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Overcoming drug resistance by regulating nuclear receptors

Taosheng Chen
Department of Chemical Biology & Therapeutics, St. Jude Children's Research Hospital, TN 38105, USA

Abstract

Drug resistance involves multiple mechanisms. Multidrug resistance (MDR) is the leading cause of treatment failure in cancer therapy. Elevated levels of MDR proteins [members of the ATP-binding cassette (ABC) transporter family] increase cellular efflux and decrease the effectiveness of chemotherapeutic agents. As a salvage approach to overcome drug resistance, inhibitors of MDR proteins have been developed, but have had limited success mainly due to undesired toxicities. Nuclear receptors (NRs), including pregnane X receptor (PXR), regulate the expression of proteins (including MDR proteins) involved in drug metabolism and drug clearance, suggesting that it is possible to overcome drug resistance by regulating NR. This review discusses the progress in the development of MDR inhibitors, with a focus on MDR1 inhibitors. Recent development of PXR antagonists to pharmacologically modulate PXR is also reviewed. The review proposes that selectively preventing the elevation of MDR levels by regulating NRs rather than non-selectively inhibiting the MDR activity by using MDR inhibitors can be a less toxic approach to overcome drug resistance during cancer therapy.

Keywords

Drug resistance; MDR1; PXR; CYP3A4; ABC transporters; Drug-metabolizing enzymes

1. Introduction

Drug resistance – the reduction in effectiveness of a drug in curing a disease or improving patient symptoms – can develop against antibiotics, antivirals, or chemotherapeutic agents for cancers. Drug resistance is a complex cellular response and target-specific and target-nonspecific mechanisms can be involved in the process.

In target-specific drug resistance, changes in a specific drug target that decrease the interaction between the target and drug might lead to drug resistance. For example, mutations in viral genes frequently lead to antiviral drug resistance [1], and loss of expression of the estrogen receptor (ER) can cause tamoxifen resistance in patients with breast cancer [2]. It can be difficult to predict, prevent, or overcome target-specific drug resistance without developing new therapeutic agents. On the other hand, in target-nonspecific drug resistance, changes in parameters not directly relevant to or dependent on the drug target contribute to drug resistance. For example, target cells or organisms might

Corresponding author: Taosheng Chen, Department of Chemical Biology & Therapeutics, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA, Taosheng.Chen@STJUDE.ORG, Tel: +1 901-595-5937, Fax: +1 901-595-5715.

produce higher levels of drug-metabolizing enzymes (DMEs) to degrade the drug or increase their efflux capacity, resulting in decreased bioavailability and reduced effectiveness of drug [3].

Cases of target-nonspecific drug resistance have several features in common, which have been targeted by various approaches in order to overcome drug resistance, especially against chemotherapeutic agents. For example, a family of ATP-dependent drug pumps, known as ATP-binding cassette (ABC) transporter proteins, can increase the resistance to chemotherapeutic agents by increasing cellular efflux. Multidrug resistance (MDR) proteins belong to the ABC transporter protein family and play an important role in maintaining normal physiologic functions that protect human tissues from drugs and other xenobiotics. Elevated levels of MDR1, a key MDR protein [also known as P-glycoprotein (P-gp) or ABCB1], have been associated with drug-mediated drug resistance in cancer [4], making inhibition of MDR1 activity a logical approach to overcome MDR1-mediated drug resistance.

This review discusses the progress made in the development of MDR1 inhibitors in overcoming drug resistance in cancer. As the primary role of MDR1 is disposition of xenobiotics, the undesired toxicities resulting from the use of MDR1 inhibitors have posed a challenge in the development of MDR1 inhibitors for clinical applications. The problems encountered and the lessons learned in developing MDR1 inhibitors as salvage therapies to reverse drug resistance are reviewed.

The expression of MDR1 as well as other proteins involved in regulating the bioavailability of drugs is regulated by nuclear receptors (NRs), a family of ligand-activated transcription factors. The pregnane X receptor (PXR) is an NR that directly regulates the expression of MDR1 and other important proteins involved in drug metabolism and resistance. PXR can be activated by xenobiotics, including drugs involved in MDR, suggesting that drug resistance can be prevented instead of being reversed. The recent progress made in developing PXR antagonists to pharmacologically modulate PXR and thereby potentially prevent the elevation of MDR1 levels is also reviewed.

Recently, a new form of MDR – drug ratio–dependent MDR – has been reported in cancer therapy, which occurs at discrete drug:drug ratios of combined chemotherapeutic agents. Drug ratio–dependent MDR can be circumvented by systematically screening a wide range of drug ratios and concentrations and encapsulating the drug combination in a liposomal delivery vehicle at optimal synergistic ratios. This has been recently reviewed [5], and will not be discussed here.

2. Drug resistance in anticancer therapies

2.1 Cancer and drug resistance

Despite years of intensive research and development, cancer remains one of the leading causes of death worldwide. In 2009, there were an estimated 1.5 million new cases of and 560,000 deaths from cancer in the US [6]. Chemotherapy is the most commonly used treatment for cancer, as surgery and radiation are often not effective in treating cancer at every location where it spreads. MDR of cancer cells to chemotherapeutic agents – a complex cellular process – is the leading cause of failure of chemotherapy and the rise in cancer-related deaths [7].

A common feature among cases of resistance to anticancer drugs is the dynamic interactions among cancer cells, the human body (the “host”) that governs the systemic drug clearance, and the therapeutic agent (Fig. 1), which can be used to develop target-nonspecific

approaches to address resistance to chemotherapeutic agents. To a healthy human body, xenobiotics or drugs are external stresses and these are disposed of via a highly regulated drug metabolism and drug clearance process. During this process, DMEs in the liver break down the drug, and NRs such as PXR and the therapeutic drugs play crucial roles in regulating the expression of DMEs [8,9]. Recently, cancer cells have also been shown to affect drug clearance by affecting the expression of DMEs [10].

2.2 Proteins involved in resistance to cancer drugs

Changes in the expression levels of DMEs that break down drugs and ABC transporters that increase cellular efflux of chemotherapeutic agents have been associated with drug resistance in many cancers [7]. Among the 48 known human ABC transporters, MDR1, the multidrug resistance-associated protein 1 (MRP1; also known as ABCC1), and the breast cancer resistance protein (BCRP; also known as ABCG2) are major contributors to the MDR phenotype. There have been intensive investments in developing compounds that can reverse the MDR phenotype. Although laboratory research has led to promising results, efforts to translate them to clinical use have been somewhat disappointing (see sections 2.3 and 2.4 for details).

2.3 Approaches used to overcome cancer drug resistance

MDR1 is known to transport several cancer drugs [7], and its activity can be pharmacologically inhibited to prevent the efflux of cancer drugs and sensitize resistant cancer cells to cancer drugs both *in vitro* [11] and in the clinical setting [12]. These early data suggested that MDR1 can be a feasible target to reverse drug resistance, which was supported by the observation that loss of both *Mdr1a* and *Mdr1b* (there are 2 rodent *Mdr1* genes but only 1 human *MDR1* gene) does not result in an obvious phenotype. Significant efforts have since led to the development of 3 generations of MDR inhibitors.

First-generation MDR1 inhibitors are compounds that have already been approved by the Food and Drug Administration (FDA) for other clinical applications. These non-specific MDR1 inhibitors, such as verapamil, quinine, and cyclosporine A, generally fail to show clinical efficacy, mainly because they have toxic side effects at doses required to inhibit MDR1 activity [13]. However, a few positive outcomes [14] encouraged the development of second-generation MDR1 inhibitors, and efforts were centered on increasing the potency for MDR1 while decreasing toxicities, using pharmacophores of the first-generation MDR1 inhibitors. PSC-833, a cyclosporine D analog with high-affinity for MDR1 and no immunosuppressive side effects, is representative of second-generation MDR1 inhibitors. However, the inhibition of MDR1 decreased the systemic clearance of drugs and increased the exposure of both normal and cancerous tissues to the toxic effect of drugs. In addition, PSC-833 and other MDR1 inhibitors inhibited cytochrome p450 3A (CYP3A) function and decreased CYP3A-mediated drug metabolism. These undesired pharmacokinetic interactions led to drug-associated adverse effects. Therefore, although PSC-833 enhanced the therapeutic effect of certain chemotherapeutic drugs (e.g., etoposide, cytarabine, and daunorubicin) in patients with acute myeloid leukemia (AML) [15], its use was associated with high rates of mortality in other phase III trials [16], and its development was therefore discontinued. The development of another second-generation MDR1 inhibitor, biricodar, was discontinued because of similar adverse effects [17]. Efforts to develop third-generation MDR1 inhibitors have focused on increasing the affinity for MDR1 and lowering pharmacokinetic interactions (i.e., not inhibiting CYP3A function and normal CYP3A-mediated drug metabolism). Therefore, unlike first- and second-generation MDR1 inhibitors, which were developed from compounds known to target other biologic functions, third-generation MDR1 inhibitors are derived from new compounds generated by combinatorial chemistry. Laniquidar, OC144-093, zosuquidar, elacridar, tariquidar and

CBT-1 are examples of third-generation MDR1 inhibitors that have a high affinity for MDR1 without having a CYP3A inhibitory effect [7]. Tariquidar was being tested in phase III clinical trials as adjunctive therapy in combination with first-line chemotherapy in patients with non-small-cell lung cancer (NSCLC), but was discontinued because of treatment-associated toxicities. It is important to note that the rationale for choosing patients with NSCLC in the studies was not clear, since there was no convincing data suggesting that the target of tariquidar, MDR1, is significantly expressed in NSCLC. In addition, the dose used for the combination therapy was higher than the maximum tolerated dose previously determined [18]. Newly exploratory trials with tariquidar are currently ongoing; zosuquidar is also being tested in phase II trials in women with metastatic and locally recurrent breast cancer [19].

Some third-generation MDR1 inhibitors are less toxic, do not affect the pharmacokinetics of anti-cancer drugs, and have better outcomes in clinical trials than first- and second-generation MDR1 inhibitors. In addition to chemical inhibitors, other MDR-reversing agents aimed at inhibiting the activity of MDR, including antibodies, have been developed [7]; however, whether the activity of MDR1 can be inhibited without causing undesired toxicity remains unclear.

2.4 Lessons learned

First- and second-generation MDR1 inhibitors have been developed based on compounds previously discovered to act on targets other than MDR1. These non-specific MDR1 inhibitors also inhibited the activity of CYP3A, affected drug metabolism and clearance, and failed in clinical trials due to undesired toxicity. Third-generation MDR1 inhibitors that are specific and potent for MDR1 and devoid of CYP3A inhibitory effect have been developed. Again, early trials in clinics failed due to undesired toxicities. The inappropriate study design of earlier trials on third-generation MDR1 inhibitors might have contributed to the failure of these trials; therefore, with appropriate study design, the approach to develop reversing agents for ABC drug transporters might have an optimistic future, suggesting that overcoming drug resistance by down-regulating MDR1 remains a feasible strategy [7]. Overcoming drug resistance by countering the elevated levels of MDR1 (due to drug-mediated over-expression) is a salvage approach. MDR1 is constitutively expressed in many normal tissues (e.g., adrenal gland, liver, kidney, intestinal mucosa, muscle, and endothelial cells of the blood brain barrier [20]) and plays an essential role in protecting normal tissues from drugs and other xenobiotics. MDR1 is over-expressed in cancer cells and causes drug resistance. MDR1 inhibitors inhibit the activity of MDR1, regardless whether it is the drug-mediated over-expressed MDR1 (which causes drug resistance) or the constitutively-expressed MDR1 (which is required for normal protecting function). To date, it has not been possible to avoid the toxicities associated with inhibition of MDR1 activity, so it remains to be studied whether drug-mediated over-expression of MDR1 can be selectively prevented. Studies on the regulation of MDR1 expression can help address the question of whether drug-mediated over-expression of MDR1 can be prevented.

The expression of MDR1 is regulated at the transcriptional level by multiple signaling mechanisms, including those mediated by hypoxia-inducible factor-1α (HIF-1α) [21], p53 [22], and even chromosomal rearrangement [23]. MDR1 expression is also regulated by epigenetic mechanisms such as methylation [24,25] and acetylation [26]. Post-transcriptional regulation of MDR1 expression by microRNA has been reported recently [27,28].

Recently, the expression of MDR1 has been shown to be regulated by xenobiotic receptor PXR [29–31], suggesting a role of NRs in regulating inducible drug resistance and a

possible new strategy to overcome drug resistance by preventing the induction of MDR1 over-expression during drug therapy instead of inhibiting the activity of total MDR1.

3. Nuclear receptors and drug resistance

3.1 Regulation of drug resistance by nuclear receptors

MDR1, MRP1, and BCRP – the ABC transporters that mediate the ATP-dependent cellular export of drugs – have high expression levels in liver, intestine, kidney, and blood-brain barrier. Their normal physiologic function is to protect the body from cytotoxicity caused by drugs or other xenobiotics. This protecting function is coordinated with the DMEs, which first break down the drugs in most cases. MDR1, MRP1, and BCRP, which partially overlap in their substrate specificity, are the major ABC transporters involved in cancer drug resistance. MDR1 was the first ABC transporter identified in Chinese hamster ovary cells selected for resistance to the cytotoxic agent colchicine [32]. MRP1 was discovered in a multi-drug-resistant human lung cancer cell line [33] and BRCP in a multi-drug-resistant human breast cancer cell line [34].

There is only 1 gene for *MDR1* in humans, but 2 genes (*Mdr1a* and *Mdr1b*) in rodents [35]. MDR1, which was first discovered as a protein associated with cancer cell resistance to cytotoxic compounds [32], was subsequently found to be expressed in normal cells from various tissues [36–39] and playing key roles such as elimination of drugs from the system by exporting drugs into the lumen of the gut [39], biliary excretion in the liver [39,40], renal elimination [41], and limiting drug uptakes into the central nervous system (CNS) [42–45]. MDR1 transports a broad range of hydrophobic compounds, including anticancer drugs, anti-HIV drugs, antibiotics, cardiac drugs, calcium channel blockers, and immunosuppressants [7,46–48].

There are 13 MRPs in humans. MRP1 was found to be amplified in multiple drug-resistant cancer cells [33]. MRP1 transports anticancer cytotoxic drugs [47,49]. BCRP also confers resistance to many anticancer drugs [49,50].

NRs have been shown to regulate the expressions of *MDR1* and *BCRP* at the transcription level. PXR [29,31] and constitutive androstane receptor (CAR) [51] bind to and activate the promoter of *MDR1*. The promoter of *BCRP* contains response elements for both ER [52] and proliferator-activated receptor γ (PPARγ) [53], suggesting the role of NR in regulating *BCRP* expression. Whether NRs regulate the expression of *MRP1* is unknown. The regulation of the *MDR1* expression by PXR has been well-studied, and is the focus of this review.

3.2 PXR and drug resistance

PXR and CAR are master xenobiotic receptors that regulate the expression of genes involved in drug metabolism and clearance, including DMEs and transporters. Although no physiologic ligand has been definitively identified for PXR, PXR can bind to many structurally diverse chemicals (a characteristic referred to as “ligand promiscuity”), including anticancer drugs such as paclitaxel [54–57]. PXR is expressed not only in normal tissues such as liver, intestine, colon, kidney, brain [58–61], breast [62], prostate [63], peripheral mononuclear blood cells [64,65], heart, bone marrow, spinal cord [66], stomach, ovary, placenta [58,67] and the immune cells [68], but also in many human cancers, including breast [62,69], prostate [63], colon [70], osteosarcoma [71], ovarian [72], and endometrial [73,74] cancers. Activation of PXR induces expression of DMEs and transporters, including MDR1, suggesting a significant role of PXR in cancer drug resistance.

NRs are ligand-activated transcription factors that regulate target gene activation [75,76]. PXR, a member of the NR superfamily, was discovered in 1998 by multiple groups [59,60,77,78]. Similar to other NRs, PXR has a highly variable N-terminal domain, a conserved DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) (Fig. 2).

Although the sub-cellular localization of un-liganded PXR remains controversial [79–82], it is clear that PXR binds to the promoter of its target gene as a heterodimer with retinoid X receptor α (RXRα) [75,83]. The consensus sequence, 5′ AG(G/T)TCA 35′, that the PXR DBD interacts with [77,78] can be arranged as direct repeats separated by 3–5 nucleotides (DR3, DR4, or DR5), everted repeats separated by 6 or 8 nucleotides (ER6 or ER8), or inverted repeats separated by 6 or no nucleotides (IR6 or IR0). Two most important PXR target genes, *CYP3A4* and *MDR1*, contain DR3/ER6 [78,84] and DR4/ER6 [31] in their promoter regions, respectively.

Depending on the ligand-regulated conformation of the LBD, the activation function 2 (AF-2) region interacts with either corepressors or coactivators, resulting in transcriptional repression or activation [75,76]. Example of coactivators are steroid receptor coactivator-1 (SRC-1), glucocorticoid receptor interacting protein 1 (GRIP1), activator for thyroid hormone and retinoid receptors (ACTR), and PPARγ coactivator 1-α (PGC-1α) [60,77,78,85]. Nuclear receptor corepressor (NCoR) and silencing mediator of retinoid and thyroid hormone receptors (SMRT) are corepressors that regulate PXR [81,86]. In the absence of PXR agonist, PXR associates with corepressors, resulting in transcriptional repression. The binding of an agonist to PXR changes its conformation, allowing coactivators to interact with the AF-2 and resulting in transcriptional activation of the target genes of PXR [75]. The ultimate outcome of transcriptional activation of a target gene for PXR depends on the PXR agonist, the promoter of the target gene for PXR, and the specific tissue- and cellular context (availability of corepressors and coactivators, cell cycle status, etc.) [71,87].

Because of its unique structure of the LBD [88,89], PXR is a “promiscuous” xenobiotics receptor that can bind to a wide variety of structurally and chemically diverse compounds [90]. Endobiotics such as endogenous steroids and bile acids [60,77,78], cholesterol, and metabolites [91] have been shown to activate PXR. In addition, xenobiotics such as antibiotics rifampicin [59], cholesterol-lowering agent SR12813 [59], anticancer drug paclitaxel [55], anti-HIV drugs, and calcium channel modulators [92], are among an expanding list of drugs that can bind to and activate PXR. The activation of PXR is likely to affect the effectiveness of many drugs.

It has been clearly demonstrated that PXR directly regulates the transcriptional activation of *MDR1*. Geick *et al*. [31] first identified a distal enhancer region −7.8 kb from the transcriptional start site of the *MDR1* promoter that mediates the induction of *MDR1* expression by rifampicin. By using LS174T, a colon cancer cell line that expresses PXR, and a reporter gene under the control of the *MDR1* promoter, Geick *et al*. showed that the promoter region between −8.0 and −7.7 kb mediates the induction by rifampicin. An electrophoretic mobility shift assay (EMSA) confirmed the binding of PXR/RXRα to 3 DR4 (I, II, and III) and an ER6/DR4(III). Mutational analysis demonstrated that DR4(I) is essential for the rifampicin-mediated induction of *MDR1* in LS174T cells. These studies elucidated the molecular mechanism responsible for PXR-mediated induction of *MDR1* expression by rifampicin. Geick *et al*. subsequently demonstrated that *MDR1* is also regulated by CAR, through the DR4(I), and, to a lesser extent, the ER6/DR(III) [51]. Interestingly, CAR also binds to the DR(II) as a monomer. Both DR4(I) and DR4(II) are required for the maximal induction of *MDR1* by CAR.

The expression of *MDR1* and *CYP3A4* is induced by PXR agonists both *in vivo* [93,94] and *in vitro* [95]. Although the level of *in vitro* induction of *MDR1* and other transporters is lower than that of *CYP3A4* and other *CYP*s [95], the *in vivo* inductions of *CYP3A4* and *MDR1* are similar [93,94,96]. The difference in *in vitro* induction might result from the significantly reduced basal expression level of *CYP3A4* and other *CYP*s, but not that of *MDR1* and other transporters, in *in vitro* systems [95,97]. These observations indicate that PXR-mediated induction of MDR1 plays important roles in modulating drug clearance, and also suggest that *in vitro* systems (e.g., cancer cell lines or isolated primary hepatocytes) might be suitable to study the regulation of *MDR1* before conducting *in vivo* experiments.

The significant role of induction of MDR1 in increasing drug clearance has also been illustrated in human volunteers treated with various PXR agonists and MDR1 substrates. PXR agonists such as rifampicin, St. John's Wort, and carbamazepine can induce the expression of intestinal MDR1 and decrease the bioavailability and plasma levels of compounds transported by MDR1 (e.g., digoxin, talinolol, and fexofenadine) [93,94,98–101]. Rifampicin can also increase digoxin clearance into bile [39], and carbamazepine can increase the renal clearance of talinolol [99]. To emphasize the role of transporters in drug clearance, digoxin, talinolol, and fexofenadine were chosen in these studies because they are transported by MDR1 but minimally metabolized by DMEs.

These studies demonstrate that induction of drug transporters such as MDR1 affects drug disposition and ultimately reduces the plasma concentration of drugs. Although induction of MDR1 and other transporters is a mechanism to protect the body against potentially toxic chemicals, it also reduces the effectiveness of therapeutic drugs in curing a disease or improving patients' symptoms, thereby contributing to drug resistance.

The undesired toxicity associated with inhibition of the physiologic function of MDR1 has limited the success of MDR1 inhibitors in clinical applications. Because MDR1 can be induced by drugs through activation of PXR, a feasible option is to pharmacologically antagonize the drug-mediated activation of PXR and PXR-induced expression of MDR1 to increase the bioavailability of drugs and minimize toxicity.

3.3 Preventing drug resistance by regulating PXR

The concept that down-regulating PXR in PXR-expressing cancers can sensitize cancer cells to chemotherapeutic agents has been proposed and investigated in several recent studies. Chen *et al.* detected the expression of PXR in both normal and cancerous prostate tissues and in prostate cancer cell lines [63]. In the prostate cancer cell line PC-3, treatment with the PXR agonist SR12813 activated PXR and increased both the expression of MDR1 and the resistance of PC-3 cells to the anticancer drugs paclitaxel and vinblastine. The targeted knock-down of PXR by using short hairpin RNA (shRNA) enhanced the sensitivity of PC-3 to paclitaxel and vinblastine, suggesting that the effectiveness of anticancer drugs can be enhanced in PXR-positive cancers by blocking the activity of PXR.

Masuyama *et al*. showed that PXR is expressed in endometrial cancer. Down-regulation of PXR by small interfering RNA (siRNA) in the endometrial cancer cell line HEC-1 decreased the expression of MDR1 and sensitized cells to anticancer agent and PXR agonist paclitaxel and cisplatin [73]. In contrast, increased expression level of PXR led to increased resistance of HEC-1 cells to paclitaxel and cisplatin.

The correlation between the activity of PXR and drug resistance observed in the studies discussed previously has also been reported in osteosarcoma [71], in which the effectiveness of etoposide was reduced due to activation of PXR. Furthermore, co-administration of PXR agonists enhanced the clearance of all-*trans*-retinoic acid (ATRA), which could potentially

contribute to ATRA resistance in the treatment of acute promyelocytic leukemia (APL) and several solid tumors [102].

Because of its ligand promiscuity, PXR can be activated by many anticancer drugs, such as tamoxifen, Taxol [55,30,103], and vincristine [55]. Most patients with cancer are usually administered many other drugs in addition to anticancer drugs while undergoing chemotherapy, which further increases the possibility of drug-mediated PXR activation. As PXR regulates the expression of proteins involved in drug metabolism and drug transport, activation of PXR can lead to undesired drug interactions. In PXR-expressing cancers, the anticancer drug that activates PXR might compromise the effectiveness of the drug itself as well as that of other drugs in combination therapy. The ability to activate PXR is therefore considered an undesirable property for a lead compound for development as a drug [104]. One approach to overcome the PXR activation of a lead compound is to chemically modify the compound to remove the PXR activating function without compromising the target activity. This has been shown to be possible in principle in a few studies. For example, paclitaxel and docetaxel, both inhibitors of microtubule disassembly, have minor structural difference and are equally potent in inhibiting microtubule depolymerization and cancer cell proliferation. However, paclitaxel, but not docetaxel, significantly activates PXR and induces *MDR1* expression [30]. Recently, Zimmermann *et al.* reported the chemical modifications of their first generation IGF-1R inhibitors to reduce PXR transactivation while maintaining potency against IGF-1R [104]. However, given the agonist promiscuity of PXR, tremendous efforts are needed in drug development programs to remove the PXR activity while maintaining the target activity for many lead compounds. In addition, it is highly likely that other properties of compounds might have also changed because of the chemical modifications to remove the PXR activating function. Furthermore, many anticancer drugs with PXR agonistic activity continue to be used in the clinical setting. In light of these considerations, efforts need to focus on developing compounds that can antagonize PXR-mediated *MDR1* expression and enhance the effectiveness of anticancer drugs.

A few compounds previously known to target various biological pathways can inhibit PXR function (Table 1). Here, PXR inhibitors refer to compounds that inhibit the agonist-mediated activation of PXR, but whether they bind to PXR is unknown. PXR antagonists refer to PXR inhibitors that have been shown to competitively bind to PXR in *in vitro* binding assays. Ecteinascidin-743 (ET-743), an antineoplastic agent, has been shown to inhibit PXR transactivation [30]. Ketoconazole, an inhibitor of CYP3A4 enzyme activity, can inhibit multiple NRs, including PXR, by disrupting the NR–coactivator interaction [105]. A-792611, an HIV protease inhibitor, inhibits PXR-mediated CYP3A4 expression [106]. Sulforaphane (SFN), an inhibitor of histone deacetylases and an inducer of phase II DMEs such as glutathione *S*-transferases (GSTs), appears to be a PXR antagonist [107]. SFN down-regulates CYP3A4 expression by directly binding to PXR and inhibiting coactivator recruitment. Coumestrol, a potent agonist of ERα and ERβ (EC_{50} 21 – 67 nM), antagonizes PXR at high concentrations (EC_{50} 12 μM) [108]. Camptothecin, an inhibitor of topoisomerase I, inhibits PXR-mediated transcriptional activation of *CYP3A4* by disrupting the interaction of PXR with SRC-1 without competing with agonist for binding to PXR [109]. The effect of camptothecin is not specific for PXR, because camptothecin also inhibits CAR-mediated, but activates vitamin D receptor (VDR)-mediated transactivation [109]. Although all known PXR inhibitors or antagonists have an activity other than inhibiting PXR, these studies suggest that it is feasible to antagonize the inducible activity of PXR and to enhance the effectiveness of drugs. In a recent study, Raynal *et al*. showed that activation of PXR reduced the chemosensitivity of colorectal cancer cells to irinotecan. Interestingly, the reduction in chemosensitivity was reversed by the PXR antagonist SFN [110].

In addition to test compounds with known bioactivity for their PXR antagonistic activity, other groups used a computational approach to study PXR antagonism. Ekins *et al*. investigated pharmacophores for both PXR agonists and antagonists, and suggested that agonists and antagonists might bind to distinct regions of PXR [111]. Ekins *et al*. used computational pharmacophore and docking tools to discover PXR antagonists in the low micromolar range [112]. In a study of the crystal structure of PXR with the agonist T-1317, Xue *et al*. suggested that because of the ligand promiscuity of PXR it may be difficult to design an effective antagonist that targets the ligand-binding pocket of PXR [113].

As several studies support the existence of PXR antagonists, the development of specific and non-toxic PXR antagonists as codrugs hold promise in order to prevent the activation of PXR and induction of MDR1 during drug therapies and thereby prevent drug resistance. Such specific PXR antagonists might have broad applications in overcoming drug resistance. For example, a PXR-like pathway regulating multidrug resistance in fungi has been reported by Thakur *et al.* [114]. The authors showed that drug resistance during treatment of fungal infections is often due to upregulation of drug efflux pumps by a fungal transcription factor that directly binds to xenobiotics, including PXR agonists, and suggest that a PXR antagonist can be used to treat multidrug-resistant fungal infections.

4. Conclusions

Drug resistance involves multiple mechanisms and targets; it is therefore impossible to overcome drug resistance by targeting a single protein. MDR1 is an important protein involved in target-nonspecific drug resistance. Inhibition of MDR1 to overcome drug resistance has had limited success due to toxicity. MDR1 expression can be regulated by several mechanisms. The recent discovery that the expression of MDR1 is induced by PXR, a xenobiotic receptor activated by many compounds, including anticancer drugs, suggests that it is possible to antagonize the drug-induced activation of PXR to prevent the drug-mediated expression of MDR1. The identification of PXR antagonists further suggests that pharmaceutical agents can be developed to enhance the efficacy of anticancer drugs. All known PXR inhibitors or antagonists have activities other than inhibiting PXR. Future studies need to focus on identifying specific PXR antagonists that target the agonist-induced activation of PXR. Such specific PXR antagonists will not interfere with the basal activity of PXR and might have minimal toxicity. Owing to the ligand promiscuity of PXR, it might be difficult to design such PXR antagonists. Large-scale high-throughput screening, using a large collection of structurally diverse compounds, might provide the most effective approach to identify and develop PXR antagonists.

Non-toxic, specific, and potent PXR antagonists can be used to improve the efficacy of anticancer drugs in PXR-positive cancers. Such specific PXR antagonists might have broad applications in overcoming drug resistance, including treating multidrug-resistant fungal infections.

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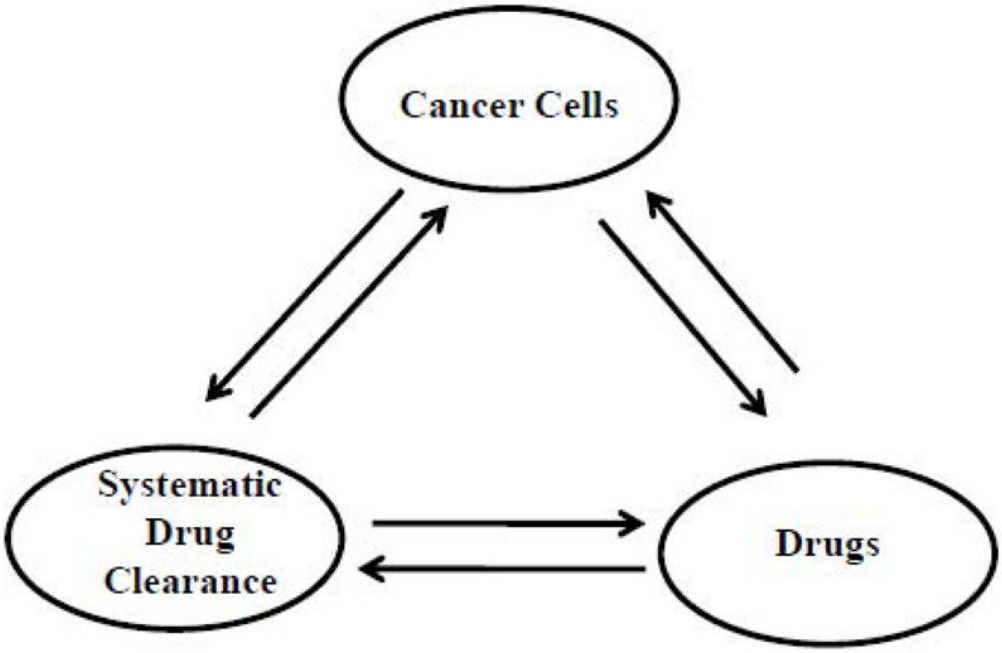


Figure 1.
The ultimate efficacy of a drug is determined by the interactions among the drug, the target cancer cells, and the drug clearance system of the human body.

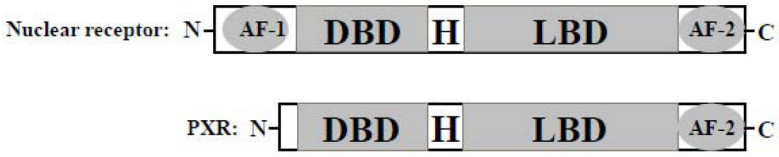
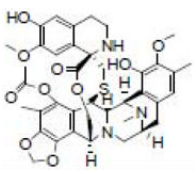
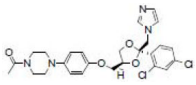
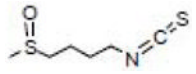
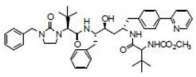
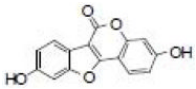
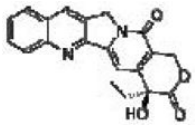


Figure 2.
A schematic comparison of the domain structures of a typical nuclear receptor and PXR. AF-1, activation function 1; DBD, DNA binding domain; H, hinge region; LBD, ligand binding domain; AF-2, transactivation function 2.

Table 1

Chemical structures and known activities of PXR inhibitors/antagonists

Compound	Structure	Other known activity	References
ET-743		Antineoplastic	Synold *et al.* [30]
Ketoconazole		Inhibiting CYP3A4 enzyme activity	Huang *et al.* [105]
Sulforaphane		Inhibiting histone deacetylases: inducing Phase II enzymes	Zhou *et al.* [107]
A-792611		Inhibiting HIV protease	Healan-Greenberg *et al.* [106]
Coumestrol		Agonist for estrogen receptors	Wang *et al.* [108]
Camptothecin		Inhibiting topoisomerase I	Chen *et al.* [109]