lipids, quantum dots, and micelles are widely used to transport anti-cancer, anti-infection, or anti-inflammatory drugs to exact target cells/tissues of patients. The size of nanoparticles greatly varies up to several hundred nm.[83,84,118-121] The assembly of nanoparticles takes place in several layers, but the surface coating is a major beneficiary step for the solubility, specificity, and stability of these nanoparticles.[122,123] The most frequently used nanovehicles for drug delivery to the target cells/tissues are bio-degradable polymeric nanoparticles. The polymers may be either natural such as gelatin, chitosan, and albumin or synthetic for example, poly (d, l-lactic acid) (PLA), poly (d, l-lactic-co-glycolic acid) (PLGA), and poly (ε-caprolactone) (PLC).[124,125] Liposome nanoparticles are also used in drug delivery systems. Liposomes may encapsulate soluble drugs and retain their natural activity by forming phospholipid bilayers and micelle spheres. It is primarily used for the delivery of those drugs which are unable to diffuse through membranes. Doxil and Daunoxome are the two nanodrugs in which doxorubicin or daunorubicin have been merged into 80-90 nm single layer liposome nanoparticles.[126] Liposomes nanoparticles show potential activity in the battle against MDR. Gold nanoparticles (AuNPs) also considered as the right choice for

Table 3. Inhibitors of P-gp, MRP2 and BCRP transporters.

P-gp/ABCB1	Atorvastatin, amlodipine, cyclosporin A, dexniguldipine, disulfiram, verapamil, quinidine, nifedipine, MS-209, GF120918, LY475776, V-104, LY335979, OC144-093, pluronic L61, PSC-833, R101933, S9788, VX-710, XR-9576
MRP2/ABCC2	Azithromycin, cyclosporin A, furosemide, glibenclamide, probenecid, MK-571
BRCP/ABCG2	Cyclosporin A, dipyridamole, elacridar, fumitremorgin C, novobiocin, ortataxel, reserpine, ritonavir, tariquidar, GF120918, VX-710, XR-9576

Data were collected from References.[32,49,68,75-82]

drug delivery and can be synthesized easily.[127] Gold nanoparticles provide their surface for targeting tumors and drug release in a controlled way.[127,128] For example, Dox-PLGA-Au has enough potential to reduce tumor growth and win the battle against MDR.[129]

Conclusions

The current review article summarizes the most recent studies of ABC superfamily transporters and their contribution to MDR in cancer chemotherapy. Almost 90% of cancer patients are facing treatment failure due to drug resistance. From the previous experimental studies, it has been confirmed that ABC drug transporters play an important role in the efflux of endogenous toxicants and xenobiotics. Therefore, some new strategies for effective chemotherapeutic treatment against MDR triggered by ABC drug transporters are required. The structural and functional understanding of ABC drug transporters has also been useful in explaining the mechanisms of MDR. The recent findings suggested that numerous signaling pathways such as protein tyrosine kinase, EGFR (epidermal growth factor receptor), MAPK (mitogen-activated protein kinase) are involved in drug resistance. Therefore, a multidisciplinary approach with a combination of technologies (genomics, epigenetics, transcriptomics, proteomics) would be a classical model in changing our therapeutic thinking and diagnosis against cancer. The ongoing research in this area is at a very early stage about the integration of these studies into everyday practice. So, the development of suitable markers for host responses will be a key factor in this research.

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