Published in final edited form as:
Curr Mol Pharmacol. 2008 June ; 1(2): 93–105.

Reversal of ABC drug transporter-mediated multidrug resistance in cancer cells: Evaluation of current strategies

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Abstract

Overexpression of ATP-binding cassette (ABC) drug transporters that actively efflux a variety of amphipathic compounds can cause multidrug resistance (MDR) in cancer cells, which is a major obstacle in the success of cancer chemotherapy. The development of synthetic small molecule compounds or the identification of natural products that block ABC transporter-mediated efflux has been the conventional approach used to combat MDR. The strategy of using chemosensitizers, however, has not been successful in clinical cancer chemotherapy. Therefore, alternative approaches to identify or to synthesize compounds that can induce selective toxicity in cancer cells overexpressing one or more ABC transporters have been undertaken. This review summarizes the recent advances in identifying strategies to restore sensitivity to chemotherapeutics in multidrug resistant cancer cells.

Keywords

ATP-binding cassette transporters; Multidrug resistance; Chemosensitizers; Modulators; Collateral sensitivity

THE FUNCTION AND SIGNIFICANCE OF ATP-BINDING CASSETTE TRANSPORTERS IN THE DEVELOPMENT OF MULTIDRUG RESISTANCE IN CANCER CELLS

Multidrug resistance (MDR) in cancer is a phenomenon that occurs when cancer cells become simultaneously resistant to structurally unrelated chemotherapeutic agents. MDR in cancer patients will eventually lead to the failure of cancer treatment. Several cellular mechanisms can be responsible for MDR, such as reduced apoptosis, advanced DNA damage repair mechanisms or altered drug metabolism. However, the most common mechanism of resistance is the active efflux of drugs by ATP-binding cassette (ABC) transporters. These transporters have important physiological roles in mammalian cells, which have been extensively reviewed [1–4].

ABC drug transporters

ABC transporters are membrane proteins consisting of both transmembrane domains (TMDs) and distinctive nucleotide-binding domains (NBDs), which generate energy from ATP hydrolysis to actively transport a variety of compounds across the membrane [4]. These

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transporters belong to the ABC protein superfamily which is subdivided into seven distinct subfamilies (ABCA-ABCG) based on sequence homology and domain organization. Among others, three members of the ABC transporter family, ABCB1 (P-glycoprotein or Pgp), ABCC1 (MRP1) and ABCG2 (MXR, BCRP), appear to play an important role in the development of MDR in cancer cells. Several members of this family are capable of actively transporting a wide range of substrates including ions, sugars, amino acids, lipids, toxins and anticancer drugs. Essentially, when these ABC drug transporters are overexpressed in cancer cells, they can confer cross-resistance to multiple drugs of differing chemical classes by actively effluxing cytotoxic drugs, thus reducing the accumulated amount of drug below the effective chemotherapeutic level and resulting in MDR [5–7]. In addition, at least 15 genetic disease conditions are associated with defects in 20 members of the ABC superfamily, such as Cystic fibrosis (ABCC7), Tangier Disease (ABCA1), Dubin-Johnson syndrome (ABCC2) and Pseudoxanthoma elasticum (ABCC6) [1].

P-glycoprotein (ABCB1)—ABCB1 was the first human ABC drug transporter identified and has been studied extensively [8]. It transports a broad variety of compounds, including some of the most popular anticancer drugs such as taxanes, anthracyclines and Vinca alkaloids [8]. Since all attempts to obtain crystals of human ABCB1 suitable for X-ray crystallography have failed thus far, the structure of ABCB1 (and other human ABC drug transporters) is predicted based on biochemical studies, mutational analysis and structural information from bacterial homologs such as Sav1866 [9]. Although a low resolution structure based on electron microscopy has been described [10,11], the predicted structure of human ABCB1 is believed to consist of 2 halves, each with one transmembrane domain (TMD) containing six transmembrane helices and 1 NBD, with helices 4, 5 and 6 in the N-terminal half and helices 10, 11 and 12 in the C-terminal half to form the transport substrate site(s) [12–14].

ABCC1 and ABCG2—ABCC1 was the first member of the MRP family that was found to be linked with MDR by Cole et al. [15]. Structurally, ABCC1 is predicted to have three TMDs containing17 transmembrane helices. Unlike ABCB1, it has one additional TMD with five transmembrane helices within its N-terminal region [16,17]. In addition to transporting its physiological substrate such as oxidized glutathione (GSSG) [18] or LTC_4 [19,20], ABCC1 is capable of transporting anticancer drugs such as anthracyclines and mitoxantrone, as well as drugs conjugated to glutathione- (GSH), sulfate- or glucuronate [21]. A more recently identified drug transporter is ABCG2, also known as ABCP [22], breast cancer resistance protein [23] or mitoxantrone resistance protein [24], which is a half-transporter with one NBD and one TMD consisting of 6 transmembrane helices. Unlike ABCB1 and ABCC1, ABCG2 must dimerize to function, and it is believed to function as a homodimer or oligomer. [25]. Similar to ABCB1 and ABCC1, ABCG2 transports a variety of drugs, including anthracyclines, mitoxantrone, topotecan, etoposide, prazosin and flavopiridol [26–30] as well as other compounds such as riboflavin [31] and sterols [32,33]. ABCG2 is expressed on stem cells of both normal and cancer lineages [34].

Clinical significance

Cancer treatment often involves the use of chemotherapeutic agents; yet these drugs are not always effective. This loss of efficacy is predominantly correlated with the overexpression of ABC drug transporters [5], which was first described in the early 1970's [35]. Various tumors such as renal cell, adrenocortical, colon and hepatocellular cancers express ABCB1 and are principally chemoresistant [36]. In contrast, exposure to chemotherapeutics causes an upregulation of ABC transporters on relapse of disease in cancers with low baseline expression of these transporters. Expression of ABC transporters is well documented in patients with leukemia; in acute myelocytic leukemia (AML) 30% of patients express ABCB1 while over 50% express ABCB1 at relapse. Plasschaert and colleagues showed that ABCG2 has higher

expression and is functionally more active in acute lymphoblastic leukemia (ALL) B-lineage than in T-lineage samples [37]. In their report, the wild type *ABCG*2 gene was found in the samples which transported Rhodamine 123. Although the expression of ABCB1 plays an important role in MDR for leukemia, there are discrepancies in studies performed to evaluate the importance of ABCG2 in this cancer (reviewed in [7]). Expression of ABCG2 has been reported in a variety of other cancers and is most prominent in colon, stomach and esophageal cancer [38].

In addition to causing multidrug resistance, ABC transporters have a great impact on the pharmacokinetics of chemotherapeutic agents. Both ABCB1 and ABCG2 are expressed at the blood-brain barrier and in the gastrointestinal tract. Expression of ABC transporters in these biological barriers limits the absorption of various compounds in these tissues. The oral bioavailability of a number of anticancer agents is altered by ABC transporter expression. [39–42]. ABCG2 has also been identified in the apical membrane of alveolar epithelial cells in lactating mammary glands of mice, cows and humans [43] and there serves to transfer drugs and xenotoxins into breast milk. Although ABCG2 plays a protective role in the mammary glands, paradoxically, carcinogens and toxins are concentrated and passed on to the infant by ABCG2. In contrast, ABCG2 localized to the placenta effluxes compounds away from the fetus and plays a role in the maternal-fetal barrier [44]. Investigators have reported a variety of compounds such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and riboflavin which have been secreted by ABCG2 into breast milk [31,43,45,46]. There have been many recent reviews which focus on the clinical significance of ABC transporters in the pharmacokinetics of drugs [6,47,48]. In the following sections, we summarize the strategies employed by researchers to combat MDR in cancer.

RE-SENSITIZING MDR CANCER CELLS TO ANTICANCER DRUGS BY DEVELOPING INHIBITORS TO ABC DRUG TRANSPORTERS

Ideally, the most direct and easiest way to restore drug sensitivity in MDR cancer cells caused by ABC drug transporters is to block transporter-mediated drug efflux. Since 1980, researchers have been searching for both broad-spectrum and specific modulators that can reverse MDR in cancer cells. Tremendous efforts have been made to discover and synthesize such inhibitors/modulators. Several examples of ABC drug transporter inhibitors which have been discovered or synthesized are listed in Table 1. In addition, alternative strategies such as regulating expression of drug transporters or using drugs that specifically target drug transporters are also discussed later.

Designing or finding potent chemosensitizers that are selective, low in intrinsic toxicity and highly effective has been more difficult than expected. However, how to tackle drug resistance using available pharmacological and structural information to select or design new inhibitors (reviewed in [3,49,50]) is now much clearer. It is agreed in principle that an inhibitor/chemosensitizer must be able to increase intracellular anticancer drug levels, restore drug sensitivity and/or interfere with photoaffinity labeling of a particular drug transporter [49]. The first ABCB1 chemosensitizer was identified in 1981 by Tsuruo et al. when the calcium channel blocker verapamil was found to re-sensitize vincristine-resistant P388 leukemia cells to vincristine and vinblastine [51]. A subsequent study presented direct evidence that verapamil restored *Vinca*-alkaloid toxicity by increasing its accumulation in resistant cells [52]. However, verapamil at its highest tolerable concentration failed to enhance vinblastine efficacy in a phase I clinical trial carried out in 1985 [53]. Several years later, the immunosuppressant cyclosporine A (CsA) was shown to completely re-sensitize a resistant variant of human T-cell acute lymphatic leukemia cell line to vincristine and daunorubicin. Furthermore, CsA was also effective against doxorubicin resistance in solid tumors [54]. It was thereafter used as a benchmark for ABCB1 inhibitors for *in vitro* studies due to its high potency and low intrinsic

toxicity [55,56]. Unfortunately, similarly to verapamil in clinical trials, CsA failed to achieve clinical inhibition of ABCB1 at the concentrations tested [57–59]. More recently, CsA was also shown to block ABCG2-mediated efflux and restore drug sensitivity in ABCG2 overexpressing cells [60,61]. It is important to note that both verapamil and CsA are transported by ABCB1 and thus, they modulate the efflux function by competing for the substrate binding site(s).

After the failure of these 1st generation ABCB1 inhibitors, the quantitative structural activity relationship approach was used to generate the 2nd generation of ABCB1 inhibitors such as SDZ PSC833 (Valspodar) and S9788. SDZ PSC833 is a non-immunosuppressive CsA derivative developed in 1991, and S9788 is a triazine that was designed based on the chemical structure of verapamil [62,63]. Disappointingly, despite being much more potent than CsA in *in vitro* testing [64], serious complications arose in clinical trials when SDZ PSC833 was used in combination with anticancer drugs [65,66]. It emerged that SDZ PSC833 partially impairs drug metabolism and elimination, significantly reduces the systemic clearance of anticancer drugs and consequently elevates toxicity [65,66]. More recently, SDZ PSC833 was tested on patients with recurring or refractory multiple myeloma, but again failed to improve the treatment [67].

GF120918 (Elacridar), OC144-093 (Ontogen), XR9576 (Tariquidar) and LY335979 (Zosuquidar) are 3rd generation ABCB1 inhibitors (see Table 1). They were synthesized in an attempt to improve on the 2nd generation inhibitors [68–71] and are reported to be more selective and work in the nanomolar concentration range [72–74]. LY335979 very potently and specifically inhibit ABCB1 function [75]. It was able to reduce tumor mass and prolong survival in mice engrafted with drug resistant human tumors [75]. On the other hand, GF120918 [68,76] and the anthranilamide derivative XR9576 [72,73,77] inhibit not only ABCB1, but also ABCG2-mediated transport. GF120918 sensitized human MDR sarcoma MES-Dx5 cells and improved topotecan bioavailability in mice [28,39]. Phase I and II clinical trials have been and are being performed on some of these 3rd generation inhibitors [78–81], and results are very promising [82–84].

ABCC1 [15] and ABCG2 [23] are more recently identified ABC drug transporters. Therefore, data on them are not as extensive as that for ABCB1. In 1995, a leukotriene LTD_4 receptor antagonist MK-571 was discovered by Gekeler et al. to inhibit ABCC1-mediated transport without any effects on ABCB1 [85]. Being low in intrinsic toxicity, relatively potent and specific, MK-571 is thus still the benchmark inhibitor to block ABCC1-mediated drug transport. Soon after the discovery of ABCG2, a fungal toxin Fumitremorgin C (FTC), was shown to inhibit ABCG2-mediated transport [86]. FTC is both highly potent and specific, but with undesirable neurotoxic effects *in vivo*. Subsequent studies resulted in the development of Ko143, a new tetracyclic analog of FTC that is even more potent and specific yet non-toxic, which is ideal for future clinical studies [87].

Furthermore, many natural products have shown promising chemosensitizing effects on ABC drug transporters. Compounds such as curcumin, ginsenoside, some polyphenols and anti-malarials are derived from natural sources and show low intrinsic toxicity. Many of them demonstrate broad-spectrum modulatory effects on more than one ABC drug transporter (Table 1). For example, plant polyphenols [88–90] and curcumin [91–93] have been reported to modulate all three major ABC drug transporters: ABCB1, ABCC1 and ABCG2. However, regardless of the source of the inhibitors, unpredictable pharmacokinetic drug interactions, simultaneous involvement of several drug transporters in tumor tissues, as well as the variability in drug transporter expression levels among individuals, remain major obstacles to using modulators to restore drug sensitivity in the clinic.

ALTERNATIVE APPROACHES TO REVERSING MDR

With the lack of success in inhibiting multidrug resistance using traditional drug inhibitors, investigators have designed novel compounds to circumvent ABC transporters by a variety of mechanisms. One popular method is to target mRNA. This can be accomplished by antisense oligonucleotides, hammerhead ribozymes, and siRNA. In addition, investigators have developed transcriptional regulators, agents to alter the plasma membrane (see Fig. (1)) as well as compounds that selectively target MDR cancer cells (Table 2).

Antisense oligonucleotides

Antisense oligonucleotides are an alternative method to inhibit the expression of ABC transporters. The mechanisms by which these oligonucleotides function are complex and have not been fully elucidated [94]. Phosphorothioate oligonucleotides are the first-generation of anti-sense molecules. They are more resistant to nucleases, but may produce pharmacological effects unrelated to the anti-sense effects. One major concern regarding these molecules is cellular uptake, and investigators have demonstrated that administration with Lipofectin is necessary to obtain partial gene silencing of ABCB1 [95]. In addition, uptake enhancing modifications such as 5′ cholesterol-conjugation produced improved silencing without the need for cationic lipids. Concentrations in the low micromolar range are necessary to reduce protein levels by half [96]. Kang et al. report that chimeric hexitol nucleic acid gapmer oligonucleotides are effective at inhibiting ABCB1 gene expression at nanomolar concentrations [97].

Hammerhead ribozymes

Ribozymes are catalytic RNAs that have intrinsic endoribonucleolytic cleavage activity which can be used to target a specific mRNA at a specific position containing a NUX motif, where N is any nucleotide and X is A, C or U. Investigators have designed ribozymes to target ABCB1 [98], ABCG2 [99], and ABCC2 [100]. These three ribozymes were then combined in a multitarget multiribozyme (MTMR) containing three trans-acting hammerhead ribozymes directed against ABCB1, ABCG2 and ABCC2, three cis-acting ABCB1 ribozymes, and three ABCB1 homologous spacer sequences [101]. This self-contained MTMR undergoes autocatalytic self-cleavage by the *cis*-acting ribozymes to free the trans-acting ribozymes to act on the targeted transcripts. This novel approach was able to cleave the ABC transporter-specific transcripts in drug-resistant cancer cell lines.

RNA interference (siRNA)

Small interfering RNAs (siRNAs) are used to target ABC transporters at the mRNA level. This double-stranded RNA, normally between 19–21 nucleotides in length, is designed to enhance the degradation of the single-stranded RNA sequence of the desired gene. Dicer, the RNAse III enzyme, processes the double-stranded RNA into siRNA that incorporates into the multiprotein RNA-induced silencing complex (RISC) which cleaves the targeted mRNA [102]. siRNA possesses several advantages over antisense oligonucleotides, which include ease of delivery, lower concentrations needed for gene silencing, and the ability to silence genes at any stage in development. The transient silencing effects of siRNA are however, one drawback of this technology. Investigators have shown that both synthetic and vector-based expression of siRNA can specifically decrease expression of ABCB1 and ABCB4 in paclitaxel-resistant ovarian cancer cells [103]. siRNA has also been utilized to modulate expression of ABCC2 and ABCC3 in primary hepatocytes [104], ABCG2 in BeWo cells [105], and ABCB1 using a combination of siRNAs [106]. The half-life of the ABCB1 mRNA (4 hours), and protein (16 hours) [107], allows for an increase in transcript and protein level to original levels 7 days after siRNA administration. To extend the silencing effects of siRNA, others have engineered an H1-RNA gene promoter-driven expression vector encoding anti-ABCB1 [108], anti-ABCC2 [109] and anti-ABCG2 [110] short hairpin RNA (shRNA), which shows the highest

efficacy to date in gene-silencing. The efficiency of gene knock-down depends on the delivery into the cell, and studies report that an adenoviral-based delivery of shRNA is superior to adenoviral delivery of ribozymes [111]. Others studies show that attenuated *Salmonella typhimurium* has potential as an in vivo delivery vector for ABCB1 siRNA in a human tongue squamous cell cancer mouse model [112]. In addition, a transposon-based Sleeping Beauty (SB)-based RNAi system produces stable and durable silencing of ABCB1 [113]. This non-viral siRNA transposon-based SB vector was utilized to show that silencing of ABCB1 causes increases in imatinib intracellular levels in chronic myeloid leukemia cells [114] and that two proteasome inhibitors used to treat relapsed or refractory multiple myeloma are substrates for ABCB1 [115]. Investigators have used a retroviral-mediated shRNAi for ABCB1 *in vivo* and provided documentation of the effect in the intact animal using bioluminescence [116]. Stein *et al*. have recently reported a complete reversal of the MDR phenotype *in vivo* using an intratumoral jet-injection of anti-ABCB1 short hairpin RNA-encoding plasmid DNA [117].

Transcriptional regulation

Investigators have also identified a number of transcriptional regulators of ABC transporters. For instance, transcriptional decoys have been employed to decrease drug resistance. In one such study, investigators used phosphorothioate-modified antisense oligonucleotides targeted at the transcriptional MED-1(Multiple start site Element Downstream) element of the human *ABCB1* gene promoter to modulate multidrug resistance [118]. The human MED-1 *cis*-element is unique because it has an inverted sequence compared to the consensus MED-1 sequence in other TATA-less promoters which allows for targeted silencing of the *ABCB1* MED-1. Investigators have also utilized *LANCL2,* a gene overexpressed in 20% of all glioblastomas, which transcriptionally suppresses *ABCB1* due to decreased *ABCB1* promoter activity [119]. Others have constructed the K2-5F repressor consisting of five Zif DNA-binding domains directed against the *ABCB1* promoter (SP1/EGR1/WT1) and two KRAB-A domains [120]; ponasterone induction of K2-5F caused reduction in cell surface expression, in total protein and in mRNA of ABCB1 [121]. Scala et. al. showed that 8-CL-c-AMP, a type I cAMP-dependent protein kinase (PKA) inhibitor, downregulated ABCB1 expression in a dose dependent manner, indicating a role for PKA in ABCB1 promoter activity [122]. Inhibition of Protein Kinase C (PKC) can also prevent ABCB1 upregulation by extracellular stimuli [123]. A natural marine product, Et743, is an inhibitor of ABCB1 transcription due to blockade of promoter activation [124]. Lastly, NF-κB has also been implicated in ABCB1 regulation [125]. Some researchers believe that ABCC1 is down regulated by p53, possibly by reducing Sp1 binding [126] while others have reported that the transcription factor complex AP-1 regulates ABCC1 [127].

Plasma membrane alterations

There are also agents that modify the plasma membrane and thus prevent ABCB1 overexpression. Fatty acid-polyethylene glycol fatty acid diesters have been used to block multidrug resistance in Ara C-treated cells [128]. These diesters work on the cell surface and were able to prevent both short-term ABCB1 expression and the multidrug resistance phenotype in cells that survived the initial treatment with chemotherapeutics.

Encapsulation of drugs

Enhancements in drug delivery systems have also been used to circumvent ABC transporters. One such advance is the use of encapsulation to deliver drugs in a tumor site-directed manner. For instance, investigators have developed poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticles to co-administer paclitaxel and ceramide, an apoptosis modulator [129]. Nanoparticles with paclitaxel and ceramide were able to sensitize multidrug resistant cells to the same concentrations of paclitaxel to which the drug-sensitive cells were susceptible.

Liposomal formulations of doxorubicin have also been successfully developed to bypass ABC transporters [130].

Antibodies

Antibodies have been used to combat multidrug resistance. Palmitoylated synthetic peptides of the extracellular loops of ABCB1 were reconstituted in liposomes with or without Lipid A, then resuspended in alum [131]. Interestingly, the mice did not show auto-immune symptoms; nevertheless, immunization increased survival half-time by 77% and efficacy of chemotherapy against P388 R cells. *In vitro* studies of such cells administered the sera of immunized mice showed similar cytotoxic results.

Drugs that are not substrates for the major ABC drug transporters

Presently, the pharmaceutical industry and the Food and Drug Administration (FDA) are aware of the alterations that ABC transporters can cause in the pharmacokinetics as well as in the efficacy of drugs. Thus, potential drug compounds are commonly tested to determine if they are substrates for ABC transporters. Consequently, medicinal chemists have been synthesizing analogues of drugs to circumvent multidrug resistance mediated by ABC transporters. For instance, camptothecin analogs were designed and tested for ABCG2 substrate specificity [132]. Compounds with low polarity were not found to be substrates for either ABCG2 or ABCB1. In addition, drugs designed to be substrates for solute carriers, a superfamily of membrane transport proteins (SLCs) recently shown to be important in the pharmacokinetics of drugs [133], may facilitate the uptake of chemotherapeutics and avoid MDR. The role of solute carriers in cancer chemotherapy has recently been reviewed elsewhere [133]. However, there are no conclusive experimental data available yet on overexpression of solute carriers in drug resistant tumor tissue.

COMPOUNDS SPECIFICALLY TARGETING MDR CANCER CELLS

Despite tremendous efforts to develop inhibitors (both specific and broad-spectrum) and to discover natural products modulators of ABC drug transporters, there are still no inhibitors currently used in clinical treatment. Verapamil, one of the first ABCB1 modulators found to effectively reverse MDR mediated by ABCB1, was also one of the first compounds to show high collateral toxicity towards ABCB1-overexpressing Chinese hamster ovary cells. This “hypersensitivity” or “collateral sensitivity” provides researchers with an alternative approach that can be used to combat MDR caused by the overexpression of ABC drug transporters. Numerous research groups are searching for compounds with substantial collateral toxicity towards MDR cell lines which can induce apoptosis more specifically in MDR cell lines. These compounds show potential for use in the clinic, either alone or in combination with already existing cancer chemotherapeutics. Examples of compounds exhibiting collateral sensitivity are listed in Table 2.

In 1987, Cano-Gauci and Riordan discovered that ABCB1-overexpressing Chinese hamster ovary (CHO) cells were hypersensitive to calcium channel blockers such as verapamil, nifedipine and the calmodulin inhibitor trifluoperazine [134]. This hypersensitivity is both calcium-independent and independent of intracellular verapamil accumulation [134]. Subsequent studies by Warr et al. in 1988 demonstrated that in addition to calcium channel blockers, vincristine-resistant CHO cells are also hypersensitive to the membrane active agent quinidine sulfate, suggesting that calcium channels are not the primary target of this hypersensitivity or collateral toxicity [135]. More recently, Karwatsky et al. focused on the mechanistic aspect of the observed hypersensitivity of MDR CHO cells to verapamil [136]. It was concluded that apoptosis was caused by elevating the production of reactive oxygen species (ROS) in MDR cells. Moreover, this collateral toxicity was correlated with high

ATPase activity, independent of p53 activity, and could be inhibited by the overexpression of Bcl-2 [136].

Recently, Ludwig et al. identified a small molecule thiosemicarbazone, NSC73306, which can induce cytotoxicity specifically in cancer cells overexpressing functional ABCB1 [137]. The hypersensitivity is correlated directly with both the increased ABCB1 function and MDR. Moreover, culturing MDR cells in the presence of NSC73306 resulted in the loss of ABCB1 expression and consequent loss of the MDR phenotype. Despite the correlation of NSC73306 cytotoxicity with the expression and function of ABCB1, there is no evidence to show direct interaction of NSC73306 with ABCB1. Considering that NSC73306 also possesses divalent metal chelation properties and that this effect is not limited to Pgp-expressing CHO cells, it is likely to have a distinct mechanism of collateral sensitivity unlike that of verapamil, even though the final phenomenon is somewhat similar. Similarly, Heffeter et. al. discovered a new lanthanum compound, KP772, with anticancer properties in both *in vitro* and *in vivo* assays [138], which also has preferential cytotoxicity towards cancer cells overexpressing either ABCB1 (Pgp), ABCC1 (MRP1) or ABCG2 [139]. It was suggested that KP772 induces significantly more apoptosis and cell cycle arrest in cells overexpressing these ABC transporters, explaining hypersensitivity in MDR cancer cells. These observations are consistent with those of Nicholson et al., who reported that a phosphatidylinositol-3-kinase inhibitor (LY294002) induced apoptosis preferentially (6-fold higher) in ABCB1-overexpressing KB-V1 cells [140] compared to drug-sensitive cells. Interestingly, comparably to NSC73306, long term exposure to KP772 resulted in the loss of ABCB1 protein expression in ABCB1-overexpressing cells, and no evidence of direct interaction of KP772 with these ABC transporters was established. Similarly, Bergman et al. observed increases in sensitivity to gemcitabine by both ABCB1- and ABCC1-overexpressing cells, which could be abolished completely by verapamil [141]. It was suggested that this hypersensitivity is due to enhanced cellular stress caused by the overexpression of ABCB1 or ABCC1, resulting in increased gemcitabine metabolism and sensitivity in these MDR cells.

In addition to the above-mentioned compounds, several compounds similar to 5-fluorouracil induce significantly greater apoptosis in MDR carcinoma cells than in drug-sensitive cells after 48 hours of exposure [142]. More recently, celecoxib, a specific inhibitor of COX-2 activity, was also shown to have the same property. Fantappie et al. demonstrated that in MDR cells celecoxib reduces expression of ABCB1, Bcl-x_L, and Bcl-2. Celecoxib also induces the translocation of Bax from the cytosol to the mitochondria and cytochrome c into the cytosol, which in turn activates caspase-3-dependent apoptosis [143].

By using a developed method that correlates the activity of candidate anticancer drugs with gene expression of ABCB1 in 60 diverse cancer cell lines used by the National Cancer Institute (NCI-60) [144], it is now possible to identify compounds that are selectively cytotoxic to ABCB1-overexpressing cancer cells. Though the exact mechanism(s) of these compounds is still unclear, several very different, but plausible mechanisms have been proposed. These agents may interfere with the PI3-kinase/PKB pathway, in turn affecting downstream intracellular signaling pathways and inducing apoptosis in MDR cells [140], or they may induce apoptosis by enhanced production of ROS in MDR cells [136]. It is feasible that different compounds have different mechanisms to achieve the same result.

CONCLUSIONS

ABC transporters provide the body vital protection from foreign compounds; however, their overexpression in cancer cells poses a major obstacle to cancer therapy. This is the main cause of treatment failure in cancer patients. Due to the clinical impact of MDR, investigators continue to search for a safe yet effective inhibitor of these transporters. Although some success

has been seen *in vitro*, until now, these results have not been translated to the clinic. A new wave of compounds that have potent collateral toxicity towards MDR cells may serve as the long sought treatment of MDR in cancer chemotherapy. By advances in understanding the molecular pharmacology of these compounds, especially their effects on signaling pathways, successful *in vivo* inhibition of MDR may soon be realized.

Acknowledgements

This research was supported by the Intramural Research Program of the National Cancer Institute, NIH, Center for Cancer Research. AMC was supported by the NIGMS Pharmacology Research Associate (PRAT) Program. We thank Dr. Zuben E. Sauna for his helpful suggestions and Mr. George Leiman for his editorial assistance.

Abbreviations

ABC
: ATP-binding cassette

CsA
: cyclosporine A

FTC
: Fumitremorgin C

MDR
: multidrug resistance

MRP
: multidrug-resistance protein

Pgp
: P-glycoprotein

TMD
: transmembrane domain

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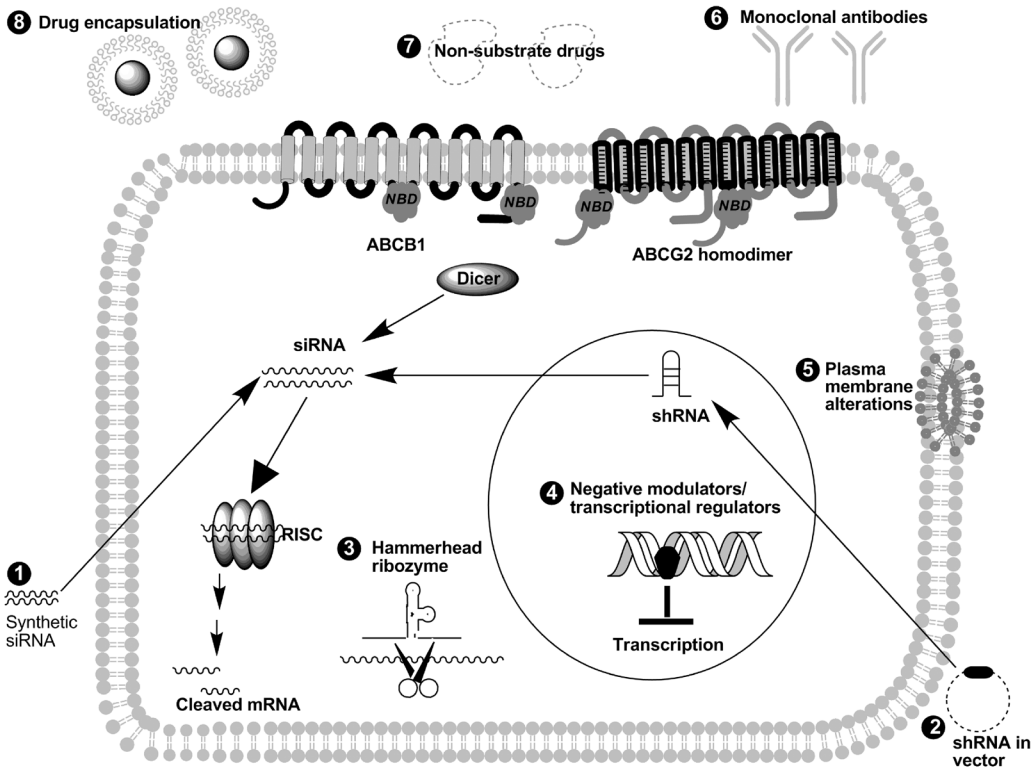

Fig. 1. Schematic of the various methods used to circumvent MDR mediated by ABC transporters

Gene silencing of ABC transporters can be performed using synthetic siRNA(1), shRNA in a vector (2), and hammerhead ribozyme (3). Numerous negative modulators/transcriptional regulators (4) can inhibit transcription of these transporters in the nucleus. MDR can also be modulated by plasma membrane alterations (5). In addition, monoclonal antibodies (6), non-substrate drugs (7), and drug encapsulation (8) have also been used to evade MDR in cancer cells.

Table 1

Inhibitors of major ABC drug transporters, ABCB1 (Pgp), ABCC1 (MRP1) and ABCG2

Modulator	ABC drug transporter(s) affected	Reference
Verapamil	ABCB1 ABCC1	[51] [145]
Cyclosporine A	ABCB1 ABCC1 ABCG2	[55,56] [145] [60,61]
SDZ PSC 833	ABCB1	[63,64]
S9788	ABCB1	[62]
MS-209	ABCB1 ABCC1	[146] [145,147]
Biricodar/VX-710	ABCB1 ABCC1 ABCG2	[148,149] [145] [149]
GF120918/Elacridar	ABCB1 ABCG2	[68] [76]
OC-144-093/ONT-093	ABCB1	[69]
Tariquidar/XR 9576	ABCB1	[70,73]
LY 335979/Zosuquidar	ABCB1	[150]
Laniquidar/R101933	ABCB1	[151]
Mitotane	ABCB1	[152]
WK-X-34	ABCB1 ABCG2	[153] [154]
Annamycin	ABCB1 ABCC1	[155] [156]
MK-571	ABCC1	[85]
Fumitremorgin C	ABCG2	[86]
Ko143	ABCG2	[87]
PK11195	ABCB1 ABCC1 ABCG2	[157] [157] [157]
Disulfiram	ABCB1 ABCC1	[158] [158]
Antimalarials: (Quinine, quinidine, mefloquine, chloroquine, primaquine, pyronaridine)	ABCB1 ABCC1	[159–161] [162,163]
Polyphenols: (Quercetin, silymarin, hesperetin, daidzein; resveratrol, naringenin, genistein)	ABCB1 ABCC1 ABCG2	[90] [89,164] [88,165]
Curcumin	ABCB1 ABCC1 ABCG2	[91] [93] [92]
Ginsenoside	ABCB1 ABCG2	[166] [167]

Table 2

Compounds that selectively target cells overexpressing ABC drug transporters

Compound (Reference)	Class of compound	Cell lines	Transporter overexpressed
Verapamil/nifedipine/calmodulin inhibitor Trifluoperazine [134–136]	Calcium channel blockers	CHO MDR cells	ABCB1
Verapamil (*S*-isomer) [168,169]	Calcium channel blocker	BHK-21	ABCC1
Bisdioxopiperazine dexrazoxane/ICRF-187 [170]	Topoisomerase II inhibitor	K/VP.5	ABCB1
Tunicamycin [171]	N-linked glycosylation inhibitor	KB-C-1	ABCB1
Gemcitabine [172]	Deoxycytidine analog	H69/DAU; NYH/VM	ABCB1
Gemcitabine [141]	Deoxycytidine analog	2R120; 2R160; SW1573/S1; SW1573/S1; GLC4/ADR; 2780AD; KB-8-5; BROmdr	ABCB1; ABCC1
Cytosine arabinoside [172]	Deoxycytidine analog	H69/DAU; NYH/VM	ABCB1
5-fluorouracil [142]	Thymidylate synthase inhibitor	KB-C-1; KB-V-1; KB-A-1	ABCB1
LY294002 [140]	PI3-kinase inhibitor	KB-V-1	ABCB1
NSC73306 [137]	Thiosemicarbazone	KB-V-1; KB-8-5; KB-8-5-11; NCI/ADR-RES	ABCB1
KP772/FFC24 [139]	Lanthanum compound	GLC4/ADR; HL60/ADR; KB-C-1; MCF-7/bcrp	ABCB1; ABCC1; ABCG2
Celecoxib [143]	COX-2 inhibitor	HT-29-dx; PN1A-NIH3T3	ABCB1
NBDHEX [173]	Glutathione-S-transferase inhibitor	CEM-VBL10; CEM-VBL100; U-2 OS/DX	ABCB1