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Modulation of Multidrug Resistance in Cancer by P-Glycoprotein

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Abstract

Multidrug resistance (MDR) is one of the main obstacles in the chemotherapy of cancer. MDR is associated with the over expression of P-glycoprotein (P-gp), resulting in increased efflux of chemotherapy from cancer cells. Inhibiting P-gp as a method to reverse MDR in cancer patients has been studied extensively, but the results have generally been disappointing. First-generation agents were limited by unacceptable toxicity, whereas second-generation agents had better tolerability but were confounded by unpredictable pharmacokinetic interactions and interactions with other transporter proteins. Third-generation inhibitors have high potency and specificity for P-gp. Furthermore, pharmacokinetic studies to date have shown no appreciable impact on drug metabolism and no clinically significant drug interactions with common chemotherapy agents. Third-generation P-gp inhibitors have shown promise in clinical trials. The continued development of these agents may establish the true therapeutic potential of P-gp-mediated MDR reversal.

Key words : ABC transporter, Multidrug resistance, P-glycoprotein, P-gp modulators

1. Introduction

Tumor cell resistance to cytotoxic drugs is considered one of the major obstacles to successful chemotherapy. Some tumors are initially resistant and never respond to cytotoxic drug treatment; others initially respond well but eventually develop and become resistant. This phenomenon may result from genetic mutations induced by the administered antitumor agent, or may represent the selection of preexisting resistant cell populations in the malignant tumor. MDR describes the simultaneous expression of cellular resistance to a variety of unrelated drugs primarily of natural origin. There have been many excellent reviews dealing with mechanisms of cellular drug resistance[1-10]. In addition to MDR-related proteins many other mechanisms of drug resistance have been documented in vitro. They are based on alterations in drug target enzymes and proteins, increased detoxification, alterations in cellular metabolism, enhanced ability to repair DNA damage, and failure to undergo apoptosis.

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Although several mechanisms are proposed for drug resistance, the best-studied mechanism of MDR is related to the over expression of P-gp, a 170 KDa ATP dependent membrane transporter that acts as a drug efflux pump[11,12]. P-gp belongs to the ATP-binding cassette (ABC) family of transporters, currently numbering 48 members that share sequence and structural homology. It is believed that, while this class of transporters has a large number of members, only 10 or so are reported to confer the drug-resistant phenotype. These transporters use the energy that is released when they hydrolyze ATP to drive the transport of various molecules across the cell membrane[13,14]. In addition to their physiologic expression in normal tissues, many are expressed and, importantly, over-expressed, in human tumors.

P-gp consists of four distinct domains. Two of these are highly hydrophobic, integral membrane domains, each of which spans the membrane six times by a-helices. The other two are hydrophilic nucleotide binding domains (NBDs). Recently, the structure of mouse ABCB1[15] has been obtained using X-ray crystallography and although this is a significant breakthrough, structural resolution was at 3.8Å in the absence of drug (Fig. 1A).

Structural information was also obtained by 'soaking' crystals in the presence of an ABCB1 inhibitor and the

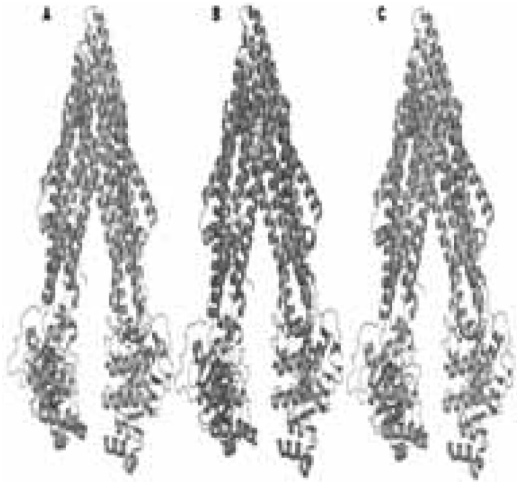

Fig. 1. Recently published crystal structures of mouse ABCB1. A) Apo inward-facing, nucleotide free conformation (PDB code: 3g5u, 3.8Å). B) Mouse ABCB1 bound with QZ59-RRR (PDB code: 3G60, 4.4Å). C) Mouse ABCB1 bound with QZ59-SSS (PDB code: 3G61, 4.3Å). Ligands are shown in CPK mode. Figures are generated using PYMOL program.

Table 1. P-gp and other transporter in MDR along with their cytotoxic substrates they transport.

Common Name	Systematic Name	Cytotoxic Substrates
P-gp	ABCB1	Actinomycin-D, Bisantrene, Daunorubicin, Docetaxel, Doxorubicin, Etoposide, Homoharringtonine, Mitoxantrone53, Paclitaxel, Teniposide, Topotecan, Vinblastine, Vincristine, Vinorelbine.
MRP1	ABCC1	Doxorubicin Epirubicin, Etoposide, Methotrexate, Vincristine, Vinorelbine.
BCRP	ABCG2	Daunorubicin, Doxorubicin, Mitoxantrone, SN-38, Topotecan.

resolution obtained was slightly lower at 4.3Å and 4.4Å. The cyclic hexa-peptide inhibitor was synthesized specifically for this study and its relationship to the established pharmacological drug interactions sites (e.g. site I for vinblastine) is presently unclear. In addition, the current level of resolution precludes atomic detail on the drug-protein interaction. Many of the residues implicated in binding to the custom-built inhibitor are, however, equivalent to those from the biochemical investigations. A great deal more analysis and functional investigation based on this structure are required. Along with P-gp, in human other proteins such as multiple resistance protein (MRP) and breast cancer resistance protein (BCRP) are also responsible for MDR development and are listed in Table 1 with their substrates. In this review, we aim to provide the reader with an up-to-date view of multidrug ABC transporter interactions.

We address, in particular, the structural features and mechanisms that allow the TMDs of ABCB1 and bacterial homolog to bind and transport toxic ions and drugs (referred to as ligands). By comparing the recently published crystal structure of ligand-bound ABCB1 with the available crystal structures of bacterial homolog in post hydrolysis states.

2. Experimental Section

2.1. Domain Organization

Multidrug ABC transporters belong to the ABC superfamily that contains 48 representatives in the human genome alone. In this superfamily, ABC exporters can be distinguished from ABC importers by the directionality of transport and distinct structural arrangements of the transmembrane domains (TMDs)[16]. All ABC transporters contain two nucleotide-binding domains (NBDs), each carrying the namesake ABC motif, and two TMDs, usually each containing six transmembrane helices (TMHs) in exporters. In Bacteria and Archaea, ABC exporters are typically expressed as half-transporters, with one NBD and one TMD on a single polypeptide chain. Two chains then assemble into a functional homo- or heterodimer. In Eukaryote, however, ABC exporters are often expressed as a single polypeptide chain containing the four domains. The human multidrug resistance P-glycoprotein ABCB1, which was first described by Danø[17] and Juliano and Ling[18].

In general, the dimeric NBDs in multidrug ABC transporters act in concert to hydrolyze ATP and provide the free energy to drive directional transport against transmembrane concentration gradients for hydrophilic substrates and against the lipid-water partition coefficient for hydrophobic substrates.

2.2. Drug Binding and Transport

Ligand specificity of multidrug ABC transporters comes, in large part, from cell biological and biochemical experimentation. One recurrent theme that emerged is that hydrophobic ligands might interact with binding sites in ABCB1 that lie within the membrane. Various studies have attempted to locate the drug-binding sites and key residues responsible for the interaction with ligands. ABCB1 is the best-studied multidrug ABC transporter. The TMDs in ABCB1 enables binding of ligands in the absence of the NBDs[19,20]. Studies of ligand-ligand interactions on ABCB1 revealed that some ligands interact with the transporter as single molecules, whereas others interact as pairs. ABCB1 contains distinct sites for transport of rhodamine 123 (R-site) and Hoechst 33342 (H-site) in addition to a modulatory site for prazosin and progesterone[21,22]. The interaction of ligand at one of the transport competent sites enhances the ligand interaction at the other site[21]. Equilibrium binding measurements on ABCB1 provided evidence for three sites for transported ligands (vinblastine, paclitaxel, and Hoechst 33342), which can interact with ligand in the absence of exogenously added nucleotide, in addition to a modulatory site for nicardipine/ GF120918 (Fig. 2)[23].

As a network of interactions between these kinetically distinguishable drug-binding sites exists, the possibility was raised that these sites are present on a common binding surface. Cross-linking studies were expanded by extensive cysteine scanning mutagenesis in which single mutants were tested for their ability to react with thiol-reactive substrates including dibromobimane[24], methanethiosulfonate-verapamil (MTS-verapamil)[25], and MTS-rhodamine B[26]. These studies showed that residues on TMHs 6, 9 and 12 form the R-site pocket, whereas the H-site is created by residues located on TMHs 4, 6, 10, 11 and 12. At 3.8Å, the crystal structure for apo ABCB1 is of moderate resolution with 65% in most favored regions, 27.5% in additional allowed regions, 7.5% in generously allowed regions and, most importantly at this resolution, 0% in disallowed regions (determined by ProCheck) (Fig. 3).

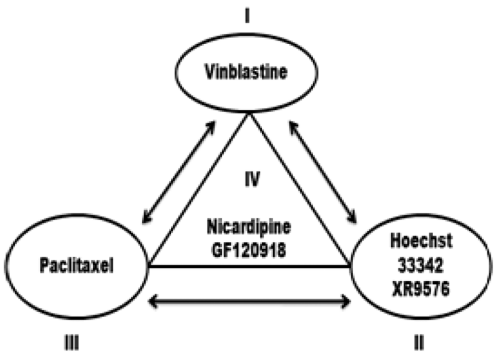

Fig. 2. Classification of drug-binding sites on P-gp[23].

The TMDs indeed form a ligand-binding chamber that is exposed to the cytoplasm and contains a laterally open gap that is accessible to the inner leaflet of the membrane (Fig. 4A). In the outward-facing nucleotide-bound conformations of the ABCB1 model (Fig. 4B) based on the templates Sav1866 from Staphylococcus aureus[27,28], the TMDs also form a lateral gap that is exposed to the outer leaflet of the bilayers and the external environment. Based on the above information we could categorize transport cycle in four steps. First, ligand enters the binding cavity from the inner membrane leaflet through the lateral gaps between the two TMDs or directly from the cytoplasm and binds the high-affinity ligand-binding site. Second, the TMDs undergo a conformational 'switch' upon binding and/or hydrolysis of ATP[29,30], closing the binding pocket to the inner leaflet of the membrane and opening it to the outer leaflet. The binding site is thought to reduce its affinity for the ligand by decreasing favorable intermolecular contacts. Third, the ligand is released at the lateral gap into the outer leaflet of the membrane and/or external aqueous environment. Fourth, after dissociation of the ligand, the TMDs reset to the inward-facing high-affinity

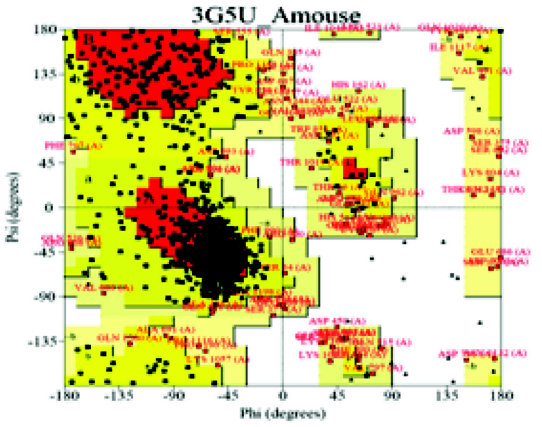

Fig. 3. Ramachandran plot calculated for apo structure of mouse ABCB1 (3G5U).

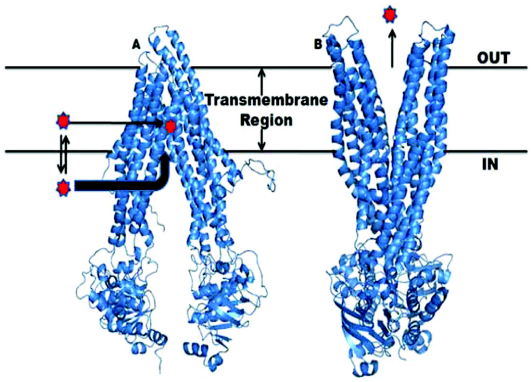

Fig. 4. Model for ligand efflux by ABCB1. ABCB1 contains a central ligand binding cavity in the transmembrane region. During transport, this binding cavity is alternately exposed to the inside and the outside surface of the membrane. (A) The inward-facing structure of ABCB1 and (B) The putative outward-facing structure exposed to the lipid bilayers and extracellular part. Ligand is shown in red star. Figures are generated using PYMOL program.

state. It is important to note that, alternating access for ABCB1 often emphasizes a two step oscillation process between inward facing and outward facing conformation (Fig. 4). The continuous process of inward-facing to outward-facing and from outward-facing to inward-facing is collectively called transport cycle.

2.3. Polyspecificity of Drug Binding Pocket

The two reported structures of ABCB1 in complex with one QZ59-RRR molecule or two QZ59-SSS molecules (Fig. 1B, C) are consistent with previous observations that ABCB1 can bind more than one ligand simultaneously[31,32]. The cavity formed by ABCB1 encloses a volume of approximately 6000Å3[15] thus providing ample space for two drug molecules. P-gp can distinguish between the stereo-isomers of cyclic peptides (Fig. 1B, C) resulting in different binding locations, orientation and stoichiometry. QZ59-RRR (Figure 2B) binds one site per transporter located at the center of the molecule between TMD 6 and TMD 12. The binding of QZ59-RRR to the “middle” site is mediated by mostly hydrophobic residues on TMDs 1, 5, 6, 7, 11, and 12. QZ59-SSS (Figure 1C) binds two sites per Pgp molecule. The QZ59-SSS molecule occupying the “upper” site is surrounded by hydrophobic aromatic residues on TMDs 1, 2, 6, 7, 11, and 12. The ligand in the “lower” site that binds to the C-terminal half of the TMD is in close proximity to TMDs 1, 5, 6, 7, 8, 9, 11, and 12.

The co-crystal structures of Pgp with QZ59 compounds demonstrate that the inward-facing conformation is competent to bind drugs. Previous studies have identified residues that interact with verapamil[33,34] are also facing in drug binding pocket and highly conserved. Analysis of structure demonstrated that upper half of the drug binding pocket contains predominantly hydrophobic/aromatic residues, the lower half of the chamber has more polar and charged residues. Hydrophobic substrates that are positively charged may bind using these residues similar to the poly-specific drug binding pockets.

2.4. P-gp Modulators

There are many studies have been reported to overcome MDR by inhibiting MDR transporters, to suppress or circumvent MDR mechanisms. The use of anticancer drugs that could escape from the ABC transporters might be a solution to avoid drug resistance. Anticancer drugs which are not the substrates of ABC transporters are alkylating drugs (cyclophosphamide), antimetabolites (5-fluorouracil), and the anthracycline modified drugs (annamycin and doxorubicin-peptide)[35]. Another method to overcome resistance to anticancer drugs is to administer compounds that would not be toxic themselves, but would inhibit ABC transporters[35,36]. The compounds that would reverse resistance against anticancer drugs are called MDR inhibitors, MDR modulators, MDR reversal agents or chemosensitizers and the process is referred as chemosensitization.

Most modulators identified interfere with P-gp by competitive or noncompetitive inhibition of its drug effluxing activity. They are normally P-gp substrates, some of them are only able to bind to the protein but are not effluxed from the cells, and can thus be considered as pure antagonists. It has been shown that various MDR type substrates and Chemosensitizers compete at a common drug binding site present in P-gp[37]. Many agents that modulate the P-gp transporter, including verapamil, cyclosporine (cyclosporine A), tamoxifen, and several calmodulin antagonists, were identified in the 1980s. These agents often produced disappointing results in vivo because their low binding affinities needed the use of high doses, resulting in unacceptable toxicity[38-40]. Many of the initial chemosensitizers identified were

themselves substrates for P-gp and thus worked by competing with the cytotoxic compounds for efflux by the P-gp pump; therefore, high serum concentrations of the chemosensitizers were necessary to produce adequate intracellular concentrations of the cytotoxic drug. In addition, many of these chemosensitizers are substrates for other transporters and enzyme systems, resulting in unpredictable pharmacokinetic interactions in the presence of chemotherapy agents. To overcome these limitations, several novel analogs of these early chemosensitizers were tested and developed, with the aim of finding P-gp modulators with less toxicity and greater potency[38,41]. The second-generation P-gp modulators include dexverapamil, dexniguldipine, valspodar (PSC 833), and biricodar (VX-710). These agents are more potent than their predecessors and also less toxic[38]. The best characterized and most studied of these agents is valspodar, a non-immunosuppressive derivative of cyclosporine D that inhibits P-gp with 10 to 20 fold greater activity than cyclosporine A[42,43]. Second-generation P-gp modulators have a better pharmacologic profile than the first-generation compounds, but they also retain some characteristics that limit their clinical usefulness. In particular, these compounds significantly inhibit the metabolism and excretion of cytotoxic agents, thus leading to drug-drug interactions. Many of the cytotoxic agents that are substrates for P-gp are also substrates for the cytochrome P450 isoenzyme 3A4. It is not surprising then that the agents that are affected by the development of MDR are also metabolized by cytochrome P450 3A4. Several of the second-generation P-gp modulators, including valspodar and biricodar, are substrates for this enzyme[44]. The competition between cytotoxic agents and these P-gp modulators for cytochrome P450 3A4 activity has resulted in unpredictable pharmacokinetic interactions. For e.g., valspodar inhibits the cytochrome P450 3A4-mediated metabolism of paclitaxel and vinblastin[45,46]. resulting in increased serum concentrations of the cytotoxic agents and potentially putting patients at risk of cytotoxic drug overexposure[47]. Similarly, in a pharmacokinetic study in patients with solid tumors, biricodar administered in a 24-hour intravenous infusion decreased the clearance of paclitaxel in a dose-dependent manner. It has been suggested that this interaction may be due in part to the inhibition of cytochrome P450 3A4 by biricodar, thereby interfering with the metabolism of paclitaxel[48]. Many of the early-generation P-gp modulators inhibited several other ABC transporters as well as the P-gp transporter. For instance, valspodar and biricodar are not specific solely to P-gp; both of these agents affect MRP1[36,49]. It is possible that this inhibition of non-target transporters may lead to greater adverse effects of anticancer drugs, including neutropenia and other myelotoxic effects[50].

Third-generation molecules that specifically and potently inhibit P-gp function have been developed by using structure-activity relationships and combinatorial chemistry, which overcome the limitations of the second generation P-gp modulators. These agents do not affect cytochrome P450 3A4 at relevant concentrations. Similarly, third-generation agents typically do not inhibit other ABC transporters[51,52]. The third generation P-gp inhibitors currently in clinical development include the anthranilamide derivative tariquidar (XR9576), diketopiperazine derivative XR9051[53], the cyclopropyldibenzosuberane zosuquidar (LY335979)[54]. Despite having diverse chemical structures and origins, these agents have in common a high potency and specificity for the P-gp transporter. One of the most promising third-generation P-gp inhibitors is tariquidar, which binds with high affinity to the P-gp transporter and potently inhibits its activity[55,56]. The cyclopropyldibenzosuberane modulator LY335979 was shown to competitively inhibit the binding of vinblastine to P-gp[57]. In clinical studies in both solid and hematologic malignancies, LY335979 showed no significant pharmacokinetic interactions with doxorubicin, etoposid, daunorubicin, vincristine, or paclitaxel[58-61]. Third-generation inhibitors of P-gp, such as tariquidar, bind with high affinity to the pump but are not themselves substrates. This induces a conformational change in the protein, thereby preventing ATP hydrolysis and transport of the cytotoxic agent out of the cell, resulting in an increased intracellular concentration.

3. Result and Discussion

During the last two decades, significant progress has been made in understanding the pharmacological and physiological role of P-gp. Concomitant use of P-gp inhibitors is hopefully an effective and safe way to perform further preclinical and clinical investigations with the hope of providing new treatment options to over-

come the MDR. The formation of the MDR is a complex and multi-factor process, so focusing attention on single aspects would be useless. In addition, this is why, to a large extent, many P-gp inhibitors have been identified but most of them failed to become MDR modulating drugs. Although P-gp inhibition seems to be a complex and difficult task, a large amount of work is still needed to optimize this strategy. The continued development of computational virtual screening techniques, 3D-QSAR studies, molecular pharmacology, and chemoinformatics, together with the technologies of chemogenomics, will deepen the understanding of P-gp's structure and efflux mechanisms. Accordingly, this should offer the opportunity for novel therapeutically effective P-gp inhibitor candidates.

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