Histol Histopathol (2013) 28: 405-420
DOI: 10.14670/HH-28.405

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

Pregnane X receptor and human malignancy

Ioannis Koutsounas, Efstratios Patsouris and Stamatios Theocharis
First Department of Pathology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

Summary. Pregnane X Receptor (PXR) is a member of the nuclear receptor superfamily, expressed in liver, intestine and other tissues. PXR exerts transcriptional regulation by binding to its DNA response elements as an heterodimer with Retinoid X Receptor (RXR). This nuclear receptor is implicated in the homeostasis of numerous endobiotics, such as glucose, lipids, steroids and bile acids. Additionally, the activation of PXR induces expression of drug metabolizing enzymes (DMEs) and transporters, including multidrug resistance protein 1 (MDR1), leading to regulation of xenobiotic metabolism and drug-drug interactions. New roles for PXR have been established in inflammatory bowel disease, bone homeostasis, liver steatosis, antifibrogenesis and oxidative stress. PXR has, additionally, a multifactorial impact on cancer, either by directly affecting cell proliferation and apoptosis or by inducing chemotherapy resistance, in colon, breast, prostate, and endometrial cancer, and in osteosarcoma. PXR polymorphisms may also have clinical significance in certain types of cancer and their treatment. Further studies are needed in order to clarify the mechanisms involved in PXR-regulated carcinogenesis. PXR down-regulation could be considered as a novel therapeutic approach to overcome chemoresistance, while future research should be mainly focused on modulating PXR status in order to increase chemotherapy effectiveness and finally improve cancer patient prognosis.

Key words: Pregnane X receptor, Xenobiotics, Metabolism, Drug-drug interactions, Cancer

Offprint requests to: Stamatios E. Theocharis, MD, PhD, First Department of Pathology, Medical School, National and Kapodistrian University of Athens, 75, Mikras Asias street, Goudi, Athens, GR11527, Greece. e-mail: theocharis@ath.forthnet.gr

Introduction

Pregnane X Receptor (PXR), a member of the nuclear receptor (NR) superfamily, was discovered in 1998 by multiple groups (Lehmann et al., 1998). Similar to other NRs, PXR is a modular protein sharing common regions, a highly variable N-terminal domain, a conserved DNA binding domain (DBD), an H region (H), and a C-terminal ligand-binding domain (LBD). PXR is expressed in liver and intestine, front line organs involved in the absorption, distribution, metabolism and elimination of xenobiotics and endobiotics, in all mammalian species (Ma et al., 2008). PXR is also expressed in other tissues (kidney, brain, breast, prostate, heart, bone marrow, spinal cord, stomach, ovary, placenta) and cells (peripheral mononuclear and other immune cells) (Dotzlaw et al., 1999; Staudinger et al., 2001; Lamba et al., 2004; Owen et al., 2004; Albermann et al., 2005).

More than 70 single-nucleotide polymorphisms (SNPs) have been identified so far, including 15 in the coding region that are non-synonymous, creating new PXR proteins. Four of the fifteen variants were located N-terminal to the DNA binding domain (A12T, E18K, P27S, and G36R) and have no significant effects on DNA-binding or transactivation compared with wild-type PXR. Three other variants were located in or near the DNA binding domain (R98C, K109N, and R122Q). The other eight variants are within the LBD of PXR (R148Q, Q158K, D163G, A370T, C379G, R381W and I403V) or close to the LBD (V140M). R148Q and Q158K are located in helix 1 of LBD (Zhang et al., 2001) (Fig. 1).

In the present review, the role of PXR in human biology and physiology, and specifically its implication in cancer are discussed. PXR and its multiple targets in humans are briefly presented in Fig. 2.

PXR ligands

PXR activation is ligand dependent; following

ligand binding, PXR forms a heterodimer with the Retinoid X Receptor (RXR) that binds to PXR response elements, located in the 5-flanking regions of PXR target genes, resulting in their transcriptional activation. Additionally, it has been suggested that PXR may act as a gene silencer (Harmsen et al., 2007).

Many PXR ligands have been identified among prescription drugs, and include the antibiotics rifampicin, clotrimazole, and ritonavir; the antineoplastic drugs cyclophosphamide, cyproterone acetate, taxol, tamoxifen, and RU486; the anti-inflammatory agents dexamethasone and indomethacin; the anti-type 2 diabetes drug troglitazone; the antihypertensive drugs nifedipine and spironolactone; and the sedatives glutethimide and phenobarbital (Table 1) (Honkakoski et al., 2003; Persson et al., 2006; Zhou et al., 2009). Commonly used herbal medicines can also activate PXR, such as St. John's wort, Gugulipid, and kava kava (Staudinger et al., 2006). Among dietary supplements, vitamins K2 and E have been established as weak PXR activators (Tabb et al., 2003). A number of environmental pollutants are also PXR ligands, such as organochlorine pesticides (Lemaire et al., 2006). Other PXR ligands include endogenous steroids such as corticosterone, 17a-hydroxyprogesterone, as well as bile acids (di Masi et al., 2009).

Activation of PXR induces expression of drug metabolizing enzymes (DMEs) and transporters, including multidrug resistance protein 1 (MDR1), suggesting a significant role of PXR in cancer drug resistance (Ma et al., 2008). PXR serves as a master transcriptional regulator of CYP3A isozymes (Quattrochi and Guzelian, 2001). Besides regulating members of the CYP families, PXR is involved in other aspects of xenobiotic metabolism, regulating carboxylesterases, alcohol dehydrogenase, glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT), sulfotransferases (SULT), and transporters such as P-glycoprotein (Pgp or ABCB1), several multidrug

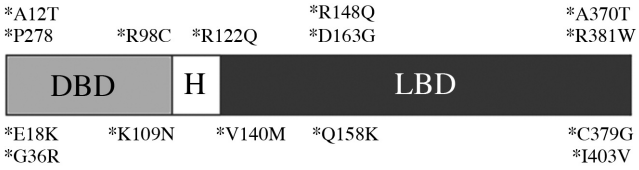

Fig. 1. PXR is a modular protein sharing common regions, a highly variable N-terminal domain, a conserved DNA binding domain (DBD), an H region (H), and a C-terminal ligand-binding domain (LBD). Several PXR allelic variants have been identified so far, including 15 in the coding region. Four of the fifteen variants were located N-terminal to the DNA binding domain (A12T, E18K, P27S, and G36R). Three other variants were located in or near the DNA binding domain (R98C, K109N, and R122Q). The other eight variants are within the LBD of PXR (R148Q, Q158K, D163G, A370T, C379G, R381W and I403V) or close to the LBD (V140M). R148Q and Q158K are located in helix 1 of LBD.

Table 1. PXR can be activated by a wide diversity of natural steroids, dietary compounds and xenobiotics. The main PXR ligands, acting either as PXR agonists (A) or antagonists (AN), are depicted in the Table.

PXR Ligand	Action	Reference
Carbamazepine	A	Willhauck et al., 2011
Clotrimazol	A	Honkakoski et al., 2003
Corticosterone	A	di Masi et al., 2009
Coumestrol	AN	Wang et al., 2008a
Cyclophosphamide	A	Chang et al., 1997
Dexamethasone	A	Honkakoski et al., 2003
Ecteinascidin-743	AN	Synold et al., 2001
17-Hydroxy-progesterone	A	di Masi et al., 2009
Hyperforin	A	Staudinger et al., 2006
Indomethacin	A	Persson et al., 2006
Ketoconazole	AN	Huang et al., 2007
Nifedipine	A	Honkakoski et al., 2003
Paclitaxel	A	Synold et al., 2001
PCN	A	Zhou et al., 2009
Phenobarbital	A	Honkakoski et al., 2003
Rifampicin	A	Lehmann et al., 1998; Lemaire et al., 2006
RU486	A	Honkakoski et al., 2003
SR12813	A	Chen et al., 2007
SFN	AN	Zhou et al., 2007
Tamoxifen	A	Desai et al., 2002
Troglitazone	A	Persson et al., 2006
Warfarin	A	Persson et al., 2006
Verapamil	A	Persson et al., 2006
Vincristine	A	Huang et al., 2006

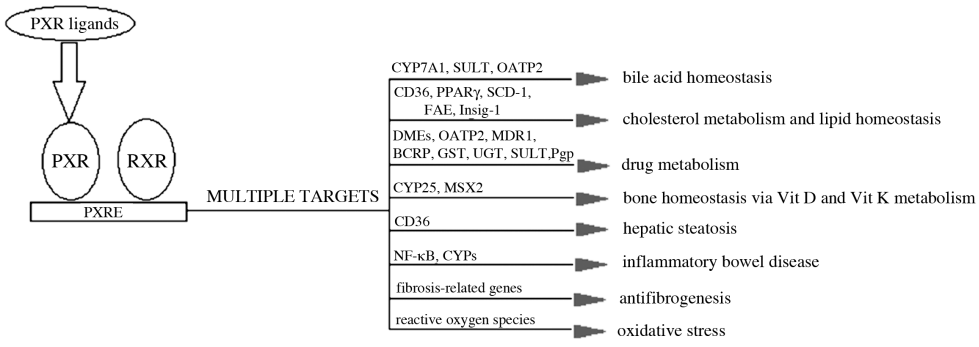

Fig. 2. PXR and its multiple targets involved in different metabolic processes.

resistance associated proteins (MRPs), and OATP2 (Rosenfeld et al., 2003).

PXR functions

In addition to important roles in cholesterol detoxification, PXR can also mediate a SREBP-independent lipogenic pathway by activating the free fatty acid (FFA) uptake transporter CD36, peroxisome proliferator-activated receptor gamma (PPARÁ), and several accessory lipogenic enzymes, such as stearoyl CoA desaturase-1 (SCD-1) and long-chain free fatty acid elongase (FAE) (Zhou et al., 2006). Additionally, PXR activation was proposed to decrease the bile acid synthesis via down-regulation of CYP7A1 and to accelerate bile acid metabolism and elimination through up-regulation of metabolic enzymes and transporters (Timsit and Negishi, 2007; Nguyen and Bouscarel, 2008). Drug-activating PXR acts like insulin and represses hepatic energy metabolism by increasing triglyceride synthesis and by decreasing b-oxidation and ketogenesis, finally leading to down-regulation of gluconeogenesis (di Masi et al., 2009). Finally, activation of PXR markedly increased plasma concentrations of corticosterone and aldosterone, and their increase was associated with the enhanced expression of adrenal steroidogenic enzymes, including CYP11A1, CYP11B1, CYP11B2, and 3b-hydroxysteroid dehydrogenase (Zhai et al., 2007).

PXR-mediated gene regulation and lipid accumulation are required for the hepatic regenerative response to liver resection, and it was suggested that PXR is essential for normal progression of liver regeneration by modulating lipid homeostasis (Dai et al., 2008; Lee et al., 2008). Very few clinical reports concerning drug-induced hepatic steatosis by PXR ligands exist. New data suggest that PXR activators inhibit transdifferentiation and proliferation of human hepatic stellate cells, and PXR may therefore be a potential target for antifibrotic therapy (Marek et al., 2005; Haughton et al., 2006).

Interestingly, budesonide, a glucocorticoid derivative frequently used as an anti-inflammatory drug for inflammatory bowel disease, has been recently identified as a PXR ligand (Maier et al., 2007). The activation of Nuclear Factor- kappa B (NF-κB) inhibits the PXR function, thereby causing a reduced expression of its target genes, while inhibition of NF-κB increases PXR activity and target genes expression (Wahli, 2008). Furthermore, PXR activation is considered to be a risk factor for oxidative stress caused by an imbalance between the production of reactive oxygen species (ROS) and detoxification of the reactive intermediates (Gong et al., 2006).

Recently, drugs such as PXR ligands have been reported to modulate the expression of the PXR target gene CYP24 both in vitro and in vivo, altering the homeostasis of 1,25(OH)2D3 (1,25-dihydroxyvitamin D3) (Pascussi et al., 2005). Overall, the role of PXR in metabolic bone disorders in humans remains unclear.

PXR-mediated drug metabolism

The expression of MDR1 as well as other proteins involved in regulating the bioavailability of drugs is regulated by NRs. PXR binds and activates the MDR1 promoter (Cerveny et al., 2007; Chen, 2010). The most common clinical implication for the activation of PXR is the occurrence of drug-drug interactions. Multiple-therapy regimens are the major reason for drug-drug interactions, especially involving patients with tuberculosis, cancer, HIV, cardiovascular disease, and diabetes. The clinical consequences of PXR mediated drug-drug interactions are generally decreased therapeutic efficacy (Ma et al., 2008). Rifampicin, a human PXR ligand used at a high dose and long term for tuberculosis treatment, activates PXR and up-regulates the PXR target gene CYP3A4, resulting in increased metabolic clearance of oral contraceptives, midazolam, and anti-HIV protease inhibitors, finally leading to decreased efficacy (Backman et al., 1996; Niemi et al., 2003; Ivanovic et al., 2008; Ma et al., 2008). Hyperforin, which is a major compound of St. John's wort, was identified as a natural ligand for PXR. St. John's wort activates PXR, up-regulates CYP3A expression, accelerating cyclosporine metabolism, and may lead to organ rejection in organ transplant patients (Murakami et al., 2006).

PXR and cancer

Drug-drug interactions in oncology

Most cancer patients are usually administered many other drugs in addition to chemotherapeutics, which further increase the possibility of drug-mediated PXR activation. As PXR regulates the expression of proteins involved in drug metabolism and 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. Drugdrug interactions can have a major impact on treatment outcome in cancer patients. These patients are at high risk of such interactions, being treated with combinations of multiple cytotoxic anticancer drugs or hormonal agents often co-administered with prophylactic antiemetics and analgesics to provide palliation. Interactions between drugs can affect the pharmacokinetics of concomitantly administered chemotherapeutic agents (Harmsen et al., 2007). The ability to activate PXR is therefore considered an undesirable property for a lead compound for development as a drug (Zimmermann et al., 2010).

Several molecular targets have been shown to be related to chemoresistance, which include efflux transporters, phases I and II detoxication enzymes, and DNA-repair enzymes. Most of these chemoresistance-

related enzymes are encoded by PXR target genes, such as Pgp, MRPs, CYP3A, UGT, and GST (Harmsen et al., 2007). Due to its ligand promiscuity, PXR can be activated by many anticancer drugs, such as tamoxifen, Taxol, vincristine and cyclophosphamide (Synold et al., 2001; Desai et al., 2002; Mani et al., 2005). Remarkably, activation of PXR induces a battery of enzymes and transporters that accelerate the metabolism and the elimination of chemotherapeutic agents, contributing to resistance to chemotherapy in breast, prostate, and endometrial cancer, as well as in osteosarcoma (Dotzlaw et al., 1999; Chen et al., 2007; Masuyama et al., 2007; Mensah-Osman et al., 2007). Assessment of PXR as a drug target may lead to drugs that improve the pharmacokinetics of other drugs by inhibiting the action of PXR, or drugs can be chemically modified so that they retain their pharmacological properties, but lack the ability to activate PXR mediated gene expression. This may ultimately provide oncologic regimens with fewer and less severe side effects and possibly enhanced antitumor activity.

Rifampicin can affect the bioavailability of concomitantly administered anticancer drugs. In one study, it was shown that rifampicin doubled the clearance of ifosfamide (Kerbusch et al., 2001). Furthermore, a decrease of the maximum observed concentration (Cmax) and area under the concentration-time curves (AUC) of imatinib when it was coadministered with rifampicin was noted (Bolton et al., 2004). Moreover, rifampicin was shown to cause sub-therapeutic doses of cyclosporine, a CYP3A4 substrate, in a pediatric patient with chronic myeloid leukaemia after bone marrow transplantation (Zelunka, 2002). Patients who received irinotecan in combination with phenytoin were also shown to have a decreased AUC of both irinotecan and its active metabolite SN-38 by 63% and 60%, respectively, most likely due to CYP3A4 induction, which is involved in the conversion of irinotecan to its inactive metabolite (Murry et al., 2002). Other studies further support the influence of anticonvulsants on the pharmacokinetics of other anticancer drugs, such as paclitaxel, topotecan, methotrexate, teniposide, and imatinib (Baker et al., 1992; Chang et al., 1998; Zamboni et al., 1998; Relling et al., 2000; Druker et al., 2001). Additionally, in a clinical trial tamoxifen reduced the plasma levels of concomitantly administered aromatase inhibitors letrozole and anastrazole, which are substrates for CYP3A4 (Dowsett et al., 2001). Treatment of primary cultures of human hepatocytes with potent nuclear receptor agonists like phenobarbital, dexamethasone and rifampicin resulted in markedly increased 4-hydroxylation of both cyclophosphamide and its isomer ifosfamide (Chang et al., 1997). In contrast to anticancer drugs like paclitaxel, tamoxifen and cyclophosphamide, which are PXR agonists, the novel marine derived anticancer agent ecteinascidin (ET)-743 (Yondelis, Trabectedin) has been shown to inhibit the transcriptional up-regulation of CYP3A4 and MDR1 by directly antagonizing PXR (Synold et al., 2001). Furthermore, pre-treatment of an osteosarcoma cell-line with ET-743 significantly enhanced the cytotoxicity of doxorubicin. The increase in cytotoxicity was associated with a down-regulation of Pgp (Mensah-Osman et al., 2007).

It was shown that paclitaxel, discodermolide, and an analogue of epothilone B, BMS-247550, induced CYP3A4 protein expression in HepG2 hepatoma cells. According to this study, hPXR activation with selective displacement of corepressors is an important mechanism by which microtubule-stabilizing drugs induce drug metabolizing enzymes both in vitro and in vivo. This study lays the basis for understanding and exploring why some paclitaxel analogues do not activate hPXR and this information may be used to guide the future development of therapeutically active but hPXR-neutral microtubule-stabilizing agents (Mani et al., 2005). 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 (Zimmermann et al., 2010). Ketoconazole, an inhibitor of CYP3A4 enzyme activity, can inhibit multiple NRs, including PXR, by disrupting the NR-coactivator interaction (Huang et al., 2007). Co-treatment with ketoconazole or its analogs, which are pure GR-antagonists, might improve the pharmaco-kinetic properties of anticancer drugs, such as paclitaxel, tamoxifen and cyclophosphamide. On the other hand, co-treatment of anticancer agents with ketoconazole could also increase adverse drug reactions as a result of impaired metabolism due to inhibition of CYP3A4 or diminished inducible expression of other bodily defence mechanisms such as efflux transporters (Wang et al., 2007). A-792611, an HIV protease inhibitor, inhibits PXR-mediated CYP3A4 expression (Healan-Greenberg et al., 2008). Sulforaphane (SFN), an histone deacetylase inhibitor and inducer of phase II DMEs such as GSTs, appears to be a PXR antagonist (Zhou et al., 2007). SFN down-regulates CYP3A4 expression by directly binding to PXR and inhibiting coactivator recruitment. Coumestrol, a potent agonist of estrogen receptor (ER)α and ERß, antagonizes PXR at high concentrations (Wang et al., 2008a). 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 the agonist for binding to PXR. The effect of camptothecin is not specific for PXR, because camptothecin also inhibits Constitutive Androstane Receptor (CAR)-mediated, but activates Vitamin D Receptor (VDR)-mediated transactivation (Chen et al., 2010). 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 drugs'effectiveness. In a recent study, Raynal et al. showed that PXR activation reduced the chemosensitivity of colorectal cancer cells to irinotecan. Interestingly, the reduction in chemosensitivity was

reversed by the PXR antagonist SFN (Raynal et al., 2010). 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 (Wang et al., 2008b). Finally, hPXR activation is related to bone demineralization and osteomalacia (Pascussi et al., 2005). Therefore, blocking unwanted PXR activation in this context, especially with other therapies like aromatase inhibitors that induce osteomalacia/osteoporosis, can be beneficial. New studies constantly contribute to literature enrichment regarding PXR involvement in drug-drug interactions in oncology.

The PXR role in different cancer types and their treatment will be further discussed in this review. PXR and its multifactorial influence on cancer is depicted in Fig. 3.

Implication of PXR in different types of cancer and their treatment

Liver cancer

Mouse nongenotoxic hepatocarcinogens phenobarbital (PB) and chlordane induce hepatomegaly characterized by hypertrophy and hyperplasia, with PXR and CAR playing key roles in these processes. The effects of PB (80 mg/kg/4 days) and chlordane (10 mg/kg/4 days) were investigated in double humanized PXR and CAR (huPXR/huCAR), double knockout PXR and CAR (PXRKO/CARKO), and wild-type (WT) C57BL/6J mice. In WT mice, both compounds caused increased liver weight, hepatocellular hypertrophy, and cell proliferation. Both compounds caused alterations to a number of cell cycle genes consistent with induction of cell proliferation in WT mice. However, these gene expression changes did not occur in PXRKO/CARKO or huPXR/huCAR mice. Liver hypertrophy without hyperplasia was demonstrated in the huPXR/huCAR animals in response to both compounds. Induction of the CAR and PXR target genes, CYP2B10 and CYP3A11, was observed in both WT and huPXR/huCAR mouse lines following treatment with PB or chlordane. In the PXRKO/CARKO mice, neither liver growth nor CYP2B10 and CYP3A11 induction was seen following PB or chlordane treatment, indicating that these effects are CAR/PXR dependent. Despite no observable increase in hepatocyte S-phase fraction, liver weight increases were demonstrated in the huPXR/huCAR mice administered PB or chlordane. This strongly suggests that the hepatomegaly observed in mice administered with such chemicals is mainly due to increased hepatocyte hypertrophy, rather than increased cell proliferation. Similarly, PB upregulates the expression of genes in the pololike kinase (Plk1)–mediated cell proliferation signaling pathway in WT mice but not huPXR/huCAR mice (Ross et al., 2010). These novel humanized mice may help to assess the human risk to rodent nongenotoxic carcinogens that act through these receptors.

In another study, the expression of PXR mRNA in the hepatoma cells was shown to be high, but still the CYP expression and induction were very poor. It was suggested that the SRC1 and TIF2 mRNA levels were lower, whereas the level of NCoR was slightly higher in hepatoma cells than in liver tissue. Additionally, in hepatoma cells, mRNA expression of several CYPs can be simultaneously enhanced by a single chimeric NR and, at least for CYP3A4, evidence was provided for its catalytic activity as well (Kublbeck et al., 2010). This study suggested that PXR, although expressed at mRNA level, is not functional or its functionality depends on the expression levels of other factors in these cells.

Hypoxia plays a key role in chemoresistance of solid tumours. In order to investigate the mechanisms involved in CYP down-regulation by hypoxia, the potential role of HIF-1a on repression of CYP3A4, which is known to be responsible for a large number of anticancer drugs (sorafenib, cyclophosphamide, tamoxifen, paclitaxel, etc.) metabolism, was analysed. Treatment with $CoCl_2$ and DFX, two HIF-1a stabilisers, and transfection with HIF-1a expression vectors lead to CYP3A4 down-regulation, evidencing that HIF-1a plays

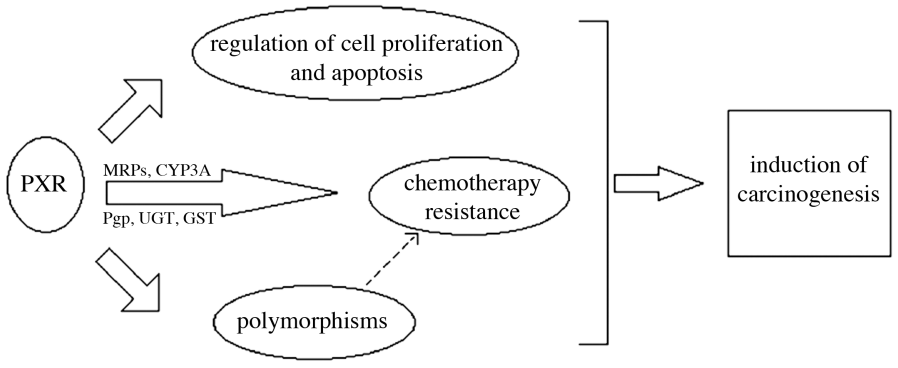

Fig. 3. PXR and its multifactorial influence on cancer. PXR may directly regulate cell proliferation and apoptosis in several cancer cell lines, induce chemotherapy resistance, or via its polymorphisms modulate cancer risk/prognosis and chemotherapy effectiveness.

a central role in CYP3A4 repression. When HepaRG cells were treated with paclitaxel, an anticancer drug widely used for treatment of malignant tumours and metabolised by CYP2C8 and CYP3A4, paclitaxel metabolism to 6a-hydroxypaclitaxel was lower, under hypoxic conditions, compared to that in normoxia (Legendre et al., 2009). Consequently, this study provided evidence that a 24-h hypoxia down-regulated several DMEs as well as PXR.

The effects of different environmental chemicals on human NRs, among them PXR, were determined using in vivo data. Hepatic histopathology, observed in rodents after two years of chronic treatment with most of the chemicals tested, was summarized by a cancer lesion progression grade. The hPXR activity pattern of chemicals weakly associated with the severity of rodent liver tumor progression. It was concluded that although such results do not prove the role of PXR activation in human liver cancer, they do have implications for its chemical biology and provide insights into putative toxicity pathways (Shah et al., 2011).

Colon cancer

Different colon cancer cell lines, among them HT29, have shown a loss of PXR expression (Xie et al., 2009). In a study, it was shown that transfected PXR significantly suppressed HT29 colon cancer cell line proliferation, as determined by cell proliferation assay and anchorage-independent assay. In a tumour xenograft model, PXR expression was retained in the HT29 cells 2 weeks after xenograft transplantation into nude mice and the tumour size was markedly suppressed by PXR. Interestingly, double staining immunofluorescence results suggest that PXR and Ki-67 expression levels were mutually exclusive, suggesting at cellular level the presence of PXR is inhibitory for colon cancer cell growth. It was also shown that PXR caused G_0/G_1 cell-cycle arrest. $p21^{WAF1/CIP1}$ expression was almost completely absent in HT29 cancer cells but highly expressed in PXR-HT29 cells. Interestingly, E2F1 expression was negatively correlated with that of p21, suggesting that its expression is downregulated by PXR presence. A slight apoptosis increase in PXR-HT29 cells, was also noted by flow cytometry analysis (Ouyang et al., 2010). Therefore, both cell-cycle regulation and apoptosis may have a role in PXR-regulated suppression of colon cancer growth. Such results suggest, according to the authors, potential applications for new therapies for colon cancer based on modulation of PXR function.

PXR activation by genetic or pharmacological means was shown to protect the PXR-overexpressing colon cancer HCT116 cells from deoxycholic acid-induced apoptosis, as well as from adriamycin-induced cell death, suggesting that the antiapoptotic effect of PXR was not bile acid specific. PXR-mediated deoxycholic acid resistance was shown to be associated with up-regulation of multiple antiapoptotic genes, including Bcl-2 associated athanogene 3 (BAG3), baculoviral IAP repeat-containing 2 (BIRC2), and myeloid cell leukemia sequence 1 (MCL-1), and down-regulation of proapoptotic genes, such as Bcl-2-antagonist/killer 1 (BAK1) and tumor protein p53 (TP53/p53). Rifampicin treatment of PXR-expressing colon cancer LS180 cells also inhibited apoptosis (Zhou et al., 2008). Consequently, activation of PXR in transgenic mice inhibited bile acid-induced colonic epithelial apoptosis and sensitized mice to dimethylhydrazine-induced colonic carcinogenesis, suggesting that the antiapoptotic effect of PXR is conserved in normal colon epithelium.

Epigenetic mechanisms involved in the regulation of PXR/CYP3A4 pathways in colon cancer cells were examined in another study. Six colon cancer cell lines were classified into two groups based on the basal level of PXR/CYP3A4 mRNA. DNA methylation status was also examined, and was reversed by the treatment of these cell lines with 5-aza-2'-deoxycytidine (5-aza-dC). DNA methylation of the CpG-rich sequence of the PXR promoter was more densely detected in the low expressing cells (Caco-2, HT29, HCT116, and SW48) than in the high expressing ones (LS180 and LoVo). This methylation was reversed by 5-aza-dC treatment, in association with re-expression of PXR and CYP3A4 mRNA. Therefore, PXR transcription was silenced by promoter methylation in the low expression cells, which most likely led to downregulation of CYP3A4 transactivation. A lower level of PXR promoter methylation was observed in colorectal cancer tissues compared with adjacent normal mucosa, suggesting upregulation of the PXR/CYP3A4 mRNAs during carcinogenesis. Additionally, PXR overexpression in colorectal cancer tissue samples was correlated with an increase in UDP glucuronosyl transferases UGT1A1, UGT1A9 and UGT1A10, and led to a marked chemoresistance to the active metabolite of irinotecan (CPT-11), approved for the treatment of metastatic colorectal cancer (Habano et al., 2011). According to this study, PXR may play a key role in colon cancer cell response to anticancer drugs by modulating expression of drug metabolizing enzymes and transporters, including UGT1A, CYP3A4 and P-glycoprotein. It was suggested that DNA methylation of the PXR promoter might be a good chemotherapy outcome and toxicity predictor in colorectal cancer.

In another study, PXR mRNA expression was quantified in a panel of 14 colon tumor samples and their matched normal tissues. PXR expression was modulated in human LS174T, SW480 and SW620 colorectal cancer cells by transfection and siRNA strategies. It was shown that PXR was strongly expressed in colon tumor samples and displayed a great expression variability. hPXR expression in human colorectal cancer cells led to a marked chemoresistance to the active metabolite of irinotecan SN38 correlated with PXR expression level. Metabolic profiles of SN38 showed a strong enhancement of SN38 glucuronidation to the inactive

SN38G metabolite in PXR-expressing cells, correlated with an increase of UDP glucuronosyl transferases UGT1A1, UGT1A9 and UGT1A10 mRNAs. These results demonstrate that tumoral metabolism of SN38 is affected by PXR and point to the potential therapeutic significance of PXR quantification in the prediction of irinotecan response. The clinical significance of this study also derives from the fact that cancer patients are often exposed to co-medications, food additives or herbal supplements able to activate PXR. Additionally, it is known that diarrhea is a major dose-limiting toxicity of irinotecan, due to SN38 accumulation in enterocytes, and it is conceivable that in situ glucuronidation by tumors and adjacent tissues depends on PXR expression levels (Gupta et al., 1994). The authors assert that PXR expression and/or activation level could help physicians in the choice of appropriate chemotherapy regimen for colorectal cancer patients, while PXR down-regulation could be considered as a novel therapeutic approach to circumvent chemoresistance to chemotherapy (Raynal et al., 2010).

The development of MDR in response to anticancer treatment is a major clinical problem, and Pgp induction is one of the main mechanisms underlying acquired MDR. Pgp is an efflux transporter that limits the cellular uptake levels of various drugs in intestine, brain, and other tissues and its induction is partly regulated by PXR (Harmsen et al., 2007). The ability of several widely used anticancer drugs to activate PXR-mediated Pgp induction was recently investigated. Pgp protein expression after treatment with several anticancer drugs was determined in both wild-type and PXR-knocked down LS180 colon cancer cells. Vincristine, tamoxifen, vinblastine, docetaxel, cyclophosphamide, Xutamide, ifosfamide and paclitaxel activated PXR-mediated Pgp induction, and were additionally shown to affect the intracellular accumulation of the Pgp probe rhodamine. Moreover, PXR activation was also shown to reduce the cytotoxic activity of the Pgp substrate doxorubicin in colon cancer cells. These results indicate that activation of PXR-mediated Pgp induction by anticancer drugs can underlie the development of acquired resistance. Since Pgp is co-expressed with PXR in important barrier tissues, such as the intestines and the liver, activation of PXR-mediated Pgp induction could affect the pharmacokinetic profile of anticancer drugs (Harmsen et al., 2010). According to this study, anticancer drugs that activate PXR might affect their own pharmacokinetics, but also that of other concomitantly administered anticancer or other drugs.

Overexpression of PXR and multidrug resistance-related protein 3 (MRP3) mRNA and protein levels were detected in human colon cancer tissues compared with those in the matched adjacent nonneoplastic colon tissues. The mRNA levels of CYP3A4 and MDR1 showed no significant differences between them, which suggested that MRP3 might play a more important role in intrinsic multidrug resistance in colon cancer than CYP3A4 and MDR1. MRP3 mRNA was significantly correlated with PXR mRNA in cancerous and nonneoplastic colon tissues. Furthermore, rifampicin treatment remarkably increased mRNA and protein levels of PXR, transcription factor SP1, and MRP3 in LS174T cells and increased survival of LS174T cells towards oxaliplatin and 5- fluorouracil. Finally, knocking down PXR via shRNAs decreased the expression of MRP3 and sensitized cells to the chemotherapeutic agents. These findings suggested an important role of PXR in human colon cancer resistance to chemotherapy, and the induction of MRP3 by PXR activation via SP1 might be involved in this process (Jiang et al., 2009). In another study, PXR-mediated induction of CYP3A4 and MDR1 gene products was shown in PXR-expressing LS180 colon adenocarcinoma cell line, and this was induced by several protease inhibitors (PIs) (Gupta et al., 2008a).

Reporter gene assays in the human colorectal adenocarcinoma cell line LS174T cells with constructs containing various lengths of the ABCB1 (Pgp) regulatory region revealed that the region containing multiple nuclear receptor binding motifs, to which PXR also binds, is essential for the VDR-mediated ABCB1 transactivation (Tachibana et al., 2009). In another study, vincristine (a ligand for ABCB1 and ABCC1-3 and a potential PXR ligand) induced ABCC2 and ABCC3 expression in LS174T colon cancer cells (Huang et al., 2006).

Ginkgo biloba is a herbal medicine commonly used to manage memory impairment. *G. biloba* extract activated mPXR and hPXR in a cell-based reporter gene assay and induced CYP3A4, CYP3A5, and ABCB1 gene expression in hPXR-expressing LS180 human colon adenocarcinoma cells. At concentrations that did not down-regulate PXR gene expression and were not cytotoxic, the hPXR antagonist L-sulforaphane decreased CYP3A4, CYP3A5, and ABCB1 gene expression in cells treated with *G. biloba* extract (Yeung et al., 2008).

Human colorectal cancer cell line Caco-2 was treated with different concentrations of three long-chain polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA). Both classes of PUFAs, omega-3 (θ-3) and omega-6 (θ-6), caused a modest but very reproducible reduction of gene expression, protein production, and pump activity of MDR1. Incubation of cells with PUFAs greatly enhanced the cytotoxicity of paclitaxel, manifested mainly through enhanced paclitaxel-induced apoptosis. Furthermore, PUFAs increased PXR mRNA level, thus implicating this transcription factor as cellular target of PUFAs in cells but not directly affecting MDR1 regulation (Kuan et al., 2011). In another study, in vivo low dietary calcium, which is involved in the etiology of colonic inflammation and cancer, significantly increased the expression of the PXR target gene CYP3A11 in the proximal colon (Nittke et al., 2008).

Finally, in a study investigating whether poly-

morphisms in different transcription factors and NRs are connected to colon carcinogenesis, no statistically significant associations between PXR polymorphisms and colorectal cancer risk were found (Andersen et al., 2010).

Esophageal cancer

PXR expression was analysed in biopsy samples of Barrett's patients. Nuclear PXR was absent in all tissue types of no-dysplasia and low-grade dysplasia patients. In high grade dysplasia and adenocarcinoma patients, PXR was highly expressed in all tissues at different progression stages. PXR immunostaining allowed an appropriate distinction between low- and high-grade dysplasia in 83% of all cases examined (van de Winkel et al., 2011).

Breast cancer

Although recent studies suggest a potential clinically relevant role of PXR in breast cancer, the pathways or target genes of PXR in breast cancer biology and progression have not yet been fully clarified. PXR is involved in the regulation of solute carrier organic anion transporter family, member 1A2 (SLCO1A2) expression. Abstract Organic Anion Transporter Polypeptides (OATPs, SLCOs) are involved in the uptake of conjugates of steroid hormones such as estrone-3-sulfate. It has been suggested that OATPs expression in breast tissues could impact breast carcinogenesis and tumor pathology. OATP1A2 is a transporter capable of mediating the cellular uptake of estrogen metabolites (Konig et al., 2006). In one study, it was shown that OATP1A2 mRNA expression is nearly 10-fold higher in cancer compared to adjacent healthy breast tissues. Treatment of breast cancer cells T47-D in vitro, with the PXR agonist rifampin, induced OATP1A2 expression in a time- and concentration-dependent manner. Additionally, OATP1A2 induction was associated with increased uptake of estrone 3-sulfate (E_1S). The rifampin response was abrogated after si-RNA targeting of PXR. Finally, the novel potent and specific PXR antagonist A-792611 was utilised to demonstrate the reversal of the rifampin effect on the cellular uptake of E_1S (Meyer zu Schwabedissen et al., 2008). Additionally, the effect of MTX treatment on expression of MRP2, breast cancer resistance protein (BCRP) and OATs in rats was examined. Four days after MTX injection 150 mg/kg, the MRP2 levels in the liver and ileum, but not in the kidney, were markedly down-regulated compared to controls. These effects of MTX were almost recovered by leucovorin, which rescues normal cells from MTX toxicity. MTX treatment also decreased PXR mRNA levels, but had no apparent effect on BCRP, cytochrome P450 2B6 and 3A1 expression levels (Shibayama et al., 2006). Finally, MRP2 mRNA was significantly increased by PCN in BALB/c mice. In contrast, BCRP mRNA expression was not significantly affected by PCN (Han and Sugiyama, 2006). Overall, according to this study, PXR activation enhances carcinogenesis and drug resistance in breast tissues.

The breast cancer resistance protein ABCG2 effluxes a variety of drugs and is believed to play an important role in multidrug resistance to chemotherapy (Robey et al., 2007). It was shown that dexamethasone and progesterone are able to strongly inhibit ABCG2 expression in progesterone receptor (PR)-positive MCF7 and PR-negative MDA-MB-231 breast cancer cells. In contrast, in the latter cells stably-transfected with PR isoforms A and B, ABCG2 expression was strongly up-regulated by dexamethasone and progesterone. In addition, two other ligands of PXR (clotrimazol, PCN) and GR were also able to down-regulate ABCG2 expression in PXR- and GR-positive MCF7 cells. ABCG2 expression inhibition by dexamethasone was associated with increased sensitivity to mitoxantrone, a known ABCG2 substrate. Consequently, by altering ABCG2 expression levels through PR-α GR-ß and/or PXR-signaling pathways in PR-positive and negative breast tumors, dexamethasone therapy might significantly contribute to increase sensitivity for ABCG2 substrates (Honorat et al., 2008). In another study, interleukin-1ß and tumor necrosis factor-α induced ABCG2 and PXR mRNAs in the MCF7 breast cancer cell line, while no significant changes to expression of the same genes in MCF7/MX cells were observed (Malekshah et al., 2011).

The transition from chemotherapy-responsive to chemotherapy-resistant breast cancer cells is mainly accompanied by the increased expression of MRPs. It was noted that tamoxifen-resistant MCF-7 (TAMR-MCF-7) cells expressed higher levels of MRP2 than control MCF-7 cells. It was also shown that MRP2 overexpression in tamoxifen-resistant breast cancer cells might result from PXR activation. In addition, the basal activities of phosphatidylinositol 3-kinase (PI3-kinase) were higher in the TAMR-MCF-7 cells than in the control cells. The inhibition of PI3-kinase significantly reduced both the PXR activity and MRP2 expression in TAMR-MCF-7 cells (Choi et al., 2007). Resistance to tamoxifen is a serious therapeutic problem in breast cancer patients, and, according to this study, PXR-mediated MRP2 induction seems to play a role in the additional acquisition of chemotherapy resistance in tamoxifen-resistant breast cancer.

The sodium iodide symporter (NIS) mediates the active iodide uptake in the thyroid gland as well as lactating breast tissue (Spitzweg and Morris, 2002). The effect of carbamazepine (CBZ), a potent PXR activator, on ATRA-induced NIS expression and therapeutic efficacy of ^{131}I in MCF-7 cells was examined in a study. Incubation with CBZ stimulated ATRA-induced iodide accumulation up to 2-fold in a concentration-dependent manner, while ATRA/Dex-stimulated iodide uptake was further stimulated up to 1.5-fold by additional CBZ treatment based on significantly increased NIS mRNA and protein levels. This stimulatory effect of CBZ was

shown to be dependent on the PI3K-Akt pathway without involvement of mTOR. In contrast, treatment with CBZ alone had no effect on functional NIS expression. Moreover, selective cytotoxicity of ^{131}I was significantly increased from approximately 20% in MCF-7 cells treated with ATRA alone to 50% after treatment with CBZ in the presence of ATRA, which was further enhanced to 90% after combined treatment with ATRA/Dex/CBZ. In conclusion, treatment with CBZ in addition to atRA or ATRA/Dex increases functional NIS expression levels, thereby significantly enhancing iodide accumulation and the selective killing effect of ^{131}I in MCF-7 cells (Willhauck et al., 2011). According to the authors, treatment with CBZ, ATRA and Dex may allow diagnostic and therapeutic application of radioiodine in breast cancer in the future.

In another study, 3,149 postmenopausal breast cancer patients and 5,489 controls from 2 German population-based case-control studies were investigated, while 33 polymorphisms located in ESR1, ESR2, PGR, PXR and AR were genotyped. Menopausal hormone therapy (HT) is associated with increased breast cancer risk among postmenopausal women (Beral, 2003). Furthermore, nuclear receptors are involved in steroid hormone- and xenobiotic-mediated signal transduction, therefore, variations within these genes may influence HT-associated breast cancer risk. In this study, risk associated with combination therapy use was shown to be significantly modified by 2 PXR polymorphisms, with reduction of risk effects in carriers of the minor PXR_rs6785049_G and PXR_rs1054191_A alleles (MARIE-GENICA, 2010).

PXR polymorphisms in healthy Asian populations (Chinese, Malay and Indian), as well as association between PXR haplotypes and hepatic mRNA expression of PXR and its downstream target genes, CYP3A4 and ABCB1, and their influence on the clearance of doxorubicin in Asian breast cancer patients, were investigated in another study. Significant interethnic variations were observed in PXR pharmacogenetics among the three Asian ethnic groups. The expression of PXR mRNA in liver tissues harboring the PXR*1B haplotype clusters was 4-fold lower compared with the non-PXR*1B (*1A + *1C) haplotype clusters. PXR*1B-bearing liver tissues were associated with significantly lower expression of CYP3A4 and ABCB1 compared with non-PXR*1B-bearing liver tissues. Doxorubicin clearance in breast cancer patients harboring the PXR*1B haplotypes was significantly lower compared with patients carrying the non-PXR*1B haplotypes (Sandanaraj et al., 2008). The authors suggest that PXR haplotype constitution could be important in influencing interindividual and interethnic variations in disposition of its putative drug substrates.

In another study by Justenhoven et al., none of the 31 investigated polymorphisms in SLCO1A2, SLCO1B1, SLCO1B3, and SLCO2B1 and none of the investigated PXR polymorphisms were associated with over-all breast cancer risk or any of the subgroup risks addressed by menopausal status, family history of breast cancer, use of oral contraceptives, use of hormone therapy, body mass index, and smoking. With respect to breast tumor characteristics, i.e., ER, PR and HER2 status, grading, tumor size, histology, and nodal status, also no association was observed. The authors suggest that it cannot be excluded that breast cancer susceptibility and/or tumor characteristics may be conferred by variants in other solute carriers (e.g., other OATPs, OATs, OCTs) and/or in ATP-binding cassette membrane transporters (e.g., ABCB and ABCC) or variants in other related genes (Justenhoven et al., 2011).

SNPs identified in PXR in an Asian female breast cancer population did not seem to have any significant effect on the clearance of docetaxel, which is a CYP3A substrate (Tham et al., 2007). Similarly, in another study in 101 Asian breast cancer patients, genotypic variability of PXR was not shown to account for variations of docetaxel and doxorubicin pharmacokinetics or pharmacodynamics (Hor et al., 2008).

The association between genetic polymorphisms of CYP2D6 and PXR, and tamoxifen pharmacokinetics and clinical outcomes in patients with breast cancer were analysed in a study. The CYP3A and CYP2D6 enzymes play a major role in converting tamoxifen into its active metabolites (Desta et al., 2004). Common alleles of CYP2D6 and PXR were identified in 202 patients treated with tamoxifen 20 mg daily for more than 8 weeks. Twelve of the 202 patients and an additional nine patients with metastatic breast cancer receiving tamoxifen were assessed for clinical outcome in correlation with genotypes. Patients carrying CYP2D6*10/*10 demonstrated significantly lower steady-state plasma concentrations of tamoxifen active metabolites 4-hydroxy-N-desmethyltamoxifen and 4-hydroxytamoxifen than did those with other genotypes, whereas no difference for PXR genotypes was found. CYP2D6*10/*10 was significantly more frequent among nonresponders. The median time to progression for patients receiving tamoxifen was shorter in those carrying CYP2D6*10/*10 than for others. Therefore, CYP2D6*10/*10 polymorphism could possibly influence the clinical outcome by tamoxifen in breast cancer patients (Lim et al., 2007).

The expression of PXR mRNA and a variant PXR mRNA, deleted in 111 nucleotides in the ligand-binding domain, was detected in both normal and neoplastic human breast tissues. The PXR mRNA level did not differ between breast tumors and their adjacent matched normal breast tissues, but did vary among breast tumors. A statistically significant inverse relationship was found between PXR and ER status, with the level of PXR mRNA expression in ER1 tumors being significantly lower than the level of PXR mRNA expression in ER2 tumors. No relationship with PR status was found (Dotzlaw et al., 1999).

Ovarian-endometrial cancer

PXR is expressed in ovarian cancer cells. In SKOV-3 ovarian carcinoma cells, PXR is functional and its

activation by cognate ligands induces PXR target genes (CYP2B6, CYP3A4, and UGT1A1) but not MDR1 and MRP2. PXR activation in SKOV-3 cells induces cell proliferation and drug resistance. In mice harboring SKOV-3 xenografts, rifampicin induced cell proliferation and tumor growth. This study suggested that PXR activation, regardless of the type of ligand agonist present, promotes the ''malignant'' phenotype of cancer cells. Chemicals are known to have multiple targets in cells and can induce growth proliferation in cancer cell lines. This is a mechanism that may not be directly related to PXR activation. These data serve as the basis for finding novel nontoxic inhibitors of PXR activation as a method to control cell growth and prevent induction of drug resistance (Gupta et al., 2008b). The clinical relevance of these findings is that PXR, which can be activated by at least 5% of the pharmacopoeia, can enhance a more aggressive state of tumors.

The relationship of the paclitaxel pharmacokinetics in 13 patients with ovarian cancer to polymorphisms in CYP2C8, CYP3A5, ABCB1, and PXR were examined. Neither the CYP3A5 A6986G (CYP3A5*3) nor the PXR C-25385T alleles were associated with altered plasma concentrations of paclitaxel and its active metabolites 6alpha-hydroxypaclitaxel and p-3'-hydroxypaclitaxel. ABCB1 T-129C, T1236C, and G2677(A,T), however, were associated with lower area under the plasma concentration-time curve (AUC) of paclitaxel (Yamaguchi et al., 2006).

PXR mediates the genomic effects of steroid hormones, including estrogen, which has been shown to contribute greatly to growth and development in endometrial cancer. In this study, various levels of PXR expression were found in endometrial cancer tissues but not normal tissues. Tissues showing high PXR expression showed significantly high expression of CYP3A4/7 and low ER expression compared with levels in tissues showing low PXR expression. Endometrial cancer cells HEC-1, which express high PXR and low ER and PR, showed a stronger transcriptional response of the PXR-CYP3A pathway to the PXR ligands than Ishikawa cells did. These data suggest that the steroid/xenobiotics metabolism in the tumor tissue through PXR-CYP3A pathway might play an important role, especially in an alternative pathway for gonadal hormone and endocrine-disrupting chemicals (EDCs) effects on endometrial cancer expressing low ER. EDCs might affect steroidogenesis in endometrial cancer tissue through the PXR-CYP3A pathway. PXR and CYP3A4 have been demonstrated to be involved in the acquisition of resistance to anticancer drugs, and paclitaxel, which is a commonly used chemotherapeutic agent, has been demonstrated to activate PXR-mediated transcription and to enhance Pgp mediated drug clearance (Synold et al., 2001). Additionally, intratumoral CYP3A4 mRNA levels might be useful as a predictor of response to docetaxel, which is another active antineoplastic drug (Miyoshi et al., 2002). Therefore, because the PXR-CYP3A4 pathway might be involved in drug clearance in endometrial cancer, PXR-CYP3A expression in endometrial cancer tissues may cause resistance to anticancer agents, which affects the prognosis of endometrial cancer patients (Masuyama et al., 2003).

In another study, whether the down-regulation of PXR affected the expression of PXR targets and PXR-mediated transcription in endometrial cancer cells was examined. In cells transfected with PXR siRNA, neither CYP3A4 nor MDR1 protein levels were increased in the presence of the PXR ligands, paclitaxel, cisplatin, estradiol, and MPA compared with cells treated with control siRNA. Moreover, no PXR mediated transactivation or augmentation of transcription by coactivators in the presence of PXR ligands was observed. It was shown that down-regulation of PXR expression caused a significant increase in cell growth inhibition and an apoptosis induction in the presence of the anticancer agents paclitaxel and cisplatin. Additionally, PXR overexpression resulted in a significant decrease of cell growth inhibition and inhibited apoptosis in the presence of paclitaxel or cisplatin (Masuyama et al., 2005, 2007). These data suggest that down-regulation of PXR could be a novel therapeutic approach for the augmentation of sensitivity to anticancer agents, or to overcome resistance to them, in the treatment of endometrial cancer.

Prostate cancer

Resistance to chemotherapy is a significant barrier to the effective management of prostate cancer. hPXR expression in both normal and cancerous prostate tissues was detected. In cancerous tissues of prostate, PXR immunoreactivity was generally elevated when compared with normal tissues. PXR immunoreactivities in cancerous tissues were scored, and those with Gleason score of 6 or grade II presented the highest levels of PXR expression. Interestingly, when tumors progress to a more advanced stage, PXR expression tends to be reduced. Pretreatment with SR12813, a potent and selective agonist of hPXR, led to nuclear translocation of PXR in PC-3 cells and increased expression of CYP3A4 and MDR1. SR12813 pretreatment increased resistance of PC-3 cells to paclitaxel and vinblastine, as assessed by viability and clonogenic survival. This study suggested that hPXR may play an important role in prostate cancer resistance to chemotherapeutics. The PXR-CYP and PXR-MDR1 pathways might be one of the main pathways for PXR to regulate drug resistance. The expression of PXR in prostate cancer raises the possibility that the intratumoral regulation of MDR1 and CYP3A4, or other DMEs, by PXR may lead to alterations in the efficacy of chemotherapeutics (Chen et al., 2007). Consequently, inhibition of PXR will be a new approch to enhance the clinical efficacy of prostate cancer chemotherapy.

The androgen receptor (AR) is a member of the NR superfamily and plays a critical role in prostate cancer development and progression. PXR was shown to be a

potent repressor of AR signaling, and PXR activators like rifampicin and methoxychlor down-regulated AR target genes i.e. PSA, which is a well-established prognostic marker of prostate cancer progression. In view of the fact that AR plays a key role in prostate cancer progression and proliferation, the present observations suggest that AR-PXR crosstalk might play a repressive role in prostate cancer conditions. This study implicated PXR as a key determinant of anti-androgen action since down-regulation of PXR diminishes the potency of the anti-androgenic drugs and enhances transcriptional actions of androgens. In addition, subcellular localization studies revealed that ligand-activated AR induces nuclear localization of PXR and the two receptors colocalize at discrete sites in nucleus and mitotic chromatin. Finally, a distinct antagonist-induced interaction between AR and PXR defining a hitherto unidentified mode of action of AR antagonist was also reported. Alteration in AR-PXR stoichiometry may explain the failures in therapeutic regimen related to endocrine mediated malignancies (Kumar et al., 2010). In this perspective, this study may help in designing and development of novel AR antagonists offering improved avenues in prostate cancer therapy.

In another study, activation of PXR by genetic or pharmacological means was sufficient to at least partially inhibit androgen-responsive prostate regeneration and prostate cancer cell proliferation, by inducing the expression of CYP3A and SULT2A1, which are enzymes important for the metabolic deactivation of androgens. In human prostate cancer cells, treatment with the PXR agonist rifampicin inhibited androgen-dependent proliferation of LAPC-4 cells but had little effect on the growth of the androgen-independent isogenic LA99 cells. Down-regulation of PXR or SULT2A1 in LAPC-4 cells by short hairpin RNA or small interfering RNA abolished the rifambicin effect, indicating that the inhibitory effect of rifampicin on androgens was PXR and SULT2A1 dependent. Moreover, the PXR agonist effect on androgen activities was abolished in PXR $^{/}$ mice (Zhang et al., 2010). Overall, the authors suggest a novel function of PXR in androgen homeostasis, which may represent a therapeutic target for hormone-dependent prostate cancer.

Osteosarcoma

Approximately 40% of osteosarcoma patients do not achieve the desired results after chemotherapy, and drug resistance is believed to be the most common reason for the failure of chemotherapy in these patients. This study showed that a variant form of PXR is expressed in osteosarcoma cells and is recognized differently by antibodies raised against different epitopes. The inability of an antibody against the N-terminal region of the protein to detect PXR in these cell lines, and the reduced molecular size of the receptor compared with wildtype, suggests a splice variant with a truncated or modified N-terminal. The N-terminal region of the PXR protein contains the AF-1 region, which plays a role in ligand-mediated transcription of target genes. The authors suggested that a mutation on the 50-flanking region of the PXR protein may have an effect on the etoposide-induced PXR-transcription of P450 3A4 in the osteosarcoma cell lines. It was also shown that PXR regulated the expression of P450 3A4 in osteosarcoma and that the induction of P450 3A4 activity via this receptor may be an important mechanism for drug resistance. The regulation of MDR1 in osteosarcomas might involve a mechanism independent of PXR because no differences in the induction of MDR1 mRNA or Pgp activity were observed, by either etoposide or rifampin. Pretreatment of osteosarcoma cells with ketoconazole, a PXR antagonist before exposure to etoposide, significantly increased the sensitivity of these cells to the selected chemotherapeutic agents (Mensah-Osman et al., 2007). Consequently, the use of compounds such as ketoconazole may be of value for the treatment of patients exhibiting high levels of PXR in their tumor biopsies.

Conclusion

PXR exerts its transcriptional regulation by binding to its DNA response elements as an heterodimer with RXR. The biological and physiological implications of PXR activation are broad, including the homeostasis of numerous endobiotics, such as glucose, lipids, steroids and bile acids. Recently, research has revealed new roles for PXR in inflammatory bowel disease, vitamin D metabolism and bone homeostasis, liver steatosis and antifibrogenesis. PXR activation results in regulation of drug-metabolizing enzymes and transporters transcription and has been established as a xenobiotic sensor that regulates xenobiotic clearance in the liver and intestine. Thus, PXR is implicated in drug metabolism and drug-drug interactions, while knowledge concerning its genetic polymorphisms may help to understand the variations in human drug response and ensure safe drug use.

PXR is involved in cancer in different ways, including direct induction of carcinogenesis in several tissue types (Fig. 3). PXR was found to be strongly expressed in colon tumor samples, while it was shown that both cell-cycle regulation and apoptosis may have a role in PXR-regulated suppression of colon cancer growth. PXR activation induced OATP1A2 expression, which enhances carcinogenesis, in breast tissues, in a time- and concentration-dependent manner. PXR is expressed in ovarian cancer cells and its activation induces cell proliferation and drug resistance, suggesting that PXR activation promotes the ''malignant'' phenotype of cancer cells. PXR mediates the genomic effects of steroid hormones, including estrogen, which has been shown to contribute greatly to growth and development in endometrial cancer. In prostate cancer,

when tumors progress to a more advanced stage, PXR expression tends to be reduced. Pretreatment with the PXR agonist SR12813 increased expression of CYP3A4 and MDR1 in prostate cancer cells. In another study, activation of PXR by genetic or pharmacological means was sufficient to at least partially inhibit androgen-responsive prostate regeneration and prostate cancer cell proliferation, by inducing the expression of CYP3A and SULT2A1.

Drug-mediated PXR activation can lead to undesired drug interactions and finally induce chemotherapy resistance. PXR activation was shown to protect PXR-expressing colon cancer cells from deoxycholic acid-induced apoptosis, as well as from adriamycin-induced cell death. PXR overexpression in colorectal cancer tissue samples led to a marked chemoresistance to the active metabolite of irinotecan, suggesting that PXR may play a key role in colon cancer cell response to anticancer drugs. PXR activation by a broad panel of chemotherapeutic agents was shown to reduce the cytotoxic activity of the Pgp substrate doxorubicin in colon cancer cells. Furthermore, rifampicin treatment increased survival of colon cancer cells towards oxaliplatin and 5-fluorouracil. PXR-mediated MRP2 induction seems to play a role in the additional acquisition of chemotherapy resistance in tamoxifen-resistant breast cancer. PXR-CYP3A expression in endometrial cancer tissues may cause resistance to anticancer agents, affecting the prognosis of endometrial cancer patients. Additionally, in endometrial cancer cells it was shown that down-regulation of PXR expression caused a significant increase in cell growth inhibition and apoptosis in the presence of the anticancer agents paclitaxel and cisplatin. hPXR activation may play an important role in prostate cancer resistance to chemotherapeutics, and the PXR-CYP and PXR-MDR1 pathways might be one of the main pathways for PXR to regulate drug resistance. Finally, pretreatment of osteosarcoma cells with the PXR antagonist ketoconazole, before exposure to etoposide, significantly increased the sensitivity of these cells to certain chemotherapeutic agents.

PXR genetic polymorphisms may also play a significant role in chemotherapy response and cancer prognosis. Doxorubicin clearance in breast cancer patients harboring the PXR*1B haplotypes was significantly lower compared with those carrying the non-PXR*1B ones. Breast cancer risk among postmenopausal women receiving menopausal hormone therapy was shown to be significantly modified by 2 PXR polymorphisms, with a reduction of risk effects in carriers of the minor PXR_rs6785049_G and PXR_rs1054191_A alleles. Neither the CYP3A5 A6986G (CYP3A5*3) nor the PXR C-25385T alleles were associated with altered plasma concentrations of paclitaxel in ovarian cancer patients.

Modulation of PXR function may offer potential applications for new therapies for colon cancer. PXR expression level could help physicians in the choice of appropriate chemotherapy regimen for colorectal cancer patients, while PXR down-regulation could be considered as a novel therapeutic approach to circumvent chemoresistance to chemotherapy. PXR activation in ovarian cancer cells induces cell proliferation and drug resistance, so blocking this process may have an impact on treatment outcome. PXR could represent a novel therapeutic approach for the augmentation of sensitivity to anticancer agents, or to overcome resistance to them, in the treatment of endometrial cancer. Additionally, inhibition of PXR could be a new approch to enhance the clinical efficacy of prostate cancer chemotherapy, while the role of PXR in androgen homeostasis may represent a therapeutic target for hormone-dependent prostate cancer. Finally, the use of PXR antagonists such as ketoconazole may be of value for the treatment of osteosarcoma patients exhibiting high levels of PXR in their tumor biopsies.

Consequently, PXR has various functions in human biology, but also possesses a crucial role in cancer and chemotherapy. Further studies are needed in order to establish the mechanisms involved in PXR-mediated carcinogenesis. Future research should be mainly focused on modulating PXR status in order to increase chemotherapy effectiveness and overall improve cancer patient outcome.

References

Albermann N., Schmitz-Winnenthal F.H., Z'Graggen K., Volk C., Hoffmann M.M., Haefeli W.E. and Weiss J. (2005). Expression of the drug transporters MDR1/ABCB1, MRP1/ABCC1, MRP2/ABCC2, BCRP/ABCG2, and PXR in peripheral blood mononuclear cells and their relationship with the expression in intestine and liver. Biochem. Pharmacol. 70, 949-958.

Andersen V., Christensen J., Overvad K., Tjonneland A. and Vogel U. (2010). Polymorphisms in NFkB, PXR, LXR and risk of colorectal cancer in a prospective study of Danes. BMC Cancer 10, 484.

Backman J.T., Olkkola K.T. and Neuvonen P.J. (1996). Rifampin drastically reduces plasma concentrations and effects of oral midazolam. Clin. Pharmacol. Ther. 59, 7-13.

Baker D.K., Relling M.V., Pui C.H., Christensen M.L., Evans W.E. and Rodman J.H. (1992). Increased teniposide clearance with concomitant anticonvulsant therapy. J. Clin. Oncol. 10, 311-315.

Beral V. (2003). Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 362, 419-427.

Bolton A.E., Peng B., Hubert M., Krebs-Brown A., Capdeville R., Keller U. and Seiberling M. (2004). Effect of rifampicin on the pharmacokinetics of imatinib mesylate (Gleevec, STI571) in healthy subjects. Cancer Chemother. Pharmacol. 53, 102-106.

Cerveny L., Svecova L., Anzenbacherova E., Vrzal R., Staud F., Dvorak Z., Ulrichova J., Anzenbacher P. and Pavek P. (2007). Valproic acid induces CYP3A4 and MDR1 gene expression by activation of constitutive androstane receptor and pregnane X receptor pathways. Drug Metab. Dispos. 35, 1032-1041.

Chang T.K., Yu L., Maurel P. and Waxman D.J. (1997). Enhanced cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response to cytochrome P-450 inducers and autoinduction by oxazaphosphorines. Cancer Res. 57, 1946-1954.

Chang S.M., Kuhn J.G., Rizzo J., Robins H.I., Schold S.C. Jr., Spence A.M., Berger M.S., Mehta M.P., Bozik M.E., Pollack I., Gilbert M., Fulton D., Rankin C., Malec M. and Prados M.D. (1998). Phase I study of paclitaxel in patients with recurrent malignant glioma: a North American Brain Tumor Consortium report. J. Clin. Oncol. 16, 2188-2194.

Chen T. (2010). Overcoming drug resistance by regulating nuclear receptors. Adv. Drug Deliv. Rev. 62, 1257-1264.

Chen Y., Tang Y., Wang M.T., Zeng S. and Nie D. (2007). Human pregnane X receptor and resistance to chemotherapy in prostate cancer. Cancer Res. 67, 10361-10367.

Chen Y., Tang Y., Robbins G.T. and Nie D. (2010). Camptothecin attenuates cytochrome P450 3A4 induction by blocking the activation of human pregnane X receptor. J. Pharmacol. Exp. Ther. 334, 999-1008.

Choi H.K., Yang J.W., Roh S.H., Han C.Y. and Kang K.W. (2007). Induction of multidrug resistance associated protein 2 in tamoxifen-resistant breast cancer cells. Endocr. Relat. Cancer 14, 293-303.

Dai G., He L., Bu P. and Wan Y.J. (2008). Pregnane X receptor is essential for normal progression of liver regeneration. Hepatology 47, 1277-1287.

Desai P.B., Nallani S.C., Sane R.S., Moore L.B., Goodwin B.J., Buckley D.J. and Buckley A.R. (2002). 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. 30, 608-612.

Desta Z., Ward B.A., Soukhova N.V. and Flockhart D.A. (2004). Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J. Pharmacol. Exp. Ther. 310, 1062-1075.

di Masi A., De Marinis E., Ascenzi P. and Marino M. (2009). Nuclear receptors CAR and PXR: Molecular, functional, and biomedical aspects. Mol. Aspects Med. 30, 297-343.

Dotzlaw H., Leygue E., Watson P. and Murphy L.C. (1999). The human orphan receptor PXR messenger RNA is expressed in both normal and neoplastic breast tissue. Clin. Cancer Res. 5, 2103-2107.

Dowsett M., Cuzick J., Howell A. and Jackson I. (2001). Pharmacokinetics of anastrozole and tamoxifen alone, and in combination, during adjuvant endocrine therapy for early breast cancer in postmenopausal women: a sub-protocol of the 'Arimidex and tamoxifen alone or in combination' (ATAC) trial. Br. J. Cancer 85, 317-324.

Druker B.J., Talpaz M., Resta D.J., Peng B., Buchdunger E., Ford J.M., Lydon N.B., Kantarjian H., Capdeville R., Ohno-Jones S. and Sawyers C.L. (2001). Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N. Engl. J. Med. 344, 1031-1037.

Gong H., Singh S.V., Singh S.P., Mu Y., Lee J.H., Saini S.P., Toma D., Ren S., Kagan V.E., Day B.W., Zimniak P. and Xie W. (2006). Orphan nuclear receptor pregnane X receptor sensitizes oxidative stress responses in transgenic mice and cancerous cells. Mol. Endocrinol. 20, 279-290.

Gupta E., Lestingi T.M., Mick R., Ramirez J., Vokes E.E. and Ratain M.J. (1994). Metabolic fate of irinotecan in humans: correlation of glucuronidation with diarrhea. Cancer Res. 54, 3723-3725.

Gupta A., Mugundu G.M., Desai P.B., Thummel K.E. and Unadkat J.D. (2008a). Intestinal human colon adenocarcinoma cell line LS180 is an excellent model to study pregnane X receptor, but not constitutive androstane receptor, mediated CYP3A4 and multidrug resistance transporter 1 induction: studies with anti-human immunodeficiency virus protease inhibitors. Drug Metab. Dispos. 36, 1172-1180.

Gupta D., Venkatesh M., Wang H., Kim S., Sinz M., Goldberg G.L., Whitney K., Longley C. and Mani S. (2008b). Expanding the roles for pregnane X receptor in cancer: proliferation and drug resistance in ovarian cancer. Clin. Cancer Res. 14, 5332-5340.

Habano W., Gamo T., Terashima J., Sugai T., Otsuka K., Wakabayashi G. and Ozawa S. (2011). Involvement of promoter methylation in the regulation of Pregnane X receptor in colon cancer cells. BMC Cancer 11, 81.

Han Y. and Sugiyama Y. (2006). Expression and regulation of breast cancer resistance protein and multidrug resistance associated protein 2 in BALB/c mice. Biol. Pharm. Bull. 29, 1032-1035.

Harmsen S., Meijerman I., Beijnen J.H. and Schellens J.H. (2007). The role of nuclear receptors in pharmacokinetic drug-drug interactions in oncology. Cancer Treat. Rev. 33, 369-380.

Harmsen S., Meijerman I., Febus C.L., Maas-Bakker R.F., Beijnen J.H. and Schellens J.H. (2010). PXR-mediated induction of P-glycoprotein by anticancer drugs in a human colon adenocarcinoma-derived cell line. Cancer Chemother. Pharmacol. 66, 765-771.

Haughton E.L., Tucker S.J., Marek C.J., Durward E., Leel V., Bascal Z., Monaghan T., Koruth M., Collie-Duguid E., Mann D.A., Trim J.E. and Wright M.C. (2006). Pregnane X receptor activators inhibit human hepatic stellate cell transdifferentiation in vitro. Gastroenterology 131, 194-209.

Healan-Greenberg C., Waring J.F., Kempf D.J., Blomme E.A., Tirona R.G. and Kim R.B. (2008). A human immunodeficiency virus protease inhibitor is a novel functional inhibitor of human pregnane X receptor. Drug Metab. Dispos. 36, 500-507.

Honkakoski P., Sueyoshi T. and Negishi M. (2003). Drug-activated nuclear receptors CAR and PXR. Ann. Med. 35, 172-182.

Honorat M., Mesnier A., Di Pietro A., Lin V., Cohen P., Dumontet C. and Payen L. (2008). Dexamethasone down-regulates ABCG2 expression levels in breast cancer cells. Biochem. Biophys. Res. Commun. 375, 308-314.

Hor S.Y., Lee S.C., Wong C.I., Lim Y.W., Lim R.C., Wang L.Z., Fan L., Guo J.Y., Lee H.S., Goh B.C. and Tan T. (2008). PXR, CAR and HNF4alpha genotypes and their association with pharmacokinetics and pharmacodynamics of docetaxel and doxorubicin in Asian patients. Pharmacogenomics J. 8, 139-146.

Huang H., Wang H., Sinz M., Zoeckler M., Staudinger J., Redinbo M.R., Teotico D.G., Locker J., Kalpana G.V. and Mani S. (2007). Inhibition of drug metabolism by blocking the activation of nuclear receptors by ketoconazole. Oncogene 26, 258-268.

Huang R., Murry D.J., Kolwankar D., Hall S.D. and Foster D.R. (2006). Vincristine transcriptional regulation of efflux drug transporters in carcinoma cell lines. Biochem. Pharmacol. 71, 1695-1704.

Ivanovic J., Nicastri E., Ascenzi P., Bellagamba R., De Marinis E., Notari S., Pucillo L.P., Tozzi V., Ippolito G. and Narciso P. (2008). Therapeutic drug monitoring in the management of HIV-infected patients. Curr. Med. Chem. 15, 1925-1939.

Jiang H., Chen K., He J., Pan F., Li J., Chen J., Chen W. and Liang H. (2009). Association of pregnane X receptor with multidrug resistance-related protein 3 and its role in human colon cancer chemoresistance. J. Gastrointest. Surg. 13, 1831-1838.

Justenhoven C., Schaeffeler E., Winter S., Baisch C., Hamann U., Harth V., Rabstein S., Spickenheuer A., Pesch B., Bruning T., Ko Y.D.,

Schwab M. and Brauch H. (2011). Polymorphisms of the nuclear receptor pregnane X receptor and organic anion transporter polypeptides 1A2, 1B1, 1B3, and 2B1 are not associated with breast cancer risk. Breast Cancer Res. Treat. 125, 563-569.

Kerbusch T., Jansen R.L., Mathot R.A., Huitema A.D., Jansen M., van Rijswijk R.E. and Beijnen J.H. (2001). Modulation of the cytochrome P450-mediated metabolism of ifosfamide by ketoconazole and rifampin. Clin. Pharmacol. Ther. 70, 132-141.

Konig J., Seithel A., Gradhand U. and Fromm M.F. (2006). Pharmacogenomics of human OATP transporters. Naunyn Schmiedebergs Arch. Pharmacol. 372, 432-443.

Kuan C.Y., Walker T.H., Luo P.G. and Chen C.F. (2011). Long-chain polyunsaturated fatty acids promote paclitaxel cytotoxicity via inhibition of the MDR1 gene in the human colon cancer Caco-2 cell line. J. Am. Coll. Nutr. 30, 265-273.

Kublbeck J., Reinisalo M., Mustonen R. and Honkakoski P. (2010). Up-regulation of CYP expression in hepatoma cells stably transfected by chimeric nuclear receptors. Eur. J. Pharm. Sci. 40, 263-272.

Kumar S., Jaiswal B., Negi S. and Tyagi R.K. (2010). Cross-talk between androgen receptor and pregnane and xenobiotic receptor reveals existence of a novel modulatory action of anti-androgenic drugs. Biochem. Pharmacol. 80, 964-976.

Lamba V., Yasuda K., Lamba J.K., Assem M., Davila J., Strom S. and Schuetz E.G. (2004). PXR (NR1I2): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. Toxicol. Appl. Pharmacol. 199, 251-265.

Lee J.H., Zhou J. and Xie W. (2008). PXR and LXR in hepatic steatosis: a new dog and an old dog with new tricks. Mol. Pharm. 5, 60-66.

Legendre C., Hori T., Loyer P., Aninat C., Ishida S., Glaise D., Lucas-Clerc C., Boudjema K., Guguen-Guillouzo C., Corlu A. and Morel F. (2009). Drug-metabolising enzymes are down-regulated by hypoxia in differentiated human hepatoma HepaRG cells: HIF-1alpha involvement in CYP3A4 repression. Eur. J. Cancer 45, 2882-2892.

Lehmann J.M., McKee D.D., Watson M.A., Willson T.M., Moore J.T. and Kliewer S.A. (1998). The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. J. Clin. Invest. 102, 1016-1023.

Lemaire G., Mnif W., Pascussi J.M., Pillon A., Rabenoelina F., Fenet H., Gomez E., Casellas C., Nicolas J.C., Cavailles V., Duchesne M.J. and Balaguer P. (2006). Identification of new human pregnane X receptor ligands among pesticides using a stable reporter cell system. Toxicol. Sci. 91, 501-509.

Lim H.S., Ju Lee H., Seok Lee K., Sook Lee E., Jang I.J. and Ro J. (2007). Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. J. Clin. Oncol. 25, 3837-3845.

Ma X., Idle J.R. and Gonzalez F.J. (2008). The pregnane X receptor: from bench to bedside. Expert Opin. Drug Metab. Toxicol. 4, 895-908.

Maier A., Zimmermann C., Beglinger C., Drewe J. and Gutmann H. (2007). Effects of budesonide on P-glycoprotein expression in intestinal cell lines. Br. J. Pharmacol. 150, 361-368.

Malekshah O.M., Bahrami A.R., Afshari J.T., Mosaffa F. and Behravan J. (2011). Correlation between PXR and ABCG2 patterns of mRNA expression in a MCF7 breast carcinoma cell derivative upon induction by proinflammatory cytokines. DNA Cell Biol. 30, 25-31.

Mani S., Huang H., Sundarababu S., Liu W., Kalpana G., Smith A.B. and Horwitz S.B. (2005). Activation of the steroid and xenobiotic receptor (human pregnane X receptor) by nontaxane microtubule-stabilizing agents. Clin. Cancer Res. 11, 6359-6369.

Marek C.J., Tucker S.J., Konstantinou D.K., Elrick L.J., Haefner D., Sigalas C., Murray G.I., Goodwin B. and Wright M.C. (2005). Pregnenolone-16alpha-carbonitrile inhibits rodent liver fibrogenesis via PXR (pregnane X receptor)-dependent and PXR-independent mechanisms. Biochem. J. 387, 601-608.

MARIE-GENICA (2010). Polymorphisms in genes of the steroid receptor superfamily modify postmenopausal breast cancer risk associated with menopausal hormone therapy. Int. J. Cancer 126, 2935-2946.

Masuyama H., Hiramatsu Y., Kodama J. and Kudo T. (2003). Expression and potential roles of pregnane X receptor in endometrial cancer. J. Clin. Endocrinol. Metab. 88, 4446-4454.

Masuyama H., Suwaki N., Tateishi Y., Nakatsukasa H., Segawa T. and Hiramatsu Y. (2005). The pregnane X receptor regulates gene expression in a ligand- and promoter-selective fashion. Mol. Endocrinol. 19, 1170-1180.

Masuyama H., Nakatsukasa H., Takamoto N. and Hiramatsu Y. (2007). Down-regulation of pregnane X receptor contributes to cell growth inhibition and apoptosis by anticancer agents in endometrial cancer cells. Mol. Pharmacol. 72, 1045-1053.

Mensah-Osman E.J., Thomas D.G., Tabb M.M., Larios J.M., Hughes D.P., Giordano T.J., Lizyness M.L., Rae J.M., Blumberg B., Hollenberg P.F. and Baker L.H. (2007). Expression levels and activation of a PXR variant are directly related to drug resistance in osteosarcoma cell lines. Cancer 109, 957-965.

Meyer zu Schwabedissen H.E., Tirona R.G., Yip C.S., Ho R.H. and Kim R.B. (2008). Interplay between the nuclear receptor pregnane X receptor and the uptake transporter organic anion transporter polypeptide 1A2 selectively enhances estrogen effects in breast cancer. Cancer Res. 68, 9338-9347.

Miyoshi Y., Ando A., Takamura Y., Taguchi T., Tamaki Y. and Noguchi S. (2002). Prediction of response to docetaxel by CYP3A4 mRNA expression in breast cancer tissues. Int. J. Cancer 97, 129-132.

Murakami Y., Tanaka T., Murakami H., Tsujimoto M., Ohtani H. and Sawada Y. (2006). Pharmacokinetic modelling of the interaction between St John's wort and ciclosporin A. Br. J. Clin. Pharmacol. 61, 671-676.

Murry D.J., Cherrick I., Salama V., Berg S., Bernstein M., Kuttesch N. and Blaney S.M. (2002). Influence of phenytoin on the disposition of irinotecan: a case report. J. Pediatr. Hematol. Oncol. 24, 130-133.

Nguyen A. and Bouscarel B. (2008). Bile acids and signal transduction: role in glucose homeostasis. Cell. Signal. 20, 2180-2197.

Niemi M., Backman J.T., Fromm M.F., Neuvonen P.J. and Kivisto K.T. (2003). Pharmacokinetic interactions with rifampicin: clinical relevance. Clin. Pharmacokinet. 42, 819-850.

Nittke T., Selig S., Kallay E. and Cross H.S. (2008). Nutritional calcium modulates colonic expression of vitamin D receptor and pregnane X receptor target genes. Mol. Nutr. Food Res. 52 (Suppl 1), S45-51.

Ouyang N., Ke S., Eagleton N., Xie Y., Chen G., Laffins B., Yao H., Zhou B. and Tian Y. (2010). Pregnane X receptor suppresses proliferation and tumourigenicity of colon cancer cells. Br. J. Cancer 102, 1753-1761.

Owen A., Chandler B., Back D.J. and Khoo S.H. (2004). Expression of pregnane-X-receptor transcript in peripheral blood mononuclear cells and correlation with MDR1 mRNA. Antivir. Ther. 9, 819-821.

Pascussi J.M., Robert A., Nguyen M., Walrant-Debray O., Garabedian M., Martin P., Pineau T., Saric J., Navarro F., Maurel P. and Vilarem M.J. (2005). Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. J. Clin. Invest.

115, 177-186.

Persson K.P., Ekehed S., Otter C., Lutz E.S., McPheat J., Masimirembwa C.M. and Andersson T.B. (2006). Evaluation of human liver slices and reporter gene assays as systems for predicting the cytochrome p450 induction potential of drugs in vivo in humans. Pharm. Res. 23, 56-69.

Quattrochi L.C. and Guzelian P.S. (2001). Cyp3A regulation: from pharmacology to nuclear receptors. Drug Metab. Dispos. 29, 615-622.

Raynal C., Pascussi J.M., Leguelinel G., Breuker C., Kantar J., Lallemant B., Poujol S., Bonnans C., Joubert D., Hollande F., Lumbroso S., Brouillet J.P. and Evrard A. (2010). Pregnane X Receptor (PXR) expression in colorectal cancer cells restricts irinotecan chemosensitivity through enhanced SN-38 glucuronidation. Mol. Cancer 9, 46.

Relling M.V., Pui C.H., Sandlund J.T., Rivera G.K., Hancock M.L., Boyett J.M., Schuetz E.G. and Evans W.E. (2000). Adverse effect of anticonvulsants on efficacy of chemotherapy for acute lymphoblastic leukaemia. Lancet 356, 285-290.

Robey R.W., Polgar O., Deeken J., To K.W. and Bates S.E. (2007). ABCG2: determining its relevance in clinical drug resistance. Cancer Metastasis Rev. 26, 39-57.

Rosenfeld J.M., Vargas R. Jr, Xie W. and Evans R.M. (2003). Genetic profiling defines the xenobiotic gene network controlled by the nuclear receptor pregnane X receptor. Mol. Endocrinol. 17, 1268-1282.

Ross J., Plummer S.M., Rode A., Scheer N., Bower C.C., Vogel O., Henderson C.J., Wolf C.R. and Elcombe C.R. (2010). Human constitutive androstane receptor (CAR) and pregnane X receptor (PXR) support the hypertrophic but not the hyperplastic response to the murine nongenotoxic hepatocarcinogens phenobarbital and chlordane in vivo. Toxicol. Sci. 116, 452-466.

Sandanaraj E., Lal S., Selvarajan V., Ooi L.L., Wong Z.W., Wong N.S., Ang P.C., Lee E.J. and Chowbay B. (2008). PXR pharmacogenetics: association of haplotypes with hepatic CYP3A4 and ABCB1 messenger RNA expression and doxorubicin clearance in Asian breast cancer patients. Clin. Cancer Res. 14, 7116-7126.

Shah I., Houck K., Judson R.S., Kavlock R.J., Martin M.T., Reif D.M., Wambaugh J. and Dix D.J. (2011). Using nuclear receptor activity to stratify hepatocarcinogens. PLoS One 6, e14584.

Shibayama Y., Ushinohama K., Ikeda R., Yoshikawa Y., Motoya T., Takeda Y. and Yamada K. (2006). Effect of methotrexate treatment on expression levels of multidrug resistance protein 2, breast cancer resistance protein and organic anion transporters Oat1, Oat2 and Oat3 in rats. Cancer Sci. 97, 1260-1266.

Spitzweg C. and Morris J.C. (2002). The sodium iodide symporter: its pathophysiological and therapeutic implications. Clin. Endocrinol. (Oxf) 57, 559-574.

Staudinger J.L., Ding X. and Lichti K. (2006). Pregnane X receptor and natural products: beyond drug-drug interactions. Expert Opin. Drug Metab. Toxicol. 2, 847-857.

Staudinger J.L., Goodwin B., Jones S.A., Hawkins-Brown D., MacKenzie K.I., LaTour A., Liu Y., Klaassen C.D., Brown K.K., Reinhard J., Willson T.M., Koller B.H. and Kliewer S.A. (2001). The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc. Natl. Acad. Sci. USA 98, 3369-3374.

Synold T.W., Dussault I. and Forman B.M. (2001). The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. Nat. Med. 7, 584-590.

Tabb M.M., Sun A., Zhou C., Grun F., Errandi J., Romero K., Pham H., Inoue S., Mallick S., Lin M., Forman B.M. and Blumberg B. (2003). Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. J. Biol. Chem. 278, 43919-43927.

Tachibana S., Yoshinari K., Chikada T., Toriyabe T., Nagata K. and Yamazoe Y. (2009). Involvement of Vitamin D receptor in the intestinal induction of human ABCB1. Drug Metab. Dispos. 37, 1604-1610.

Tham L.S., Holford N.H., Hor S.Y., Tan T., Wang L., Lim R.C., Lee H.S., Lee S.C. and Goh B.C. (2007). Lack of association of single-nucleotide polymorphisms in pregnane X receptor, hepatic nuclear factor 4alpha, and constitutive androstane receptor with docetaxel pharmacokinetics. Clin. Cancer Res. 13, 7126-7132.

Timsit Y.E. and Negishi M. (2007). CAR and PXR: the xenobiotic-sensing receptors. Steroids 72, 231-246.

van de Winkel A., van Zoest K.P., van Dekken H., Moons L.M., Kuipers E.J. and van der Laan L.J. (2011). Differential expression of the nuclear receptors farnesoid X receptor (FXR) and pregnane X receptor (PXR) for grading dysplasia in patients with Barrett's oesophagus. Histopathology 58, 246-253.

Wahli W. (2008). A gut feeling of the PXR, PPAR and NF-kappaB connection. J. Intern. Med. 263, 613-619.

Wang H., Huang H., Li H., Teotico D.G., Sinz M., Baker S.D., Staudinger J., Kalpana G., Redinbo M.R. and Mani S. (2007). Activated pregnenolone X-receptor is a target for ketoconazole and its analogs. Clin. Cancer Res. 13, 2488-2495.

Wang H., Li H., Moore L.B., Johnson M.D., Maglich J.M., Goodwin B., Ittoop O.R., Wisely B., Creech K., Parks D.J., Collins J.L., Willson T.M., Kalpana G.V., Venkatesh M., Xie W., Cho S.Y., Roboz J., Redinbo M., Moore J.T. and Mani S. (2008a). The phytoestrogen coumestrol is a naturally occurring antagonist of the human pregnane X receptor. Mol. Endocrinol. 22, 838-857.

Wang T., Ma X., Krausz K.W., Idle J.R. and Gonzalez F.J. (2008b). Role of pregnane X receptor in control of all-trans retinoic acid (ATRA) metabolism and its potential contribution to ATRA resistance. J. Pharmacol. Exp. Ther. 324, 674-684.

Willhauck M.J., Okane D.J., Wunderlich N., Goke B. and Spitzweg C. (2011). Stimulation of retinoic acid-induced functional sodium iodide symporter (NIS) expression and cytotoxicity of (1)(3)(1)I by carbamazepine in breast cancer cells. Breast Cancer Res. Treat. 125, 377-386.

Xie Y., Ke S., Ouyang N., He J., Xie W., Bedford M.T. and Tian Y. (2009). Epigenetic regulation of transcriptional activity of pregnane X receptor by protein arginine methyltransferase 1. J. Biol. Chem. 284, 9199-9205.

Yamaguchi H., Hishinuma T., Endo N., Tsukamoto H., Kishikawa Y., Sato M., Murai Y., Hiratsuka M., Ito K., Okamura C., Yaegashi N., Suzuki N., Tomioka Y. and Goto J. (2006). Genetic variation in ABCB1 influences paclitaxel pharmacokinetics in Japanese patients with ovarian cancer. Int. J. Gynecol. Cancer 16, 979-985.

Yeung E.Y., Sueyoshi T., Negishi M. and Chang T.K. (2008). Identification of Ginkgo biloba as a novel activator of pregnane X receptor. Drug Metab. Dispos. 36, 2270-2276.

Zamboni W.C., Gajjar A.J., Heideman R.L., Beijnen J.H., Rosing H., Houghton P.J. and Stewart C.F. (1998). Phenytoin alters the disposition of topotecan and N-desmethyl topotecan in a patient with medulloblastoma. Clin. Cancer Res. 4, 783-789.

Zelunka E.J. (2002). Intravenous cyclosporine-rifampin interaction in a pediatric bone marrow transplant recipient. Pharmacotherapy 22, 387-390.

Zhai Y., Pai H.V., Zhou J., Amico J.A., Vollmer R.R. and Xie W. (2007). Activation of pregnane X receptor disrupts glucocorticoid and mineralocorticoid homeostasis. Mol. Endocrinol. 21, 138-147.

Zhang B., Cheng Q., Ou Z., Lee J.H., Xu M., Kochhar U., Ren S., Huang M., Pflug B.R. and Xie W. (2010). Pregnane X receptor as a therapeutic target to inhibit androgen activity. Endocrinology 151, 5721-5729.

Zhang J., Kuehl P., Green E.D., Touchman J.W., Watkins P.B., Daly A., Hall S.D., Maurel P., Relling M., Brimer C., Yasuda K., Wrighton S.A., Hancock M., Kim R.B., Strom S., Thummel K., Russell C.G., Hudson J.R. Jr, Schuetz E.G. and Boguski M.S. (2001). The human pregnane x receptor: Genomic structure and identification and functional characterization of natural allelic variants. Pharmacogenetics 11, 555-572.

Zhou C., Tabb M.M., Nelson E.L., Grun F., Verma S., Sadatrafiei A., Lin M., Mallick S., Forman B.M., Thummel K.E. and Blumberg B. (2006). Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. J. Clin. Invest. 116, 2280-2289.

Zhou C., Poulton E.J., Grun F., Bammler T.K., Blumberg B., Thummel K.E. and Eaton D.L. (2007). The dietary isothiocyanate sulforaphane is an antagonist of the human steroid and xenobiotic nuclear receptor. Mol. Pharmacol. 71, 220-229.

Zhou C., Verma S. and Blumberg B. (2009). The steroid and xenobiotic receptor (SXR), beyond xenobiotic metabolism. Nucl. Recept. Signal. 7, e001.

Zhou J., Liu M., Zhai Y. and Xie W. (2008). The antiapoptotic role of pregnane X receptor in human colon cancer cells. Mol. Endocrinol. 22, 868-880.

Zimmermann K., Wittman M.D., Saulnier M.G., Velaparthi U., Sang X., Frennesson D.B., Struzynski C., Seitz S.P., He L., Carboni J.M., Li A., Greer A.F., Gottardis M., Attar R.M., Yang Z., Balimane P., Discenza L.N., Lee F.Y., Sinz M., Kim S. and Vyas D. (2010). SAR of PXR transactivation in benzimidazole-based IGF-1R kinase inhibitors. Bioorg. Med. Chem. Lett. 20, 1744-1748.

Accepted November 15, 2012