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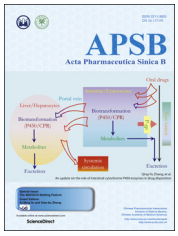

REVIEW

PXR variants: the impact on drug metabolism and therapeutic responses

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Abstract The pregnane X receptor (PXR) plays an important and diverse role in mediating xenobiotic induction of drug-metabolizing enzymes and transporters. Several protein isoforms of PXR exist, and they have differential transcriptional activity upon target genes; transcript variants 3 (PXR3) and 4 (PXR4) do not induce target gene expression, whereas transcript variants 1 (PXR1) and 2 (PXR2) respond to agonist by activating target gene expression. PXR protein variants also display differences in protein–protein interactions; PXR1 interacts with p53, whereas PXR3 does not. Furthermore, the transcript variants of PXR that encode these protein isoforms are differentially regulated by methylation and deletions in the respective promoters of the variants, and their expression differs in various human cancers and also in cancerous tissue compared to adjacent normal tissues. *PXR1* and *PXR4* mRNA are downregulated by methylation in cancerous tissue and have divergent effects on cellular proliferation when ectopically overexpressed. Additional detailed and comparative mechanistic studies are required to predict the effect of PXR transcript variant expression on carcinogenesis, therapeutic response, and the development of toxicity.

Abbreviations: AF, activating function; BAMCA, bacterial artificial chromosome array–based methylated CpG island amplification; CYP, cytochrome P450; GST, glutathione *S*-transferase; MDR, multidrug resistance protein; NHR, nuclear hormone receptor; P-gp, P-glycoprotein; PXR1, PXR transcript variant 1 (434 residues); PXR2, transcript variant 2 (473 residues); PXR3, transcript variant 3 (397 residues); PXR4, transcript variant 4 (322 residues; AK122990); RACE, 5′ rapid amplification of cDNA ends; shRNA, short hairpin RNA; siRNA, small interfering RNA; UGT, UDP-glucuronosyltransferase; UTR, untranslated region

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1. Introduction

The pregnane X receptor (PXR), also known as NR1I2 (nuclear receptor subfamily 1, group I, member 2), SXR (steroid and xenobiotic sensing receptor), or PAR (pregnane activated receptor), regulates the expression of proteins involved in all three phases of drug metabolism and transport. PXR is a nuclear hormone receptor (NHR), one of a class of proteins characterized by a DNA-binding domain and activating function domains 1 and 2 (AF-1 and AF-2) that are relatively conserved in different species and, in PXR specifically, by a promiscuous ligand-binding domain (LBD)[1–4]. NHRs bind to specific DNA sequences and bring the DNA molecule into the preferential steric conditions for transcription of target genes; in the case of PXR, these are predominantly genes involved in metabolizing xenobiotics. The LBD of PXR interacts with ligands to stabilize the protein (and recruit binding proteins, such as RXR, retinoic X receptor) and enable the recruitment of coactivators to the AF-2 region, resulting in further stabilization of the protein while in complex with DNA[1–6]. PXR regulates the expression of phase I enzymes, including cytochrome P450 enzymes (CYPs) CYP3A4, CYP2B6, CYP2C9, and CYP2C19; phase II enzymes, including UDP-glucuronosyltransferase 1 family polypeptide A1 (UGT1A1), UGT1A2, and sulfotransferase 2 A (SULT2A); and phase III transporters, including ATP-binding cassette transporter ABCB1 (also known as MDR1 or P-gp), multiple organic anion transporters (OATs), and multidrug-resistance protein 3 (MRP3)[2–4,7,8]. PXR is mainly expressed in the liver and the intestines[9]. The recent identification of PXR as a potential therapeutic target in several diseases underscores the necessity of fully describing all variants of the receptor and their effect on physiologic and pathophysiologic processes[10–14]. PXR has been implicated in the pathophysiology of bone disease[11], inflammatory disorders[13,14], and dyslipidemias[10], in addition to its roles in hepatotoxicity and hepatic fibrosis[15–18]. Because some PXR ligands are species-specific, a mouse model in which mouse PXR is replaced with human PXR (hPXR) enables examination of hPXR function *in vivo*[19–22]; however, mouse models that are engineered to express only PXR variant 1 mRNA (*PXR1*) and protein fail to account for the multiple PXR transcripts. Therefore, a mouse model in which the entire *hPXR* gene is inserted into the mouse genome was created[21,23].

Ligand activation of PXR results in the induction of target genes by a broad range of structurally dissimilar xenobiotics, leading to the metabolism of an even greater range of compounds than those that directly activate xenobiotic metabolism *via* activation of PXR (Table 1[24–31]). Analgesics [NSAIDs (nonsteroidalanti- inflammatory drugs) and non-NSAIDs], protease inhibitors, antibacterials, anticonvulsants, glucocorticoids, and statins activate PXR (Table 1). PXR is also activated by a structurally and functionally diverse set of ligands implicated in a range of disease states. PXR target genes display considerable interindividual variation in expression profile and enzymatic activity[32–34]. Administration of xenobiotics to patients might cause adverse drug responses such as hepatotoxicity[15,17,35,36]. Interindividual variability in drug response or nonresponse may result, in part, from variation in total PXR protein expression, single-nucleotide polymorphisms in coding or promoter regions of *PXR*, and variation in the relative expression of PXR isoforms[9,32]. Interindividual variability in drug metabolism, therapeutic response, and the incidence and degree of dose-limiting toxicity may reflect, in part, variability in the expression levels of *PXR* transcript variants. There is considerable variability in *PXR* transcript expression in human liver and intestines; some patients express low levels of a common variant, whereas others express high levels of uncommon variants[7,9,37–39]. These variants display altered transactivation activity towards target genes[7,39]. In addition, PXR1 and PXR2 have separate transcription start sites[40].

2. PXR and chemotherapeutic metabolism

Distinct from its less investigated direct role in cellular proliferation and senescence, PXR can play seemingly dual roles in the development of resistance to chemotherapeutic agents. For example, after treatment with a PXR agonist, an inactive anticancer prodrug is metabolized to a greater degree to an active metabolite which may confer the anticancer chemotherapeutic activity[41]. Conversely, PXR activation may enhance the metabolism of the active forms of a drug into less active metabolites or excreted, with a resultant increase in resistance to chemotherapy[28,42–60]. The role of PXR in chemotherapeutic metabolism has been elegantly reviewed by Zhuo[61].

Cyclophosphamide and ifosfamide are prodrugs that are converted by CYP3A4 and CYP2B6 to active 4-hydroxy metabolites[41]. Indeed, the treatment with rifampicin and dexamethasone, both agonists of PXR, increases the metabolism of the prodrugs to their 4-hydroxy form to confer anticancer activity[41]. Irinotecan is a prodrug that is metabolized by carboxylesterase to its active form SN38 and is further metabolized by UGT1A1 and, to a lesser extent, CYP3A4 to an inactive form, SN38 glucuronide (SN38G)[57,58]. PXR protein overexpression, and activation by rifampicin and SN38 itself, results in increased metabolism to inactive SN38G[42,43]. Tamoxifen is metabolized to 4-hydroxytamoxifen, a more active metabolite, by CYP2D6[62]. Conversely, tamoxifen is metabolized by CYP3A4 to *N*-desmethyltamoxifen, a metabolite with very low activity[63]. PXR protein overexpression and activation by rifampicin, SR12813, tamoxifen, and 4-hydroxytamoxifen lead to increased metabolism to *N*-desmethyltamoxifen and efflux which may result in increased resistance to chemotherapy[48,49,60]. Paclitaxel is metabolized by CYP2C8 and CYP3A4 to inactive metabolites[64,65]. *PXR* knockdown by small interfering RNA (siRNA) or short hairpin RNA (shRNA) results in increased sensitivity to therapy[51,59], whereas the activation of PXR by rifampicin, paclitaxel, or SR12813 leads to increased metabolism and efflux of paclitaxel, resulting in increased resistance to chemotherapy[44,50,51,59]. Doxorubicin is metabolized directly by the Cyp3a family or by carbonyl reductases to doxorubicinol and is further metabolized by Cyp3a enzymes to inactive aglycone metabolites[66]. Additionally, transcriptional activation of PXR by either overexpressing a constitutively active PXR protein or binding of rifampicin to the wild-type hPXR results in decreased sensitivity to doxorubicin[39,67]. Vinblastine is metabolized by CYP3A4 and CYP3A5 to inactive metabolites[68]. Pretreatment with a PXR agonist decreases the sensitivity of cancer cells to vinblastine treatment, whereas siRNA knockdown of *PXR* increases their sensitivity to vinblastine[51,56]. Together, these results clearly demonstrate the roles of PXR in regulating the efficacy of chemotherapeutic agents.

3. Structural, functional, and expression characteristics of *PXR* transcript variants

The *PXR* gene located on chromosome 3 encodes multiple transcript variants, resulting in structurally and functionally

Table 1 Examples of hPXR agonists.

Compound	Description (indication, drug class, etc.)	Reference
12-Ketolithocholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
3-Keto-7α,12α-dihydroxy-5α-cholanic acid	Bile salt found in sea lamprey	Krasowski et al. (2005)[24]
5α-Cyprinol 27-sulfate	Bile salt found in zebrafish	Krasowski et al. (2005)[24]
5β-Pregnane-3,20-dione	Steroid hormone	Jones et al. (2000)[25]
5β-Scymnol 27-sulfate	Bile salt found in cartilaginous fish	Krasowski et al. (2005)[24]
7,12-Diketolithocholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
7-Ketodeoxycholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
7-Ketolithocholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
Carbemazepine	Epilepsy	Luo et al. (2002)[26]
Cholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
Clotrimazole	Anti-fungal	Xie et al. (2000)[27]; Luo et al. (2002)[26]; Xie et al. (2003)[28]; Jones et al. (2000)[25]
Corticosterone	Glucocorticoid	Jones et al. (2000)[25]
Cyproterone acetate	Antineoplastic (prostate); androgen disorders, steroidal anti-androgen	Jones et al. (2000)[25]
Deoxycholic acid	Bile salt found in rabbit	Krasowski et al. (2005)[24]
Dexamethasone	Glucocorticoid	Xie et al. (2000)[27]; Luo et al. (2002)[26]
Dexamethasone-*t*-butyl acetate	Glucocorticoid	Synold et al. (2001)[29]; Luo et al. (2002)[26]
Estradiol	Steroid hormone	Luo et al. (2002)[26]
Glycolithocholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
Glycolithocholic acid 3-sulfate	Bile salt found in humans	Krasowski et al. (2005)[24]
Hyodeoxycholic acid	Bile salt found in many mammals	Krasowski et al. (2005)[24]
Hyperforin	St. John's Wort; herbal supplement commonly used for depression	Luo et al. (2002)[26]
Lithocholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
Lithocholic acid 3-sulfate	Bile salt found in humans	Krasowski et al. (2005)[24]
Lithocholic acid acetate	Bile salt found in humans	Krasowski et al. (2005)[24]
Lithocholic acid acetate methyl ester	Bile salt found in humans	Krasowski et al. (2005)[24]
Mifepristone (RU486)	Pregnancy termination, steroidal antiprogesterone	Xie et al. (2000)[27]; Jones et al. (2000)[25]
Nonylphenol	Anthropogenic environmental estrogen	Mota et al. (2011)[30]
Paclitaxel (Taxol)	Anti-neoplastic	Synold et al. (2001)[29]; Luo et al. (2002)[26]
Petromyzonol 24-sulfate	Bile salt found in sea lamprey	Krasowski et al. (2005)[24]
Phenobarbital	Epilepsy	Jones et al. (2000)[25]; Luo et al., (2002)[26]
Pheytoin	Seizure disorders	Luo et al. (2002)[26]
Piperine	Component of the spice, black pepper	Wang et al. (2013)[31]
Pregnenolone	Steroid hormone	Jones et al. (2000)[25]
Progesterone	Steroid hormone	Jones et al. (2000)[25]
Rifampicin	A component of the first line anti-tuberculosis therapy	Jones et al. (2000)[25]; Synold et al. (2001)[29]; Xie et al. (2000)[27]; Xie et al. (2003)[28]; Luo et al. (2002)[26]
Ritonavir	HIV, protease inhibitor	Luo et al. (2002)[26]
Spironolactone	Diuretic, steroidal anti-mineralocorticoid	Jones et al. (2000)[25]
SR12813	Dyslipidemia, HMG-CoA inhibitor	Jones et al. (2000)[25]; Synold et al. (2001)[29]
Sulfadimidine	Antibiotic, sulfonamide	Luo et al. (2002)[26]
Sulfinpyrazone	Gout	Luo et al. (2002)[26]
Taurochenodeoxycholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
Taurohyodeoxycholic acid	Bile salt found in many mammals	Krasowski et al. (2005)[24]
Taurolithocholic acid	Bile salt found in humans	Krasowski et al. (2005)[24]
Trans-nonachlor (Chlordane)	Pesticide	Jones et al. (2000)[25]
Troglitazone	Diabetes (withdrawn), thiazolidinedione	Jones et al. (2000)[25]
Troleandomycin	Antibiotic, macrolide	Luo et al. (2002)[26]
α-Muricholic acid	Bile salt found in rat	Krasowski et al. (2005)[24]

distinct proteins[3,7,37–39,69,70]. The identification of 9 splicing and transcript variants of *PXR* in human livers has led to the postulation that these variants, which may have different transactivation activity, contribute to interindividual variability in the expression of drug-metabolizing enzymes and efflux transporters, such as CYP3A4 and P-gp[69]. Recent work has focused on four of these mRNA transcript variants. The *hPXR* gene (NC_000003.12, Fig. 1) is located on chromosome 3q12-q13.3 and consists of approximately 38,000 base pairs[69]. *PXR1* mRNA possesses the noncoding exon 1a of the *hPXR* gene and is transcribed into an mRNA of approximately 4400 nucleotides (NM_003889), which is then translated into a protein of 434 amino acids using a CTG start codon in exon 2 (NP_003880). *PXR3* mRNA, a splicing variant of *PXR1*, originates from exon 1a of the *hPXR* gene, which

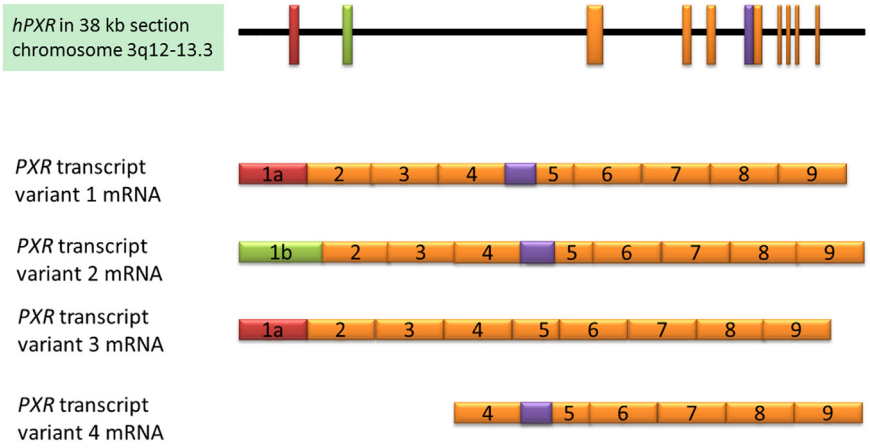

Figure 1 The human *PXR* gene locus and mRNA transcripts 1–4. Exons are represented as rectangles; the region of exon 5 that is not expressed in variant 3 is shown in purple, and the alternative positions of exon 1 are shown in red and green.

contains a 111-base pair (bp) deletion at the 5′ end of exon 5, due to preferential usage of a cryptic splice acceptor site within exon 5, and is transcribed into an mRNA of approximately 4300 nucleotides (NM_033013), which is translated into a protein of 397 amino acids (NP_148934) with a deletion of 37 amino acids in the LBD of variant 1[37,69]. The 37-amino acid deletion in human PXR3 corresponds to a 41-amino acid deletion in the LBD of the corresponding mouse PXR variant[3]. *PXR2* mRNA originates from the exon 1b of the *hPXR* gene and is transcribed into an mRNA of 2800 nucleotides (NM_022002), which is translated into a protein of 473 amino acids using a ATG start codon in exon 1b (NP_071285) that contains an additional 39 amino acids at the N-terminus, as compared to PXR1[69,71,72]. *PXR1* and *PXR2* variants share exon 2 through exon 9 therefore they have identical LBD and DNA-binding domain. Many other PXR variants have been described, including PXR4, also known as short PXR (sPXR), a newly identified 37-kDa short PXR protein containing 322 amino acids that is translated from exon 4 to exon 9 (cDNA AK122990)[72]. The naming of different *PXR* transcript variants and their corresponding abbreviations, though mostly consistent, varies between authors; please refer to the "Abbreviations" section for the naming scheme used throughout this article.

PXR splice and transcript variants differ in their effects on target gene transcription[7,38,39,70]. PXR3 lacks the activity of PXR1 in terms of both gene transactivation activity and protein–protein interactions[7,38,39,70,73], whereas the protein encoded by the *PXR2* mRNA has comparable activity to the main protein, PXR1[7,38,70]. Similarly to PXR3, PXR4 has been shown to lack gene transactivational activity, but, in contrast to PXR3, it has an intact LBD[72]. These observations and the lack of exhaustive study into the functions of *PXR* transcript variants highlight the necessity of investigating the functions of these variant proteins and transcripts and their effects on drug metabolism.

The *PXR* transcript variants possess distinct and overlapping functionality. PXR2 was as effective as PXR1 in mediating transcription of *CYP3A7*[7]. Transcription of the *UGT1A* family increased in cells transfected with *PXR1* and *PXR2*, but this was not consistent between *UGT1A* family members: Whereas *UGT1A1*, *UGT1A3*, *UGT1A4*, and *UGT1A6* mRNA were induced to various extents by PXR1 and PXR2 in response to rifampicin treatments, *UGT1A9* mRNA was not induced by rifampicin[7]. PXR3 did not activate *CYP3A7* or *UGT1A* mRNA expression[7], but when *PXR3* was cotransfected with *PXR1*, it displayed a dominant negative effect on PXR1 activation of CYP3A4 induced by rifampicin[39]. Although PXR3 binds to PXR response element, it does not activate the expression of a luciferase reporter construct under the control of the *CYP3A4* promoter (CYP-Luc)[39]. In addition, it binds to corepressors but not coactivators[39]. One study[69] found 9 variants of *PXR1*, 7 of which had deletions in exon 5. PXR3 results from splicing of *PXR1*, whereas PXR2 is generated by an alternate transcription start site and has a first exon distinct from that of *PXR1*. No publications exist that further investigate the *PXR* variants 5–9. Stable transfection of HepG2 and LS180 cells with *PXR1*, but not with *PXR3*, resulted in an increase in *CYP3A4*, *MDR1*, *CYP2B6*, and *UGT1A1* mRNA in both the absence and presence of the PXR agonist rifampicin[38,39].

The expression profiles of the main *PXR* transcript variants have not been fully elucidated, nor have the implications of the 5′ diversity among the transcripts been fully investigated. There are data available on the organism-wide expression profile for variants 1 and 3, but the expression of other variants has been quantified only in the liver[9,38,40,72]. Quantification of *PXR2* mRNA in a larger sample ($n = 56$) found that *PXR2* represented, on average, approximately 15% of the total *PXR* transcripts in the liver, although this proportion was as high as 60% in some cases[38]. The average mRNA level of *PXR3*, which lacks the transactivation activity of PXR1 on inducing CYP3A4[70] accounts for 7% of the total *PXR* transcripts in the liver[9]. As *PXR* transcript variants 1 to 4 have identical 3′ regions, it would be predicted that similar regulatory mechanisms act on this region in all variants. However, the 5′ region of the *PXR* transcripts differs between *PXR1* and *PXR2*[40,71,74], and this 5′ diversity may contribute to differential regulation by transregulatory factors at both the genomic (*i.e.*, gene) and post-transcriptional (*i.e.*, mRNA) levels[74]. When 5′ rapid amplification of cDNA ends (RACE) sequencing was performed to map the 5′ untranslated region (UTR) of *PXR* transcripts, putative transcription factor binding sites were found upstream of the *PXR2* first exon that were distinct from those in the upstream region of the *PXR1* first exon[74]. As mentioned previously, *PXR1* contains a noncoding exon 1a and uses a CTG start codon located in exon 2[74]. However, *PXR2* contains exon 1b and an ATG start site that results in a protein with an N-terminal addition of 39 amino acids (as compared to variant 1)[74]. The alternating use of exons 1a and 1b results in distinct 5′ regions in *PXR1* and *PXR2*[74]. The characterization of the upstream region of *PXR2* revealed a promoter region 1.5 kb upstream of the major

PXR2 transcript transcription start site that displayed gene transactivation activity when placed upstream from a luciferase gene in a reporter construct[75]. A search of the TRANSFAC transcription factor database (http://www.gene-regulation.com/pub/databases.html) also revealed consensus binding sites for hepatocyte nuclear factor 1 (HNF1), HNF3β, and HNF4 in this 1.5-kb region that is required for activating luciferase expression[75]. Further characterization of this promoter region by sequential deletion revealed that a putative HNF1 response element was required for luciferase expression[75]. Additionally, putative TATA boxes and consensus sites for HNF-3β, octamer factor 1 (Oct-1), CCAAT/enhancer binding protein β (C/EBPβ), and glucocorticoid receptor (GR), were found upstream of the major transcription start site identified for *PXR1*[74]. In another study, the region upstream of the transcription start site of *PXR1* containing putative transcription factor binding sites displayed promoter activity when placed upstream of a luciferase gene and transfected into HepG2 cells[71]. The authors also mapped the minimal essential region for promoter activity to a 160-bp region upstream of the transcription initiation site, showing that this region also binds to nuclear proteins and that mutations of this region disrupt protein binding and reduce promoter activity. Alternative promoter use would account for the multiple 5′ ends of the transcripts for *PXR1* and *PXR2*[74]. The 5′ regions for *PXR1* and *PXR2* were later extended, and new proximal promoters were identified[40]. Additionally, the exon 3 region with the CpG island also displays promoter activity by luciferase reporter assay[76], suggesting that transcription factors may be able to regulate *PXR4* differently from the other variants, as *PXR4* lacks exon 3.

Interestingly, a 6-bp deletion ($^{-133}$GAGAAG^{-128}) spanning the putative HNF1 binding sequence upstream of the *PXR2* transcription start site[74] was reported in a Japanese population[75]. This deletion was associated with a complete loss of promoter activity when conjugated to a luciferase gene and transfected into HepG2 cells. The allele frequency of the deletion variant was 28% in both healthy control subjects and patients with aspirin-induced asthma (AIA), and almost half of the population sampled had this 6-bp deletion, either heterozygously or homozygously. The same 6-bp deletion was further investigated in a Chinese population in healthy controls and in patients with hepatic carcinoma[38]. In this study, the allelic frequency, as determined by analyzing blood samples ($n = 177$), in healthy controls (22%) was significantly different from that in patients with hepatic carcinoma (38%). This research group also reported that *PXR2* mRNA represented an average of 15% of the total *PXR* mRNA expression, with a range from 1% to 60%. The 6-bp deletion reduces the levels of *PXR2* and, thus, of total *PXR* and of *MDR1* and *CYP3A4* mRNA expression[38]. The significantly higher allelic frequency of the 6-bp deletion in patients with hepatic carcinoma might also suggest its correlation with carcinoma formation, possibly as a result of the reduced capacity for detoxification.

4. Relation of *PXR* variant expression and activity in the context of chemotherapeutic response and carcinogenesis

Organism-wide expression profiling studies have revealed an incomplete correlation between the expression of PXR target genes and that of the major transcript variant, PXR1 (434 residues). PXR2 (473 residues), which regulates similar target genes to PXR1, may contribute to this incomplete correlation; however, an organism-wide expression profile for PXR2 has not been attempted[9]. It is possible that the interindividual and tissue variation in target gene expression may be related to the tissue-specific expression of specific *PXR* transcript variants. For example, PXR1 and PXR3 are, in some instances, not expressed in tissues that express CYP3A4, whereas PXR2 may be expressed in those tissues. Similarly, Caco-2 cells only express *PXR1* mRNA, whereas HepG2 cells, human jejunum, and human hepatocytes express *PXR1*, *PXR2*, and *PXR3* mRNA[7]. In addition to tissue-specific expression, the *PXR* transcript variants also display divergent effects on target gene transcription. PXR3 binds to the PXR response element but fails to activate the expression of PXR target genes in response to PXR ligands[39]. Instead, it functions as a dominant negative interfering with PXR1[39]. PXR1 and PXR2, but not PXR3, induce *CYP3A7*, *UGT1A1*, *UGT1A3*, and *UGT1A4* mRNA in HepG2 and Caco-2 cells in response to rifampicin[7]. These data suggested that both PXR1 and PXR2, although they may be differentially expressed, contribute to the overall PXR activity[7]. Conversely, PXR3 and PXR4 lack transcriptional activity in inducing target gene expression[39,72]. Thus, the relative expression levels of the *PXR* transcript variants may directly affect chemotherapeutic metabolism.

A recent report describes the inverse correlation between the expression level of PXR4 and the methylation status of a CpG island upstream of exon 3, as well as the effect of overexpressed PXR4 protein in reducing cell proliferation markers[72]. The CpG island was reported to be methylated in various cell lines and normal colon tissue, and the methylation status of this region did not affect *PXR1* mRNA expression[77]. PXR4 contains 322 amino acids, is homologous to the LBD of PXR1 and PXR2, retains ligand and coregulator binding capacity, and is detectable in the nucleus, but it lacks target gene transactivation activity[72]. These investigators also found *PXR4* to represent approximately 10% of the total *PXR* mRNA expressed in human livers. Overexpression of PXR4 in HepG2 cells decreased cellular proliferation and rifampicin-induced expression of CYP3A4 and FGF19[72]. The authors postulated that PXR4 was a tumor suppressor functioning as a dominant negative against PXR1. Although no significant difference in expression of *PXR4* mRNA was observed in normal tissue and hepatocellular carcinoma or adenoma, the authors did observe differences in expression of *PXR4* mRNA among certain tumor subtypes. For example, *PXR4* mRNA was decreased in inflammatory hepatocellular adenomas compared to normal livers or noninflammatory subtypes. Interestingly, higher levels of *PXR4* mRNA expression were associated with favorable prognostic indicators and greater disease-free survival, whereas lower levels of expression in subtypes of hepatocellular carcinoma were associated with poor prognoses. The authors also observed decreased expression of PXR4 in the inflammatory subtype of hepatocellular adenoma that is believed to progress to hepatocellular carcinoma as a result of inflammatory activation *via* the JAK/STAT pathway. These observations suggest that the suppressive effect of PXR4 on PXR target genes may also extend to interactions with the NF-κB pathway, a pathway known to interact with PXR[14,72,78,79]. PXR4 may thus serve as a prognostic marker in hepatic carcinoma, as well as playing a role in the malignant transformation of hepatic carcinomas.

The *PXR* variants may also display differential behavior in protein–protein interactions. It has been reported that p53 interacts with PXR1 and represses PXR-mediated transactivation of the *CYP3A4* promoter. However, PXR3 does not interact with p53[73]. The p53 protein, mutated in many cancers, is a tumor suppressor that leads to cell cycle arrest, DNA repair, and apoptosis when activated by a wide array of

genotoxic stresses[80,81]. Interestingly, while both the wild-type and mutated forms of p53 interact with PXR, only the wild-type form inhibits PXR activity[73]. Therefore, cell populations with a mutant p53 protein will have higher levels of PXR transcriptional activity and drug-metabolizing enzymes, such as CYP3A4, possibly rendering these populations less responsive to chemotherapeutic agents that are metabolized by CYP3A4. Whether other PXR protein variants interact with p53 has not been investigated.

As discussed earlier, the differential regulation of PXR1 and PXR2 resulting from their different 5′ UTRs may contribute to the differential expression of the variants and the levels of total PXR and its target genes, which might be associated with differential risk of carcinoma formation and the response of cancer to chemotherapeutic agents.

Methylation of the *PXR* promoter was first reported in detail in neuroblastoma[76]. Neuroblastoma is the most common solid tumor in children, with advanced stages having an incidence of mortality greater than 60%[82,83]. Normal adrenal tissue, the tissue of origin of neuroblastoma, expresses *PXR1* mRNA exclusively[76]. Hypermethylation in CpG-rich exons and promoter regions has been observed in many cancers and can lead to the transcriptional inactivation of tumor suppressors[84]. *PXR* mRNA expression was analyzed across a panel of 19 neuroblastoma cell lines, and no PXR was detected in 14 of the cell lines[76]. By using bacterial artificial chromosome array-based methylated CpG island amplification (BAMCA), the promoter of *PXR* was found to be methylated in several neuroblastoma cell lines and primary tumors[76]. Specifically, *PXR* mRNA was expressed in the low-grade tumors but not in the advanced neuroblastoma cell lines analyzed. However, *PXR* mRNA expression increased in the advanced neuroblastoma cell lines after they were treated with 5-aza-Cyd, an inhibitor of DNA methyltransferase[76]. The authors performed bisulfite sequencing in the exon 1a region and in a region of exon 3. Methylation of the exon 3 region was more frequently detected in advanced tumors, tumors from patients with a poor prognosis, and tumors from patients who were more than 1 year of age. The methylation status of exon 1a did not correlate with *PXR* mRNA expression. However, hypermethylation of the exon 3 region was detected in cell lines lacking *PXR* mRNA expression (IMR32 and SH-SY5Y), whereas hypomethylation, as well as *PXR* mRNA expression, was detected in a normal lymphoblast cell line and two neuroblastoma cell lines (SK-N-AS and SK-N-KP). The exon 3 region with the CpG island exhibited promoter activity. Additionally, there was no detectable difference in the methylation status of the exon 1a region among these cell lines. *PXR1* and *PXR2* mRNA were expressed in cell lines in which the *PXR* promoter is unmethylated, but not in cell lines with methylated *PXR* promoters. In cell lines with methylated *PXR* promoter, the expression of *PXR1* mRNA, but not of *PXR2* mRNA, was restored by the treatment with 5-aza-Cyd. *PXR2* was detected in the hypomethylated (SJ-N-KP and SK-N-AS) cell lines and not in the hypermethylated ones (SJ-N-GC and SMS-KAN), whereas *PXR3* mRNA was not expressed in any of the cell lines, regardless of their methylation status or whether they were treated with 5-aza-Cyd. Consequently, the methylation status of separate regions of the *PXR* gene differentially regulates the expression of specific *PXR* variants. In this study, the ectopic overexpression of PXR decreased cell proliferation, suggesting that PXR functions as a tumor suppressor[76]. However, whether this finding is specific to neuroblastoma cell lines is unclear.

In colon cancer cell lines, *PXR* promoter methylation was associated with *PXR* mRNA and *CYP3A4* mRNA expression levels[77]. Moreover, the *PXR* promoter was less methylated and *PXR* and *CYP3A4* mRNA expression levels were correspondingly higher in cancerous colon tissues than in adjacent normal tissues. In a xenograft model of colon cancer, PXR activation correlates with the growth of both human colon tumor cell lines and primary human colon cancer tissue. PXR potentially functions as an oncogene[85]. In colon cancer cell lines (LS180, LoVo, Caco-2, HCT116, HT29, and SW48), *PXR* and *CYP3A4* mRNA levels were associated with hypermethylation of a CpG-rich sequence in the promoter of *PXR* and were increased after treatment with 5-aza-dC[77]. The cell lines that express high levels of PXR1 and low levels of PXR2 and PXR3 have mostly methylated CpG-rich sequences in exon 1b and in the intron of the 3′ end of exon 1b. However, a mostly unmethylated CpG-rich sequence exists in exon 1a and in the intron of the immediate 3′ end of exon 1a in the cell lines with higher expression of PXR (LS180 and LoVo)[77]. Thus, it would seem that the mRNA expression of different *PXR* variants could be differentially regulated by methylation. It would be expected that methylation of the CpG-rich sequences in and around exon 1a might specifically affect the expression of *PXR1* mRNA and *PXR3* mRNA, whereas the methylation status of and near exon 1b would affect the expression patterns of *PXR2* mRNA. In colon cancer cells excised from colonic tissue samples, the CpG island within the exon 3 region is the most methylated region of the *PXR* gene, whereas the CpG-rich sequence around exon 1a is the most unmethylated region[77]. The CpG-rich sequence around exon 1b is methylated in these colon cancer cells, and this may correlate with low *PXR2* mRNA expression. Additionally, the methylation of the CpG island within exon 3 seen in cell lines[77] may be associated with low expression of PXR4 and, subsequently, with higher expression observed after treatment with a demethylating agent[72]. Therefore, determination of the protumor effect of PXR in colon cancer may involve a complex series of events that requires a specific relative expression pattern of multiple transcript variants. Additional analysis of the methylation of *PXR* variants in different tumor types is warranted to further establish the correlation between methylation and expression of PXR and tumor development, as well as tumor response to chemotherapeutic agents.

5. Conclusions

It is clinically and scientifically important to elucidate the functions of the *PXR* variants in order to predict the outcome of ligand activation of PXR and the role of PXR in various model systems. PXR is activated by structurally and functionally diverse ligands that are associated with and prescribed in a multitude of disease states. A greater understanding of the roles of the *hPXR* transcript variants would greatly contribute to predicting therapeutic failure of cotherapies arising from xenosensor-mediated decreases in drug bioavailability or increases in toxicity. For example, high overall PXR expression and low PXR3 (which functions in a dominant negative manner) expression may result in decreased drug bioavailability whereas high overall PXR expression and high PXR3 expression (compared to transcriptionally active variants) would be expected to result in increased drug bioavailability. PXR1 plays a well-described role in the detoxifying drug metabolism pathways of cancer therapeutics and can cause treatment failure at high levels of expression. PXR2 has similar effect on target gene transactivation but has not been fully characterized in terms of coregulator recruitment, ligand response,

protein–protein interactions, or organism-wide tissue expression. PXR3 has been reported to recruit corepressors but does not similarly participate in the protein–protein interactions associated with PXR1 or transactivate PXR target genes. However, it is unknown whether the dominant negative effect of PXR3 plays a significant pathophysiologic or physiologic role. PXR4 has been reported to compete with PXR1 in binding to ligand and coactivators to exert a dominant negative effect, as has PXR3 with respect to PXR1 transactivational activity. Several of these transcripts have been implicated in cancer pathogenesis, with PXR1 expression being a negative prognostic factor in some but not all cancers because of its role in drug metabolism and transport. There have been many contradictory reports concerning the effects of PXR1 on cellular proliferation and apoptosis; these effects may be more clearly defined by investigating the roles of the variants in these various model systems. *PXR* polymorphisms, the use of alternate promoters, and splicing variations have not been fully elucidated in terms of expression across disease states and transactivation profiles of target genes. Future studies investigating the role of PXR on drug metabolism would be remiss not to evaluate multiple transcript variants and the resultant proteins.

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References

1. Watkins RE, Davis-Searles PR, Lambert MH, Redinbo MR. Coactivator binding promotes the specific interaction between ligand and the pregnane X receptor. *J Mol Biol* 2003;**331**:815–28.
2. Zollner G, Wagner M, Trauner M. Nuclear receptors as drug targets in cholestasis and drug-induced hepatotoxicity. *Pharmacol Ther* 2010;**126**:228–43.
3. Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, et al. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* 1998;**92**:73–82.
4. Lehmann JM, McKee DD, Watson MA, Willson TM, Moore JT, Kliewer SA. The human orphan nuclear receptor PXR is activated by compounds that regulate *CYP3A4* gene expression and cause drug interactions. *J Clin Invest* 1998;**102**:1016–23.
5. Chen T. Nuclear receptor drug discovery. *Curr Opin Chem Biol* 2008;**12**:418–26.
6. Wang YM, Ong SS, Chai SC, Chen T. Role of CAR and PXR in xenobiotic sensing and metabolism. *Expert Opin Drug Metab Toxicol* 2012;**8**:803–17.
7. Gardner-Stephen D, Heydel JM, Goyal A, Lu Y, Xie W, Lindblom T, et al. Human *PXR* variants and their differential effects on the regulation of human UDP-glucuronosyltransferase gene expression. *Drug Metab Dispos* 2004;**32**:340–7.
8. Aleksunes LM, Klaassen CD. Coordinated regulation of hepatic phase I and II drug-metabolizing genes and transporters using *AhR*-, *CAR*-, *PXR*-, *PPARα*-, and *Nrf2*-null mice. *Drug Metab Dispos* 2012;**40**:1366–79.
9. Lamba V, Yasuda K, Lamba JK, Assem M, Davila J, Strom S, et al. *PXR* (*NR1I2*): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. *Toxicol Appl Pharmacol* 2004;**199**:251–65.
10. Ma Y, Liu D. Activation of pregnane X receptor by pregnenolone 16*α*-carbonitrile prevents high-fat diet–induced obesity in AKR/J mice. *PLoS One* 2012;**7**:e38734.
11. Azuma K, Casey SC, Ito M, Urano T, Horie K, Ouchi Y, et al. Pregnane X receptor knockout mice display osteopenia with reduced bone formation and enhanced bone resorption. *J Endocrinol* 2010;**207**:257–63.
12. Helsley RN, Sui Y, Ai N, Park SH, Welsh WJ, Zhou C. Pregnane X receptor mediates dyslipidemia induced by the HIV protease inhibitor amprenavir in mice. *Mol Pharmacol* 2013;**83**:1190–9.
13. Cheng J, Shah YM, Ma X, Pang X, Tanaka T, Kodama T, et al. Therapeutic role of rifaximin in inflammatory bowel disease: clinical implication of human pregnane X receptor activation. *J Pharmacol Exp Ther* 2010;**335**:32–41.
14. Shah YM, Ma X, Morimura K, Kim I, Gonzalez FJ. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-*κ*B target gene expression. *Am J Physiol Gastrointest Liver Physiol* 2007;**292**:G1114–22.
15. Hawkins MT, Lewis JH. Latest advances in predicting DILI in human subjects: focus on biomarkers. *Expert Opin Drug Metab Toxicol* 2012;**8**:1521–30.
16. Marschall HU, Wagner M, Zollner G, Trauner M. Clinical hepatotoxicity. Regulation and treatment with inducers of transport and cofactors. *Mol Pharm* 2007;**4**:895–910.
17. Andrews E, Armstrong M, Tugwood J, Swan D, Glaves P, Pirmohamed M, et al. A role for the pregnane X receptor in flucloxacillin-induced liver injury. *Hepatology* 2010;**51**:1656–64.
18. Wang YM, Chai SC, Brewer CT, Chen T. Pregnane X receptor and drug-induced liver injury. *Expert Opin Drug Metab Toxicol* 2014;**10**:1521–32.
19. Gong H, Sinz MW, Feng Y, Chen T, Venkataramanan R, Xie W. Animal models of xenobiotic receptors in drug metabolism and diseases. *Methods Enzymol* 2005;**400**:598–618.
20. Ma X, Shah Y, Cheung C, Guo GL, Feigenbaum L, Krausz KW, et al. The PREgnane X receptor gene-humanized mouse: a model for investigating drug–drug interactions mediated by cytochromes P450 3A. *Drug Metab Dispos* 2007;**35**:194–200.
21. Scheer N, Ross J, Rode A, Zevnik B, Niehaves S, Faust N, et al. A novel panel of mouse models to evaluate the role of human pregnane X receptor and constitutive androstane receptor in drug response. *J Clin Invest* 2008;**118**:3228–39.
22. Cheng J, Ma X, Gonzalez FJ. Pregnane X receptor- and CYP3A4-humanized mouse models and their applications. *Br J Pharmacol* 2011;**163**:461–8.
23. Scheer N, Ross J, Kapelyukh Y, Rode A, Wolf CR. *In vivo* responses of the human and murine pregnane X receptor to dexamethasone in mice. *Drug Metab Dispos* 2010;**38**:1046–53.
24. Krasowski MD, Yasuda K, Hagey LR, Schuetz EG. Evolution of the pregnane X receptor: adaptation to cross-species differences in biliary bile salts. *Mol Endocrinol* 2005;**19**:1720–39.
25. Jones SA, Moore LB, Shenk JL, Wisely GB, Hamilton GA, McKee DD, et al. The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol Endocrinol* 2000;**14**:27–39.
26. Luo G, Cunningham M, Kim S, Burn T, Lin J, Sinz M, et al. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab Dispos* 2002;**30**:795–804.
27. Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, et al. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev* 2000;**14**:3014–23.
28. Xie W, Yeuh MF, Radominska-Pandya A, Saini SP, Negishi Y, Bottroff BS, et al. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. *Proc Natl Acad Sci U S A* 2003;**100**:4150–5.
29. Synold TW, Dussault I, Forman BM. The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med* 2001;**7**:584–90.

30. Mota LC, Barfield C, Hernandez JP, Baldwin WS, Nonylphenol-mediated CYP. induction is PXR-dependent: the use of humanized mice and human hepatocytes suggests that hPXR is less sensitive than mouse PXR to nonylphenol treatment. *Toxicol Appl Pharmacol* 2011;**252**:259–67.
31. Wang YM, Lin W, Chai SC, Wu J, Ong SS, Schuetz EG, et al. Piperine activates human pregnane X receptor to induce the expression of cytochrome P450 3A4 and multidrug resistance protein 1. *Toxicol Appl Pharmacol* 2013;**272**:96–107.
32. Zhang J, Kuehl P, Green ED, Touchman JW, Watkins PB, Daly A, et al. The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics* 2001;**11**:555–72.
33. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* 2002;**54**:1271–94.
34. Lamba V, Panetta JC, Strom S, Schuetz EG. Genetic predictors of interindividual variability in hepatic CYP3A4 expression. *J Pharmacol Exp Ther* 2010;**332**:1088–99.
35. Roberts RA, Ganey PE, Ju C, Kamendulis LM, Rusyn I, Klaunig JE. Role of the Kupffer cell in mediating hepatic toxicity and carcinogenesis. *Toxicol Sci* 2007;**96**:2–15.
36. James LP, Mayeux PR, Hinson JA. Acetaminophen-induced hepatotoxicity. *Drug Metab Dispos* 2003;**31**:1499–506.
37. Dotzlaw H, Leygue E, Watson P, Murphy LC. The human orphan receptor *PXR* messenger RNA is expressed in both normal and neoplastic breast tissue. *Clin Cancer Res* 1999;**5**:2103–7.
38. Liu Y, Ji W, Yin Y, Fan L, Zhang J, Yun H, et al. The effects of splicing variant of *PXR PAR-2* on *CYP3A4* and *MDR1* mRNA expressions. *Clin Chim Acta* 2009;**403**:142–4.
39. Lin YS, Yasuda K, Assem M, Cline C, Barber J, Li CW, et al. The major human pregnane X receptor (*PXR*) splice variant, *PXR.2*, exhibits significantly diminished ligand-activated transcriptional regulation. *Drug Metab Dispos* 2009;**37**:1295–304.
40. Tompkins LM, Sit TL, Wallace AD. Unique transcription start sites and distinct promoter regions differentiate the pregnane X receptor (PXR) isoforms PXR 1 and PXR 2. *Drug Metab Dispos* 2008;**36**:923–9.
41. Chang TK, Yu L, Maurel P, Waxman DJ. Enhanced cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response to cytochrome P-450 inducers and autoinduction by oxazaphosphorines. *Cancer Res* 1997;**57**:1946–54.
42. Raynal C, Pascussi JM, Leguelinel G, Breuker C, Kantar J, Lallemant B, et al. Pregnane X Receptor (PXR) expression in colorectal cancer cells restricts irinotecan chemosensitivity through enhanced SN-38 glucuronidation. *Mol Cancer* 2010;**9**:46.
43. Basseville A, Preisser L, de Carne Trecesson S, Boisdron-Celle M, Gamelin E, Coqueret O, et al. Irinotecan induces steroid and xenobiotic receptor (SXR) signaling to detoxification pathway in colon cancer cells. *Mol Cancer* 2011;**10**:80.
44. Gupta D, Venkatesh M, Wang H, Kim S, Sinz M, Goldberg GL, et al. Expanding the roles for pregnane X receptor in cancer: proliferation and drug resistance in ovarian cancer. *Clin Cancer Res* 2008;**14**:5332–40.
45. Yonemori K, Takeda Y, Toyota E, Kobayashi N, Kudo K. Potential interactions between irinotecan and rifampin in a patient with small-cell lung cancer. *Int J Clin Oncol* 2004;**9**:206–9.
46. Miki Y, Suzuki T, Kitada K, Yabuki N, Shibuya R, Moriya T, et al. Expression of the steroid and xenobiotic receptor and its possible target gene, organic anion transporting polypeptide-A, in human breast carcinoma. *Cancer Res* 2006;**66**:535–42.
47. Choi HK, Yang JW, Roh SH, Han CY, Kang KW. Induction of multidrug resistance associated protein 2 in tamoxifen-resistant breast cancer cells. *Endocr Relat Cancer* 2007;**14**:293–303.
48. Nagaoka R, Iwasaki T, Rokutanda N, Takeshita A, Koibuchi Y, Horiguchi J, et al. Tamoxifen activates *CYP3A4* and *MDR1* genes through steroid and xenobiotic receptor in breast cancer cells. *Endocrine* 2006;**30**:261–8.
49. Sane RS, Buckley DJ, Buckley AR, Nallani SC, Desai PB. Role of human pregnane X receptor in tamoxifen- and 4-hydroxytamoxifen-mediated CYP3A4 induction in primary human hepatocytes and LS174T cells. *Drug Metab Dispos* 2008;**36**:946–54.
50. Nallani SC, Goodwin B, Maglich JM, Buckley DJ, Buckley AR, Desai PB. Induction of cytochrome P450 3A by paclitaxel in mice: pivotal role of the nuclear xenobiotic receptor, pregnane X receptor. *Drug Metab Dispos* 2003;**31**:681–4.
51. Chen Y, Tang Y, Wang MT, Zeng S, Nie D. Human pregnane X receptor and resistance to chemotherapy in prostate cancer. *Cancer Res* 2007;**67**:10361–7.
52. Masuyama H, Suwaki N, Tateishi Y, Nakatsukasa H, Segawa T, Hiramatsu Y. The pregnane X receptor regulates gene expression in a ligand- and promoter-selective fashion. *Mol Endocrinol* 2005;**19**:1170–80.
53. Zhou J, Liu M, Zhai Y, Xie W. The antiapoptotic role of pregnane X receptor in human colon cancer cells. *Mol Endocrinol* 2008;**22**:868–80.
54. Sandanaraj E, Lal S, Selvarajan V, Ooi LL, Wong ZW, Wong NS, et al. PXR pharmacogenetics: association of haplotypes with hepatic *CYP3A4* and *ABCB1* messenger RNA expression and doxorubicin clearance in Asian breast cancer patients. *Clin Cancer Res* 2008;**14**:7116–26.
55. Huang R, Murry DJ, Kolwankar D, Hall SD, Foster DR. Vincristine transcriptional regulation of efflux drug transporters in carcinoma cell lines. *Biochem Pharmacol* 2006;**71**:1695–704.
56. Smith NF, Mani S, Schuetz EG, Yasuda K, Sissung TM, Bates SE, et al. Induction of CYP3A4 by vinblastine: role of the nuclear receptor NR1I2. *Ann Pharmacother* 2010;**44**:1709–17.
57. Haaz MC, Rivory L, Riche C, Vernillet L, Robert J. Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. *Cancer Res* 1998;**58**:468–72.
58. Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G, et al. Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin Cancer Res* 2001;**7**:2182–94.
59. Masuyama H, Nakatsukasa H, Takamoto N, Hiramatsu Y. Down-regulation of pregnane X receptor contributes to cell growth inhibition and apoptosis by anticancer agents in endometrial cancer cells. *Mol Pharmacol* 2007;**72**:1045–53.
60. Desai PB, Nallani SC, Sane RS, Moore LB, Goodwin BJ, Buckley DJ, et al. Induction of cytochrome P450 3A4 in primary human hepatocytes and activation of the human pregnane X receptor by tamoxifen and 4-hydroxytamoxifen. *Drug Metab Dispos* 2002;**30**:608–12.
61. Zhuo W, Hu L, Lv J, Wang H, Zhou H, Fan L. Role of pregnane X receptor in chemotherapeutic treatment. *Cancer Chemother Pharmacol* 2014;**74**:217–27.
62. Dehal SS, Kupfer D. CYP2D6 catalyzes tamoxifen 4-hydroxylation in human liver. *Cancer Res* 1997;**57**:3402–6.
63. Jacolot F, Simon I, Dreano Y, Beaune P, Riche C, Berthou F. Identification of the cytochrome P450 IIIA family as the enzymes involved in the *N*-demethylation of tamoxifen in human liver microsomes. *Biochem Pharmacol* 1991;**41**:1911–9.
64. Harris JW, Rahman A, Kim BR, Guengerich FP, Collins JM. Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. *Cancer Res* 1994;**54**:4026–35.
65. Rahman A, Korzekwa KR, Grogan J, Gonzalez FJ, Harris JW. Selective biotransformation of taxol to 6α-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res* 1994;**54**:5543–6.
66. Lee HJ, Lee MG. Effects of dexamethasone on the pharmacokinetics of adriamycin after intravenous administration to rats. *Res Commun Mol Pathol Pharmacol* 1999;**105**:87–96.
67. Harmsen S, Meijerman I, Febus CL, Maas-Bakker RF, Beijnen JH, Schellens JH. PXR-mediated induction of P-glycoprotein by anticancer drugs in a human colon adenocarcinoma-derived cell line. *Cancer Chemother Pharmacol* 2010;**66**:765–71.
68. Leveque D, Jehl F. Molecular pharmacokinetics of catharanthus (vinca) alkaloids. *J Clin Pharmacol* 2007;**47**:579–88.
69. Fukuen S, Fukuda T, Matsuda H, Sumida A, Yamamoto I, Inaba T, et al. Identification of the novel splicing variants for the *hPXR* in human livers. *Biochem Biophys Res Commun* 2002;**298**:433–8.

70. Hustert E, Zibat A, Presecan-Siedel E, Eiselt R, Mueller R, Fuss C, et al. Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos* 2001;**29**:1454–9.
71. Kurose K, Ikeda S, Koyano S, Tohkin M, Hasegawa R, Sawada J. Identification of regulatory sites in the human *PXR* (*NR1I2*) promoter region. *Mol Cell Biochem* 2006;**281**:35–43.
72. Breuker C, Planque C, Rajabi F, Nault JC, Couchy G, Zucman-Rossi J, et al. Characterization of a novel PXR isoform with potential dominant-negative properties. *J Hepatol* 2014;**61**:609–16.
73. Elias A, Wu J, Chen T. Tumor suppressor protein p53 negatively regulates human pregnane X receptor activity. *Mol Pharmacol* 2013;**83**:1229–36.
74. Kurose K, Koyano S, Ikeda S, Tohkin M, Hasegawa R, Sawada J. 5′ diversity of human hepatic *PXR* (*NR1I2*) transcripts and identification of the major transcription initiation site. *Mol Cell Biochem* 2005;**273**:79–85.
75. Uno Y, Sakamoto Y, Yoshida K, Hasegawa T, Hasegawa Y, Koshino T, et al. Characterization of six base pair deletion in the putative HNF1-binding site of human *PXR* promoter. *J Hum Genet* 2003;**48**:594–7.
76. Misawa A, Inoue J, Sugino Y, Hosoi H, Sugimoto T, Hosoda F, et al. Methylation-associated silencing of the nuclear receptor 1I2 gene in advanced-type neuroblastomas, identified by bacterial artificial chromosome array-based methylated CpG island amplification. *Cancer Res* 2005;**65**:10233–42.
77. Habano W, Gamo T, Terashima J, Sugai T, Otsuka K, Wakabayashi G, et al. Involvement of promoter methylation in the regulation of pregnane X receptor in colon cancer cells. *BMC Cancer* 2011;**11**:81.
78. Gu X, Ke S, Liu D, Sheng T, Thomas PE, Rabson AB, et al. Role of NF-κB in regulation of PXR-mediated gene expression: a mechanism for the suppression of cytochrome P-450 3A4 by proinflammatory agents. *J Biol Chem* 2006;**281**:17882–9.
79. Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, et al. Mutual repression between steroid and xenobiotic receptor and NF-κB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest* 2006;**116**:2280–9.
80. Muller PA, Vousden KH. p53 mutations in cancer. *Nat Cell Biol* 2013;**15**:2–8.
81. Appella E, Anderson CW. Post-translational modifications and activation of p53 by genotoxic stresses. *Eur J Biochem* 2001;**268**:2764–72.
82. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 2003;**3**:203–16.
83. Westermann F, Schwab M. Genetic parameters of neuroblastomas. *Cancer Lett* 2002;**184**:127–47.
84. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv Cancer Res* 1998;**72**:141–96.
85. Wang H, Venkatesh M, Li H, Goetz R, Mukherjee S, Biswas A, et al. Pregnane X receptor activation induces FGF19-dependent tumor aggressiveness in humans and mice. *J Clin Invest* 2011;**121**:3220–32.