View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by IntechOpen

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

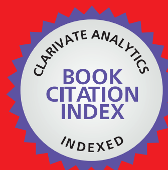
135M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

13

Pregnane X Receptor in Drug Development

Su Sien Ong[1], Yue-Ming Wang[1], Sergio C. Chai[1,2] and Taosheng Chen[2]
St. Jude Children's Research Hospital
USA

1. Introduction

Benzoate X receptor (BXR) was identified in *Xenopus* and named BXR for its recognition of benzoates as substrates (Blumberg et al. 1998a). Subsequently, a related mouse gene was identified based on its sequence homology to other known nuclear receptors (NRs) and named pregnane X receptor (PXR) for its activation by pregnane steroids (Kliewer et al. 1998). Upon its identification, the human PXR gene was first given the name SXR for steroid and xenobiotics receptor but was subsequently coined human PXR (hPXR) (Bertilsson et al. 1998; Blumberg et al. 1998b; Lehmann et al. 1998). PXR is a broad-spectrum xenobiotic sensor and master transcriptional regulator of xenobiotic detoxification and metabolism genes, capable of being activated by structurally diverse ligands, including many commercially marketed chemotherapeutics. Upon ligand engagement, PXR binds to the promoter regions of its target genes as a heterodimer with another NR, retinoic X receptor (RXR) to initiate gene transcription (Bertilsson et al. 1998; Kliewer et al. 1998). Target genes of PXR include genes for phase I and phase II drug metabolizing enzymes (DMEs) and phase III ATP binding cassette (ABC) drug transporters. The two most important target genes of PXR are cytochrome P450 3A4 (*CYP3A4*) and multidrug resistance 1 (*MDR1*). CYP3A4 is most abundantly expressed in the liver and is the primary contributor to metabolizing most of the currently marketed therapeutic agents (Ingelman-Sundberg 2004). MDR1 is involved in drug resistance. Induction of CYP3A4 and MDR1 contributes to clinical drug-drug interactions and drug resistance. In addition, PXR plays roles in many other important physiologic and pathologic processes, such as those in bone disorders, liver diseases, inflammation, and cancers. Topics in this chapter include structural-functional analysis of PXR, regulation of PXR and its target genes, physiologic and pathologic functions of PXR, and relevant drug discovery techniques for PXR. Importantly, this chapter highlights PXR as an appealing target for both the development of novel drugs and the improvement of current drug therapies.

2. Structure of Pregnane X Receptor

PXR shares common structural features that are characteristic of NRs (Ingraham & Redinbo 2005). A DNA-binding domain (DBD) residing at the amino terminus allows for the NR to

[1] These co-first authors contribute equally: the first draft was prepared by S.S.O. (sections 1, 3, 7.3. and 8), S.C.C. (sections 2 and 7.1.-7.2.), and Y-M.W. (sections 4-6 and 7.4.) and assembled by S.S.O.;
[2] Corresponding authors

bind to hormone response elements (HREs), facilitated by the presence of two zinc-finger motifs. The ligand-binding domain (LBD) is the most prominent feature in PXR. Crystal structures of the LBD shed light on the promiscuity of PXR, which depict a flexible and substantially large cavity, with a volume > 1600 Å (Fig. 1) (Watkins et al. 2001). According to the 3-D structure, the LBD comprises three sets of α-helices: α1/α3, α4/α5/α8, and α7/α10. In addition, a layer of five stranded anti-parallel β-sheets includes two novel β-strands not observed in other NRs: β1 and β1′. In contrast to other NRs of known structures, PXR contains an insert of approximately 60 amino acids between helices α1 and α3, which contribute to the formation of the novel helix α2, β1, and β1′. In hPXR, a flexible loop encompassing residues 309-321 replaces helix α6 (Orans et al. 2005).

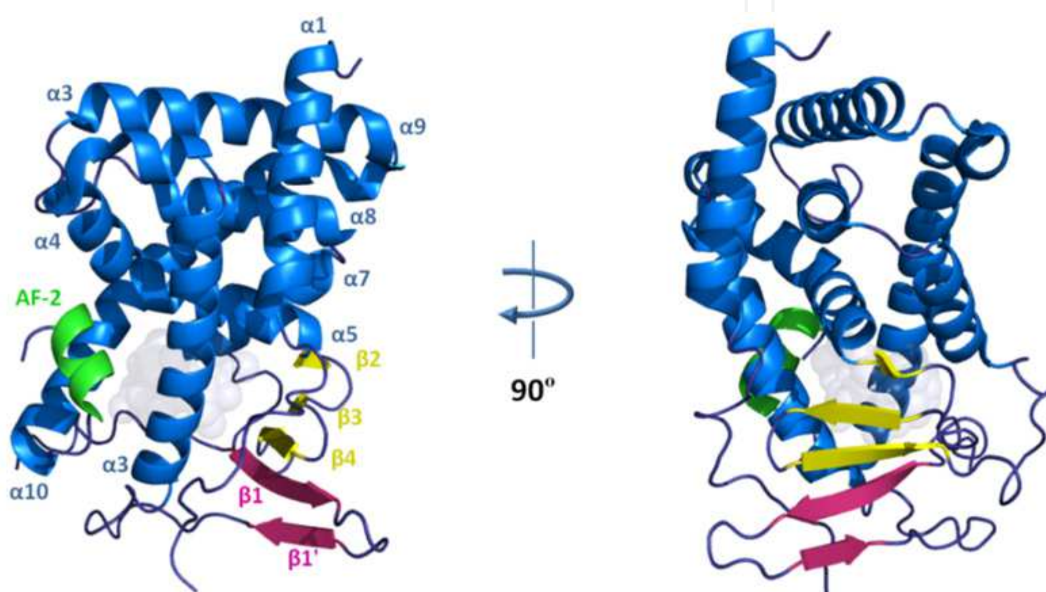


Fig. 1. Crystal structure of the hPXR LBD (PDB code 1ILH). The α-helices are rendered in blue, β-sheets present in other NRs are shown in yellow, β-sheets unique to PXR are represented in magenta, and the AF-2 helix is depicted in green. The three orientations of the agonist SR12813 are shown as transparent spheres, indicating the ligand-binding cavity.

PXR was shown to homodimerize in solution (Noble et al. 2006). Crystal structures indicate the involvement of the terminal β1′ strands from each monomer to form the dimer interface, supported by six intermolecular hydrogen bonds (Fig. 2). In addition, Trp223 and Tyr225 from each monomer interlock to form a tryptophan zipper, the first to be observed in a native protein. The Trp223Ala, Tyr225Ala double mutant prevents homodimerization without affecting protein folding. The mutant retained ligand and DNA binding capabilities but exhibited a much reduced CYP3A4 induction in response to PXR agonists SR12813 and rifampicin. This impairment was believed to be due to a disruption of coactivator recruitment.

PXR interacts with p160/SRC coactivators such as the steroid receptor coactivator 1 (SRC-1) through the active conformation of the ligand-dependent activating function 2 (AF-2) helix within the LBD (Watkins et al. 2003a). These coactivators contain three LXXLL motifs (L=Leu, X=any other amino acid), which also adopt α-helical conformations and interact with the NR via a “charge clamp”. A 25-mer SRC-1 peptide containing the second LXXLL motif was co-crystallized with hPXR (Fig. 2). The LXXLL region of the peptide is buried in a

groove on the surface of the PXR LBD composed of AF-2, α3, and α4. Binding of the SRC-1 peptide stabilized the LBD. Not surprisingly, coactivator peptides were reported to be required for stable expression and purification of the protein in bacterial systems.

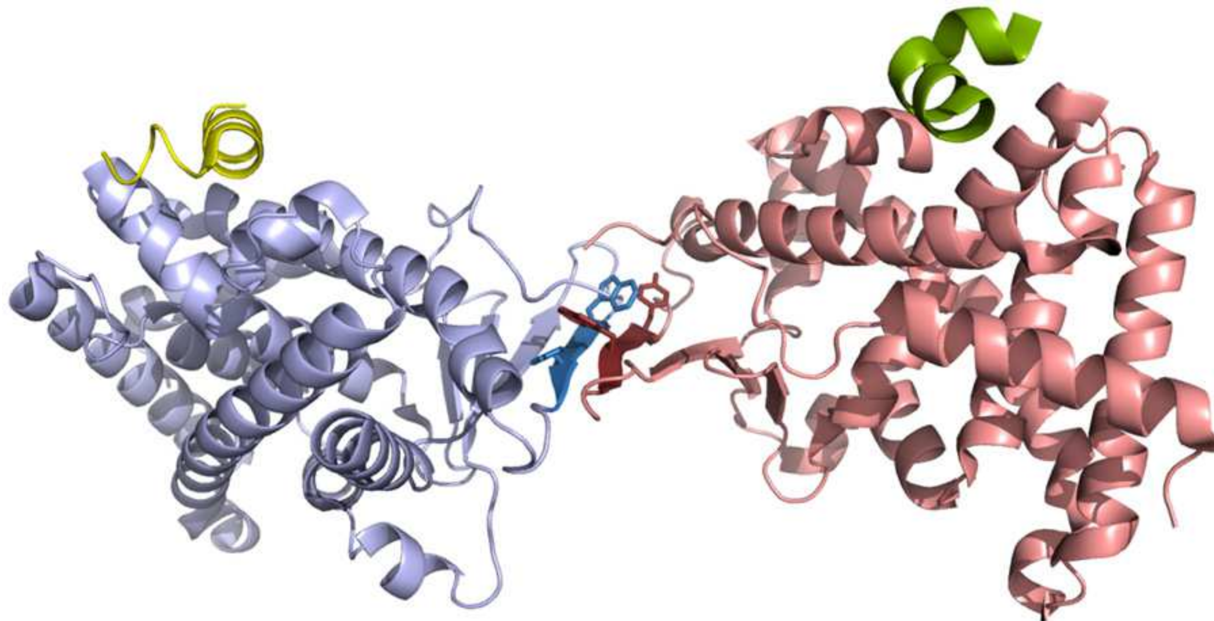


Fig. 2. Structure of hPXR illustrating the homodimerization of the receptor (PDB code 1NRL). Each monomer is represented as either marine blue or salmon red, with the respective β1′ rendered as blue or red, respectively. Trp223 and Tyr225 from each monomer are represented as sticks. The SRC-1 peptides are shown as either yellow or green.

The ligand-binding pocket is formed in large part by non-polar residues, creating a mostly hydrophobic and uncharged cavity (Fig. 3A). Of the four charged amino acid side chains present in the pocket (Glu321, His327, His407, and Arg410), a salt bridge was observed between Glu321 and Arg410. Another salt bridge occurs between Asp205 and Arg413 surrounding the ligand-binding pocket. The amino acid residues participating in these electrostatic interactions were shown to be important in the basal activity of PXR based on mutagenesis and cell-based reporter assays (Watkins et al. 2001). Some of these residues along four polar residues (Ser208, Ser247, Cys284, and Gln285) can form critical interactions with the ligand. The structural models also provide insights into the marked differences in the activation of PXR across species. For instance, SR12813 selectively activates hPXR over the mouse PXR (mPXR). When residues that are unique and are involved in SR12813 binding to hPXR were incorporated in mPXR, the mouse-human hybrid PXR responded efficiently to SR12813.

Several x-ray structures of the PXR LBD in complex with agonists have been reported. The first complex showed the cholesterol-lowering drug SR12813 in three distinct positions within the cavity (Watkins et al. 2001). A more recent crystallographically determined structure involving PXR-SR12813 in complex with an SRC-1 coactivator peptide revealed a single agonist binding mode (Fig. 3B) (Watkins et al. 2003a). Thus, Redinbo and coworkers argued that the PXR ligand-binding pocket can accommodate ligands in multiple positions, and upon coactivator binding, the ligand is stabilized into a single active orientation. In this “active” state, SR12813 interacts with Ser247 and His407 through hydrogen bonding involving 11 hydrophobic amino acid side chains. The other PXR agonists that were

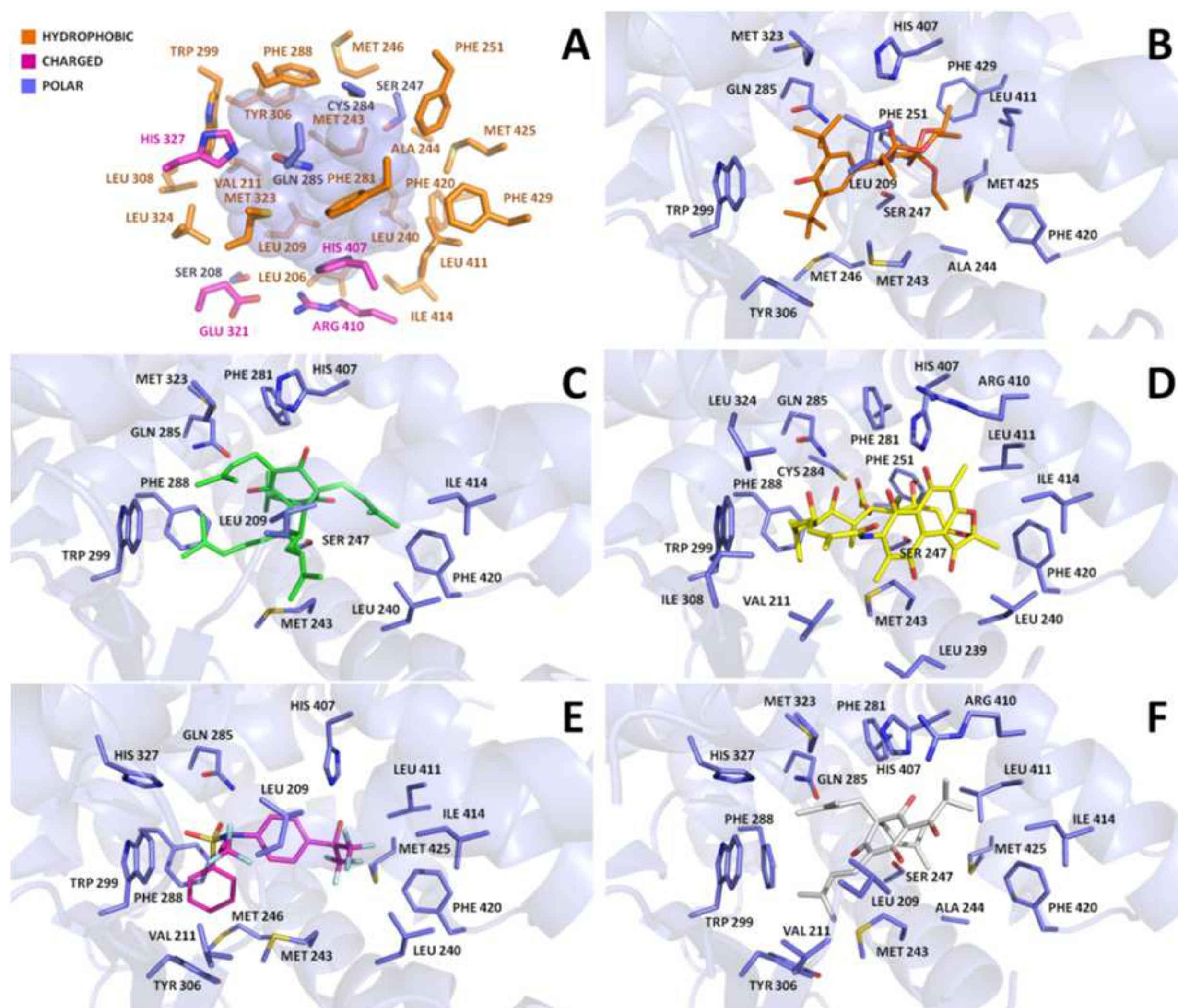


Fig. 3. A) Stick representation of the amino acids forming the ligand-binding pocket of hPXR (PDB code 1ILH). The carbon atoms of the hydrophobic residues are shown in orange, charged residues in magenta and polar residues in marine blue. The areas occupied by the three binding modes of the agonist SR12813 are shown as transparent spheres. B) hPXR LBD in complex with SR12813 in a single orientation (PDB code 1NRL). Carbon atoms of the ligand are shown in orange. C) hPXR LBD in complex with hyperforin (PDB code 1M13). Carbon atoms of the ligand are shown in green. D) hPXR LBD in complex with rifampicin (PDB code 1SKX). Carbon atoms of the ligand are shown in yellow. E) hPXR LBD in complex with TO901317 (PDB code 2O9I). Carbon atoms of the ligand are shown in magenta. F) hPXR LBD in complex with colupulone (PDB code 2QNV). Carbon atoms of the ligand are shown in gray. For panels B-F, the structures are viewed from the same orientation. The carbon atoms of the LBD residues are illustrated in marine blue. For panels A-F, oxygen, nitrogen, and sulfur atoms are depicted in red, blue, and yellow, respectively.

examined by crystallography in complex with hPXR also reveal the importance of hydrogen bond interactions of the ligand and the residues forming the cavity, in addition to the extended hydrophobic contacts. The active component of the herbal antidepressant St. John's wort, hyperforin, forms hydrogen bonds with Ser247, Gln285, and His407 (Fig. 3C) (Watkins et al. 2003b). The antibiotic rifampicin, one of the largest known PXR ligands, also

forms hydrogen bonds with Ser247, Gln285, and His407 (Fig. 3D) (Chrencik et al. 2005). The liver X receptor (LXR) agonist T0901317 was shown to interact with the hPXR LBD through polar interactions with Gln285 and His407 (Fig. 3E) (Xue et al. 2007). A third hydrogen bond involved His327, which has not been observed to interact with ligands in previous structures. The hops constituent colupulone forms hydrogen bonds with His407 and bonds to Gln285 through a water molecule (Fig. 3F) (Teotico et al. 2008).

The PXR-ligand interaction appears to be a dynamic process, leading to structural changes that quite possibly alter the interaction between PXR and its coactivator or corepressor. In contrast to the apo-PXR, binding of hyperforin changes the pocket shape and increases its volume from 1294 to 1544 Å. In addition, a novel α-helix (α6) is observed in place of the unordered loop in the 317-321 region. Interestingly, binding of rifampicin increases the disorder of three flexible loops neighboring the ligand cavity. Binding of colupulone or rifampicin increases the thermal motion in various regions of PXR, including the AF2 region and LBD sections formed by β1 and β1′.

The growing number of crystal structures will prove to be invaluable in uncovering the complex relationship among ligand, receptor, coregulators, and target DNA. Structural information from all the complexes obtained to date reveals a large and expandable ligand-binding pocket that can harbor ligands of varying sizes, with different chemical and structural properties, thus explaining the promiscuity of PXR in contrast to other NRs.

3. Regulation of Pregnane X Receptor and its target genes

The expression profile of PXR was initially thought to be limited to the liver, colon, and small intestines (Bertilsson et al. 1998; Blumberg et al. 1998b; Lehmann et al. 1998) but has since been found to have a much wider range, including the brain, bone marrow, peripheral blood mononuclear cells (PBMCs) (Albermann et al. 2005; Bauer et al. 2004; Lamba et al. 2004), ovaries (Masuyama et al. 2001), and T lymphocytes (Dubrac et al. 2010). Regulation of PXR is perhaps best studied in the liver. The adult liver is the primary organ of xenobiotic metabolism and elimination. In contrast, fetal liver is mainly involved in hematopoiesis. Toward the late stages of fetal development, the liver ceases to be the main organ for hematopoiesis and begins to express genes associated with xenobiotic detoxification, such as cytochrome P450 (CYP). During this stage, expression of PXR is transcriptionally regulated by another NR, hepatocyte nuclear factor 4α (HNF-4α) (Kamiya et al. 2003; Li et al. 2000). A closer examination of the promoter region of PXR revealed binding sites for HNF-4α. Indeed, disruption of the HNF-4α gene led to reduced expression of PXR and Cyp3a11 (Cyp3a11 is the mouse ortholog of human CYP3A4) in fetal mouse livers (Kamiya et al. 2003). Interestingly, expression of genes transcriptionally regulated by HNF-4α in fetal livers, including PXR, remained unperturbed in adult livers derived from HNF-4α–null mice, suggesting that the regulation of these genes by HNF-4α is developmentally restricted (Hayhurst et al. 2001; Kamiya et al. 2003). Although PXR is no longer subject to HNF-4α regulation in adult livers, it is still tightly regulated at many levels, as discussed below.

3.1 Transcriptional regulation of Pregnane X Receptor

3.1.1 Histone methylation

Hypermethylated CpG-rich promoter regions of key tumor suppressor genes that result in transcriptional silencing are often detected in many human cancers (Baylin et al. 1998).

Silencing of PXR through the hypermethylation of its CpG-rich promoter regions is associated with aggressive neuroblastoma (Misawa et al. 2005), while a decrease in methylation of PXR promoter is correlated with colorectal cancer (Habano et al. 2011). The restoration of PXR expression in neuroblastoma cells results in growth suppression of the cells, suggesting a tumor suppressive property of PXR (Misawa et al. 2005). However, overexpression of PXR in colorectal cancer through promoter hypomethylation suggests a tumor promoting property of PXR. Indeed, chemoresistance to irinotecan (CPT-11), a topoisomerase I inhibitor currently used for the treatment of metastatic colorectal cancer, results from increased PXR expression (Raynal et al. 2010). The role of increased expression of PXR during carcinogenesis could be explained, in part, by its antiapoptotic property independent of xenobiotic enzyme regulation (Zhou et al. 2008b). Conversely, PXR has also been shown to suppress proliferation and tumorigenicity of colon cancer cells (Ouyang et al. 2010). The role of PXR in carcinogenesis remains to be fully elucidated.

3.2 Post-transcriptional regulation of Pregnane X Receptor

3.2.1 Regulation by miRNA

Post-transcriptional regulation of PXR involves the role of microRNA (miRNA). A growing list of proteins, including NRs and CYPs, exhibit similar patterns of regulation by miRNA (Nakajima & Yokoi 2011). miRNAs are a family of non-coding RNA of ~22 nucleotides in length that regulate protein expression either through the attenuation of protein translation or mRNA degradation (Bartel 2004). An online search using the miRBase Target database revealed potential recognition sites for 16 miRNAs in the 3′ -untranslated region (UTR) of the hPXR, including miR-148a, miR-560, and miR-192 (Takagi et al. 2008). miR-148a, a highly abundant miRNA selectively expressed in the liver, was shown to modulate the expression of PXR. Overexpression of miR-148a results in a decrease in PXR levels and a concomitant decrease in *CYP3A4* mRNA induction (Takagi et al. 2008). Regulation of PXR through miRNA presents an exciting avenue that warrants further investigation.

3.3 Post-translational regulation of Pregnane X Receptor

3.3.1 Phosphorylation

Phosphorylation of PXR is often inhibitory (Pondugula et al. 2009b). Treatment of primary rat and human hepatocytes with protein kinase A (PKA) activator leads to the attenuation of *Cyp3A1* (Cyp3A1 is the rat ortholog of human CYP3A4) and *CYP3A4* mRNA levels, respectively. This can be attributed, in part, to the phosphorylation of PXR by PKA (Ding & Staudinger 2005a; Lichti-Kaiser et al. 2009b). However, a similar treatment of mouse hepatocytes resulted in an increase in *Cyp3a11* mRNA levels, suggesting species specificity within PXR (Lichti-Kaiser et al. 2009b).

Work from our laboratory demonstrated that the treatment of HepG2 cells with flavonoids leads to an increase in CYP expression through modulating the activity of cyclin-dependent kinase 5 (Cdk5). We further showed PXR to be a substrate for Cdk5 in *in vitro* kinase assays, suggesting that Cdk5 may modulate the activity of PXR through inhibitory phosphorylation (Dong et al. 2010). Cdk2 was also shown to attenuate PXR activity, in part, through inhibitory phosphorylation of PXR (Lin et al. 2008). The negative regulation of PXR by the cell-cycle–regulated Cdk2 suggests that PXR is subject to cell cycle regulation.

PXR was also reported to be a substrate in *in vitro* kinase assays for a panel of kinases; protein kinase C (PKC) (Ding and Staudinger 2005b), 70 kDa ribosomal S6 kinase (p70S6K),

glycogen synthase kinase 3 (GSK3), and casein kinase II (CK2) (Lichti-Kaiser et al. 2009b), further suggesting that PXR may be modulated by a wide range of protein kinases. However, to this end, *in vivo* phosphorylation of PXR remains undetectable. It is possible that the level of phosphorylation of PXR is below current detection limits.

A systematic approach to mutating serine/threonine (S/T) residues to aspartic acid (D) revealed that Ser8Asp, Thr57Asp, Ser208Asp, and Thr408Asp resulted in a decrease in PXR transactivation (Lichti-Kaiser et al. 2009a). Notably, we determined the phosphomimetic mutant of PXR, Thr57Asp, also exhibited an altered pattern of subcellular localization (Pondugula et al. 2009a). Collectively, phosphorylation of PXR confers a negative regulatory effect, possibly through altering its pattern of subcellular localization or affecting its interaction with corepressors or coactivators.

3.3.2 Ubiquitination, SUMOylation, and acetylation

PXR degradation plays a pivotal role in its regulation, although little is known about the mechanism behind the regulation of its stability. A semi-quantitative approach determined that the half-life of unliganded PXR is less than 4 hours. Binding of PXR to a subset of its ligand increases its half-life, in part due to the disruption of its interaction with suppressor for gal1 (SUG1), a component of the proteasome (Masuyama et al. 2002; Masuyama et al. 2005). Recent work from the Staudinger group further demonstrated an increase in ubiquitinated PXR following inhibition of the 26S proteasome with MG132 (Staudinger et al. 2011). Preliminary work from our laboratory demonstrated that PXR mainly undergoes a lysine-48 (Lys-48) polyubiquitin linkage, which signals its degradation. Proteasomal inhibition also resulted in the inhibition of PXR transactivation, suggesting interplay between PXR and the ubiquitin pathway (Staudinger et al. 2011). However, it is noteworthy that many coregulators of PXR are also subject to regulation through the proteasomal pathway (Lonard & O'Malley 2009). More work will need to be done to dissect the role of ubiquitination on PXR.

It was first observed that patients undergoing long-term treatment with rifampicin exhibit suppression of the inflammatory response in the liver through repression of nuclear factor kappa B (NF-κB) activation (Gu et al. 2006; Paunescu 1970). Activation of the inflammatory response in the liver significantly attenuates the SUMOylation of ligand-bound PXR. SUMOylation of PXR was shown to occur mainly through the SUMO2/3 chains. Although preliminary, the culmination of these studies will shed light on the role of SUMOylation of PXR and its crosstalk with the inflammatory response pathway.

Lysine acetylation was first identified and studied as histone modification and, as such, these lysine modifying enzymes were coined histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Yang & Seto 2008). Since then, lysine acetylation has been shown to extend beyond histones. NRs such as androgen receptor and estrogen receptor undergo acetylation, which modulates the NRs' ligand sensitivity (Fu et al. 2004). Little is known about PXR acetylation, but a recent report showed the presence of an acetylated species of PXR (Biswas et al. 2011). Acetylation of PXR was shown to modulate its activity, and ligand-induced deacetylation of PXR is modulated by SIRT1 (Biswas et al. 2011).

3.4 Pregnane X Receptor coregulators

Coregulators were first identified and described merely as adaptors to stabilize gene transcription. Their known functions have since expanded to include histone remodeling,

transcription initiation, RNA elongation, and protein degradation (McKenna & O'Malley 2002). Coregulators are broadly divided into coactivators and corepressors, molecules that lead to enhanced and attenuated gene transcription, respectively (McKenna & O'Malley 2002). Unliganded PXR interacts with corepressors, small heterodimer partner (SHP), silencing mediator for retinoid and thyroid receptors (SMRT), and nuclear receptor corepressor (NCoR) to inhibit gene transcription (Ourlin et al. 2003; Takeshita et al. 2002). Ligand binding to PXR in turn results in the dissociation of corepressors and the association of coactivators, such as SRC-1/NCOA1. Structure-based analysis has shown that coactivator binding further promotes the interaction between PXR and its ligand (Watkins et al. 2003a). Other coactivators of PXR include SRC-2 (GRIP1), nuclear receptor interacting protein 1 (NRIP1), peroxisome proliferator-activated receptor-gamma (PPARγ) coactivator (PGC-1), and forkhead transcription receptor (FHKR/FOXO1) (Moore et al. 2006).

3.5 Pregnane X Receptor regulation of target genes

PXR is a ligand-activated transcription factor, which upon ligand engagement becomes activated and binds to DNA in the nucleus to regulate gene transcription. Although its LBD exhibits flexibility and variability, the DBD is highly conserved across species. Several studies identified PXR response elements to be AG(G/T)TCA-like everted repeats (ERs) separated by 6 or 8 bp (ER-6 and ER-8) and direct repeats (DRs) separated by 3 or 4 bp (DR-3 and DR-4) (Kliewer et al. 2002). Next-generation sequencing techniques on the mouse cistrome using chromatin immunoprecipitation (ChIP-on-chip) and ChIP sequencing (ChIP-Seq) further revealed an *in vivo* preferred binding site to include a novel DR-(5n+4) pattern (Cui et al. 2010). ChIP studies using cryopreserved primary human hepatocytes treated with rifampicin revealed the upregulation of genes involved in drug metabolism and clearance, which is in agreement with studies performed in cell lines (Hariparsad et al. 2009). Importantly, results from a recent study suggest that while the presence of low concentrations of two different agonists, pregnenolone-16α-carbonitrile (PCN) and lithocholic acid, leads to a similar transcriptome response in primary rat hepatocytes, a divergence in the transcriptome response was observed with higher concentrations of the agonists. This observation suggests that although PXR can be activated by a wide range of agonists, downstream response may be more selective for these agonists. A negative feedback loop was also shown to be in effect for PXR, whereby ligand-induced activation of PXR inhibits the transcription of the PXR gene, limiting the cellular concentration of PXR in the presence of a high concentration of agonist (Bailey et al. 2011).

4. Physiologic functions of Pregnane X Receptor

In recent years, numerous studies have revealed the mechanisms of PXR-mediated induction of DMEs and drug transporters by which xenobiotics is detoxified. In addition, PXR also regulates endobiotic metabolism, which is important for maintaining homeostasis of cholesterol, bile acids, lipids, steroid hormone, and glucose in the human body.

4.1 Pregnane X Receptor in xenobiotic metabolism

DMEs and drug transporters play crucial roles in xenobiotic detoxification and elimination. Phase I CYPs belong to the monooxygenase superfamily and are highly expressed in the liver and intestine. They catalyze the first step of detoxification of aliphatic or lipophilic

compounds through hydroxylation or oxidation reactions and convert these compounds into more soluble derivatives (Guengerich 2001; Nebert & Gonzalez 1987; Willson & Kliewer 2002). Phase II conjugation reactions are catalyzed by a large group of transferases, such as sulfotransferase (SULT), glutathione-*S*-transferase (GST), and UDP-glucuronosyltransferase (UGT) (McCarver & Hines 2002). Polar functional groups are conjugated onto xenobiotics and endobiotics to generate water-soluble, inactive metabolites (McCarver & Hines 2002), which can then be excreted from the cell in a process regulated by ABC and solute carrier (SLC) family transporters (Ayrton & Morgan 2001; El-Sheikh et al. 2008). These transporters, together with phase I and II DMEs, orchestrate the xenobiotic metabolism process.

PXR regulates the metabolism and elimination of xenobiotics by regulating the expression of DMEs and drug transporters. Phase I DME genes regulated by hPXR include *CYP3A4*, *CYP2C8*, *CYP2C9*, and *CYP2B6* (Ferguson et al. 2005; Gerbal-Chaloin et al. 2001; Goodwin et al. 2001; Kliewer et al. 1998; Lehmann et al. 1998). Phase II DME genes regulated by PXR include SULTs (Alnouti & Klaassen 2008), GSTs (Higgins & Hayes 2011) and UGTs (Chen et al. 2003). PXR also regulates the expression of ABC transporters and organic anion-transporting polypeptides (OATPs/SLC) responsible for the influx/efflux of xenobiotics across the cell membrane in the liver and intestine, including multidrug resistance 1 (MDR1/P-gp) (Geick et al. 2001), multidrug resistance associated proteins (MRPs) (Kast et al. 2002; Teng et al. 2003) and multiple OATPs (Meyer zu Schwabedissen et al. 2008).

4.2 Pregnane X Receptor in endobiotic metabolism

Cholesterol is an essential component of the cell membrane and is important in producing bile acids, steroid hormones, and vitamin D. However, oxidized cholesterol contributes to the development of atherosclerosis (Ross 1999). Hence, cholesterol metabolism and transportation are important in the control of cholesterol homeostasis and protection against atherosclerosis (Repa et al. 2000). The biotransformation and transportation of cholesterol in most tissues are catalyzed mainly by mitochondrial sterol 27-hydroxylase, CYP27A. A recent study revealed that upon ligand activation, PXR can induce CYP27A expression and further increase the expression of cholesterol efflux transporters ABCA1 and ABCG1 in intestinal cells but not in hepatocytes (Li et al. 2007). Another study showed that PXR activation can induce hypercholesterolemia in wild-type mice associated with an elevated level of proteins in the liver related to cholesterol transportation and metabolism, such as CD36, ApoA-IV, and CYP39A1 (Zhou et al. 2009). In addition, previous clinical studies have shown that long-term treatment of patients with PXR agonists led to an elevation in cholesterol levels and was associated with an increased risk of cardiovascular disease (Carr et al. 1998; Eiris et al. 1995; Khogali et al. 1974; Lutjohann et al. 2004). These data strongly implicate PXR in mediating cholesterol homeostasis.

Bile acids play major roles in cholesterol metabolism and excretion. They are produced in the liver by CYPs-mediated oxidation of cholesterol (Ihunnah et al. 2011). Accumulation of bile acid is mainly responsible for cholestatic liver injury (Allen et al. 2011). Therefore, bile acid levels need to be tightly controlled to avoid cellular toxicity. It has been reported that PXR plays a central role in bile acid synthesis, metabolism, and transportation. PXR affects the biosynthesis of bile acids by negatively regulating the expression of Cyp7a1 (Staudinger et al. 2001), a rate-limiting enzyme in bile acid biosynthesis (Saini et al. 2004). PXR regulates bile acid metabolism by regulating the expression of CYP3A (Xie et al. 2001) and SULT2A

(Sonoda et al. 2002). In addition to bile acid synthesis and metabolism, PXR also regulates the expression of bile acid transporters (Staudinger et al. 2001; Teng & Piquette-Miller 2007; Wagner et al. 2005).

Hepatic lipid homeostasis relies on the balance of lipid uptake and synthesis (lipogenesis), lipid catabolism (β-oxidation), and secretion. Recent studies showed that activation of PXR regulates lipogenesis independent of the activation of lipogenic transcriptional factor sterol regulatory element-binding protein 1c (SREBP-1c) and is associated with the induction of the free fatty acid uptake transporter CD36, PPARγ, and stearoyl-CoA desaturase-1 (SCD-1) (Zhou et al. 2008a; Zhou et al. 2006). Moreover, PXR also regulates the expression of other hepatic genes related to lipid homeostasis, including ApoA-IV, oxysterol 7α-hydroxylase (CYP39A1), and 7-dehydrocholesterol reductase (DHCR7) (Zhou et al. 2009). In addition, PXR affects lipid homeostasis by regulating lipid catabolism. Treatment with PCN increased levels of hepatic triglycerides in PXR+/+ mice but not in PXR−/− mice (Nakamura et al. 2007). Further investigation revealed that direct interaction of PXR with FoxA2 appeared to be the underlying mechanism by which activation of PXR repressed the expression of carnitine palmitoyltransferase 1A (CPT1A) and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), two key enzymes involved in beta-oxidation and ketogenesis, respectively (Nakamura et al. 2007).

Glucocorticoids, a class of steroid hormones, perform numerous physiologic functions in growth, development, and metabolic events. Both genetic and pharmacologic activation of PXR increased the plasma levels of corticosterone and aldosterone, the mouse equivalence of primary glucocorticoid and mineralocorticoid, respectively (Zhai et al. 2007). This increase was accompanied by the activation of adrenal steroidogenic enzymes, such as CYP11 and 3β-hydroxysteroid dehydrogenase (Zhai et al. 2007). Transgenic mice carrying an activated PXR (VP-PXR) exhibited normal ACTH secretion in the pituitary and intact suppression of dexamethasone by corticosterone, indicating a functional hypothalamus–pituitary–adrenal axis despite severely disrupted adrenal steroid homeostasis (Zhai et al. 2007). Clinical observations in patients undergoing rifampicin treatment revealed ACTH-independent hypercortisolism, which may result in a misdiagnosis of Cushing's syndrome, suggesting that PXR may have broad implications in steroid homeostasis (Zhai et al. 2007).

Blood glucose levels in the body are tightly controlled through multiple processes, including gluconeogenesis, glycogenolysis, and glycogenesis. Importantly, PXR has been shown to modulate these processes. Firstly, the expression of PEPCK and G6Pase, two important rate-limiting enzymes in gluconeogenesis and glycogenolysis, are reduced in VP-hPXR transgenic mice following sustained activation of PXR in the liver (Zhou et al. 2006). Second, the PXR agonist PCN downregulates G6Pase gene expression in wild-type but not PXR−/− mice (Kodama et al. 2007). Third, cross-talk between PXR, CREB, and FOXO1 can also affect gluconeogenesis (Konno et al. 2008). It has been reported that ligand-activated PXR can bind to phosphorylated CREB and FOXO1 and further suppress their transcriptional activity, leading to the suppression of G6Pase and PEPCK1 gene expression and decreased gluconeogenesis (Kodama et al. 2004; Konno et al. 2008). These results suggest that PXR can regulate glucose levels by controlling gluconeogenesis.

5. Pregnane X Receptor as a novel target for drug development

5.1 Pregnane X Receptor in hepatic steatosis

Hepatic steatosis, also known as fatty liver, is a reversible process manifested as abnormal accumulation of lipids in the liver. Hepatic steatosis is associated with a multitude of

diseases, such as cardiovascular disease, obesity, diabetes, cancer, and liver diseases (Diehl 2010). Recent studies suggested that activation of PXR could contribute to the process of hepatic steatosis (Zhou et al. 2006). As discussed in section 4.2., PXR-humanized mice treated with rifampicin exhibited hepatic lipid accumulation. The PXR-mediated triglyceride accumulation was independent of SREBP-1c, but was linked to elevated levels of CD36 and several accessory lipogenic enzymes, such as SCD-1 and long chain free fatty acid elongase (Zhou et al. 2006). PXR directly regulates the transcription of CD36 by binding to its promoter (Zhou et al. 2008a; Zhou et al. 2006). Recently, S14, which plays an important role in the induction of lipogenic enzymes, was identified as a novel transcriptional target of PXR (Moreau et al. 2009). PXR mediates lipogenesis through the induction of S14 expression. Collectively, these studies suggested that abnormal activation of PXR may greatly contribute to the pathogenesis of hepatic steatosis.

5.2 Pregnane X Receptor in bone disorders

Vitamin D plays important roles in bone homeostasis since it regulates the absorption and excretion of calcium, a major component in bone development and maintenance. The physiologically active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25$(OH)_2D_3$), binds to the vitamin D receptor (VDR) to exert its function (Jurutka et al. 2007). CYP24 and CYP3A4 can catabolize 1,25$(OH)_2D_3$ into its inactive form in the liver and intestine (Pascussi et al. 2005; Xu et al. 2006). Recently, *in vivo* and *in vitro* studies identified CYP24 as a PXR target gene, suggesting that PXR activation can alter the homeostasis of 1,25$(OH)_2D_3$ and affect VDR activation through modulating CYP24 and CYP3A4 gene expression (Pascussi et al. 2005; Xu et al. 2006). In addition, it has long been known that prolonged treatment with antiepileptic drugs, many of which are PXR agonists, may lead to vitamin D deficiency (Andress et al. 2002). These results suggest that the activation of PXR may contribute to osteomalacia, the softening of the bones caused by defective bone mineralization.

However, the role of PXR in bone disorders is still unclear. PXR knockout mice displayed marked osteopenia with enhanced bone resorption and reduced bone formation in the trabecular bones and decreased thickness in the cortical bones (Azuma et al. 2010). Additionally, vitamin K was recently identified as a PXR ligand and exerts PXR-dependent biological functions in the bone. It has been reported that vitamin K stimulated several PXR target genes expressed in bone, such as tsukushi, matrilin-2, CD14, and Msx2 which are involved in osteoblast differentiation (Ichikawa et al. 2006; Igarashi et al. 2007). Furthermore, vitamin K supplementation increases bone density *in vivo* and is currently in clinical use to manage osteoporosis (Fang et al. 2011). These opposing data suggest PXR also plays roles in bone disorders.

5.3 Pregnane X Receptor in inflammatory bowel disease

Inflammatory bowel disease (IBD) refers to an inflammation of the intestinal tract. In IBD patients, the expression of PXR and its target genes were significantly reduced in the intestine, which led to the deregulation of PXR activity and xenobiotic metabolism, contributing to the pathogenesis of this disease (Dring et al. 2006; Langmann et al. 2004). Two recent studies have demonstrated the efficacy of rifaximin, an activator of hPXR, in the treatment of IBD (Mencarelli et al. 2010; Shah et al. 2007). In both a human colon cell line and IBD mouse models, induced by either dextran sulfate sodium or trinitrobenzene sulfonic acid, rifaximin showed protective and therapeutic activity associated with the

induction of PXR target genes related to intestinal detoxification (Mencarelli et al. 2010; Shah et al. 2007). This therapeutic effect of rifaximin was not observed in PXR knockdown colon epithelial cells or in PXR-null IBD mice (Shah et al. 2007). Other studies showed that rifaximin-activated PXR also inhibited the NF-κB signaling cascade, resulting in the suppression of the NF-κB-mediated proinflammatory response (Gu et al. 2006). These data suggest that PXR is a potential therapeutic target in the prevention and treatment of human IBD.

5.4 Pregnane X Receptor in cancer and chemotherapy

It has been well documented that PXR is expressed in many human cancers, including breast (Dotzlaw et al. 1999; Miki et al. 2006), prostate (Chen et al. 2007), colon (Ouyang et al. 2010; Raynal et al. 2010; Zhou et al. 2008b), osteosarcoma (Mensah-Osman et al. 2007), ovarian (Gupta et al. 2008), and endometrial cancers (Masuyama et al. 2003; Masuyama et al. 2007). Due to its ligand promiscuity, PXR can be activated by many anticancer drugs, including cyclophosphamide, tamoxifen, taxol, vincristine, and vinblastine (Koyano et al. 2002; Poso & Honkakoski 2006; Smith et al. 2010; Synold et al. 2001). Moreover, cancer patients are usually treated with combination therapy in addition to anticancer drugs, which also increases the possibility of drug-mediated PXR activation. Accordingly, recent studies support the idea that activation of PXR may compromise the effectiveness of anticancer drugs and contribute to acquired multi-drug resistance during anticancer chemotherapy (Chen, 2010). In PXR-expressing cancer cells such as prostate, colon, and endometrial cancer, PXR agonists can lead to increased resistance of cancer cells to chemotherapeutic agents, while the cancer cells can be sensitized to these anticancer agents by knockdown of PXR (Chen et al. 2007; Masuyama et al. 2007; Ouyang et al. 2010). On the other hand, PXR-mediated chemoresistance originating from inducible activity of PXR can also be blocked by pharmacologic intervention, leading to enhanced efficacy of chemotherapy. A recent study demonstrated that the reduced chemosensitivity of colorectal cancer cells to irinotecan was reversed by the PXR antagonist sulforaphane, while the activation of PXR decreased the effectiveness of this drug (Raynal et al. 2010). Thus the concept has been proposed to tackle resistance to anticancer drug by pharmacologically antagonizing regulating PXR (Chen 2010). The discovery and development of nontoxic, specific, and potent PXR antagonists will provide an effective way to improve the efficacy of anticancer drugs for the treatment of PXR-positive cancers.

6. Pregnane X Receptor as a target for improvement on current drug therapies

Adverse drug reactions (ADRs) induced by drug-drug interactions are major clinical problems, significantly contributing to mortality and morbidity (Wilke et al. 2007). PXR activation plays a crucial role in drug-drug interactions by inducing the expression of DMEs and drug transporters. Many prescription drugs have been found to bind to PXR, induce PXR target gene expression, and affect the metabolism and pharmacokinetics of the drugs. These drugs include calcium channel blockers felodipine, isradipine, lacidipine, nicardipine, and nifedipine (Xiao et al. 2011), HIV protease inhibitors (Dussault et al. 2001), anti-inflammatory agent dexamethasone (Pascussi et al. 2000), and others. Common herbal medicines can also activate PXR, such as licorice (Mu et al. 2006), gugulipid (Brobst et al.

2004), Ginkgo biloba (Yeung et al. 2008), and St. John’s wort (Moore et al. 2000). Since activated PXR induces the expression of CYPs, it is conceivable that the activation of PXR can lead to undesirable drug–drug interactions in a large number of pharmaceutical drugs.

PXR contributes to ADRs by decreasing therapeutic efficacy and by increasing drug toxicity (Ma et al. 2008b). When co-administrated with rifampicin, the antihypertensive drug verapamil and anti-HIV protease inhibitors showed dramatically decreased efficacy because of increased drug metabolism and clearance caused by PXR-induced CYP3A4 expression (Fuhr 2000; Niemi et al. 2003). Another example comes from St. John's wort, an herbal medicine containing PXR agonist hyperforin. Long-term consumption of St. John's wort induces PXR-mediated CYP3A expression, resulting in increased metabolism and reduced efficacy of many therapeutic drugs in combination therapy, such as amitriptyline, cyclosporine, digoxin, indinavir, irinotecan, warfarin, phenprocoumon, alprazolam, dextromethorphan, simvastatin, and oral contraceptives (Mai et al. 2004; Moore et al. 2000). Besides decreasing drug efficacy, PXR also plays an important role in drug-induced toxicity. A recent study showed that pretreatment with PCN significantly enhanced acetaminophen-induced hepatotoxicity in mice, probably by inducing CYP3A and hence converting acetaminophen to its toxic intermediate metabolite, N-acetyl-p-benzoquinone imine (Cheng et al. 2009; Guo et al. 2004).

Efforts have been made to overcome PXR-induced drug-drug interactions in drug development. One approach is to chemically modify the lead compound to minimize its PXR activating function without compromising its pharmacologic activity against the therapeutic target. This approach was illustrated in a recent study on the first generation of IGF-1R inhibitors (Velaparthi et al. 2008; Zimmermann et al. 2010). However, due to PXR ligand promiscuity, tremendous chemistry efforts will be needed to remove the PXR activating function of many lead compounds. In addition, many commercially marketed drugs with PXR agonistic activity are still in the clinical use. In light of these considerations, another approach is to discover and develop new compounds that can antagonize PXR activity as co-therapeutics (Chen 2008; Chen 2010; Venkatesh et al. 2011). The feasibility of this approach was supported by recent studies that showed that the effectiveness of drugs can be enhanced by antagonizing the inducible activity of PXR with several pharmacologic interventions, such as ecteinascidin-743 (Synold et al. 2001), ketoconazole (Huang et al. 2007), sulforaphane (Zhou et al. 2007), A-792611 (Healan-Greenberg et al. 2008), and coumestrol (Wang et al. 2008a). These studies shed light on the development of PXR antagonists as an effective way to minimize PXR-induced ADRs.

7. Drug discovery techniques for Pregnane X Receptor

7.1 Structure-based modeling for predicting Pregnane X Receptor ligands

Computational studies have become useful tools geared toward understanding and predicting PXR-ligand interactions, a major focus being the determination of features within the ligand that allow for biomolecular recognition. This information can be used to predict potential drug clearance or resistance or to develop chemical PXR modulators. The promiscuity of PXR was investigated using computational solvent mapping to determine hot spots on the protein surface (Ngan et al. 2009). These studies identified five well-defined hot spots on all sides of the binding pocket, with the most important one being formed by the residues Phe288, Trp299, and Tyr306. *In silico* pharmacophore and docking analysis yielded PXR antagonists, some of which may bind to the AF-2 domain of PXR (Ekins et al.

2008). Quantitative structural activity relationships (QSARs) have been applied using data from various sources to identify ligand structural features that contribute to biological activity (Jacobs 2004). To encompass a broader spectrum of compounds, molecular descriptors for PXR agonistic effects were predicted using machine learning approaches (Ung et al. 2007). To obtain a more consistent set of biological data, Xiao *et al.* cross-evaluated compounds with varying degrees of PXR activation using an *in silico* modeling analysis with several biological assays. They concluded that potent ligands interact with residues in the PXR ligand-binding cavity through critical hydrogen bonds and π-π contacts. Molecules with low molecular weight or mismatched shapes appeared to be weak binders (Xiao et al. 2011).

7.2 Biochemical assays

Several biochemical assays have been developed to study the direct interaction of ligands with PXR. Stable and highly purified protein preparations are required to obtain reliable results. However, as with many other NRs, PXR proves to be a difficult protein to purify. Full-length recombinant hPXR expressed in *E. coli* cells was first purified as inclusion bodies, followed by resolubilization, to be used for the generation of polyclonal antibodies (Saradhi et al. 2005). Aside from this single report, only the LBD of PXR is commonly expressed and purified from bacterial systems to be used in biochemical assays and crystallographic experiments. To increase protein stability, an SRC-1 coactivator peptide is co-expressed and co-purified with PXR (Watkins et al. 2003a), or the peptide is tethered to the LBD (Wang et al. 2008b). Due to inconsistencies in the co-expression of PXR and SRC-1 peptide at an equal ratio, it is believed that the tethered protein offers the better alternative.

The scintillation proximity assay (SPA) has become a powerful technology to study PXR-ligand interactions. Microspheres or beads incorporating a scintillant and designed to bind the target protein is incubated with radiolabeled ligands. Commercially available beads come in different core materials, such as polyvinyltoluene (PVT) or yttrium silicate (YSi), coated with coupling molecules to capture the target protein. Jones *et al*. described a novel SPA assay that utilizes the tritium-labeled PXR agonist SR12813 (Jones et al. 2000). In this protocol, biotinylated hPXR was bound to streptavidin-coated PVT beads. Various PXR activators were tested for their ability to compete with the [^{3}H]SR12813.

The affinity selection-mass spectrometry system (ALIS) utilizes liquid chromatography in tandem with mass spectrometric detection. PXR homogenates are pre-incubated with the desired compound, and the complex is separated rapidly (e.g., <20 s) from the unbound compound by a size-exclusion column (Xiao et al. 2011). The protein-ligand complex elutes at the void volume and is subsequently trapped in a collection loop, with the separation being monitored by UV absorbance. The complex is injected into a reversed-phase column at high temperature (e.g., 60 °C) with the acidic mobile phase containing 0.1% formic acid. Under these conditions, the ligand dissociates from the protein and is detected and quantified by the mass analyzer within the desired mass range. Elution from the reversed-phase column follows a standard gradient procedure with increasing non-polar solvent. Using this system, binding affinities can be obtained by performing dilutions of the ligand at constant protein concentrations. The advantage of this method is that the direct binding of the ligand to the protein can be evaluated.

In the temperature-dependent circular dichroism (TdCD) method, PXR-ligand solutions are allowed to equilibrate at room temperature (Xiao et al. 2011). The ellipticity is then monitored by circular dichroism spectroscopy at 220 nm and at increasing temperature (e.g.,

2°C/min). In the reported experiment, a single unfolding transition phase was observed for the apo PXR (PXR was tethered to an SRC-1 peptide) with a melting temperature (T_m) of 41.5°C. In the presence of rifampicin, the T_m value increased to 49°C. Hypothetical K_d values can be extracted based on T_m shifts. A relatively good correlation (r^2=0.72) was shown between the data obtained by this assay and that of a reporter gene cell-based assay.
Fluorescence polarization is a widely used technique to assess protein-peptide interactions and was applied to measure the recruitment of the coactivator SRC-1 upon ligand binding to PXR. A relatively small fluorescently labeled SRC-1 peptide emits the absorbed polarized radiation in a different direction from that of the incident light due to the fast tumbling rate. The binding of the peptide to the PXR protein increases the effective mass, resulting in a slower tumbling rate, leading to an increase in polarized light. A fluorescein-labeled SRC-1 peptide containing the amino acid sequence ILRKLLQE was used as a probe to test for direct interactions between bile acid intermediates and mouse PXR LBD fused to GST (Goodwin et al. 2003). In the presence of agonist, an increase in fluorescence polarization indicated ligand-dependent recruitment of SRC-1.
Several other techniques have been broadly used to evaluate the binding affinity and molecular recognitions of protein-ligand systems (Jecklin et al. 2009). Initially limited to probing only large biomolecules, label-free tools have become sophisticated in the analysis of protein interactions with small molecules. Surface plasmon resonance (SPR) can provide data in real time, with concomitant determination of kinetic descriptors and binding affinities. Isothermal titration calorimetry (ITC) is considered to be a true label-free technique for investigating thermodynamic profiles and affinity measurements. Major drawbacks include lack of sensitivity, which requires higher concentrations of either protein or compound. This could lead to solubility issues and challenges in the analysis of weak binders. In the case of SPR technology, immobilization conditions could cause artifacts due to conformational changes. Another obvious limitation in these methods is the need for relatively pure and stable purified PXR in large amounts.

7.3 Cell-based assays

Cell-based assays to determine PXR transactivation and CYP induction are especially useful, eliminating the need for primary hepatocytes while still providing a physiologically relevant environment. The first reporter-based assay described the use of chloramphenicol acetyltransferase (CAT) as a readout (Quattrochi et al. 1995). This method was subsequently improved upon by substituting luciferase for CAT; with the expression of luciferase being controlled by the PXR response element (PXRE) (Raucy et al. 2002). This model system has since been widely used in many laboratory settings, including high-throughput screening for potential PXR agonists and antagonists (Dong et al. 2010; Zhu et al. 2004).
Another cell-based assay gaining a foothold in the area of transcription factor is the mammalian two-hybrid system to study protein-protein interactions in cells. This assay is especially useful in determining the interaction between PXR and its coregulators. Physical interactions between two proteins of interest are detected through a simple end-point luciferase readout, which provides a semi-quantitative method for detecting protein-protein interactions. This method is superior to the traditional immunoprecipitation method because the spatial regulation of these protein interactions is preserved.
The subcellular localization of PXR in the cells remains unclear, with reports showing both nuclear and cytosolic localization. As such, efforts to incorporate high-content imaging-

based screening for PXR remain a challenge. Our laboratory is currently developing a fusion protein of PXR containing a photoconvertible fluorophore. With this tool in hand, we will be able to investigate the kinetics of PXR's nuclear/cytoplasmic translocation.

7.4 Animal models

The mouse models for PXR offer several advantages over cell-based systems by providing *in vivo* context to reveal physiologic functions. Although cell-based *in vitro* systems, such as a reporter assay, are useful for identifying PXR ligands, the ultimate goal is to translate these *in vitro* findings into *in vivo* models with clinical relevance. The development of mouse models for PXR, such as PXR-null mice and PXR-humanized mice, provides useful tools to achieve this goal.

To better understand PXR-dependent signaling *in vivo*, two PXR-null mouse models were generated using similar approaches by disruption of PXR alleles with homologous recombination (Staudinger et al. 2001; Xie et al. 2000a). These PXR-null mice did not exhibit any apparent phenotypic changes, with normal development, growth, and reproduction. Extensive serum analysis did not reveal any significant changes in multiple serum biochemical profile, such as cholesterol, triglyceride, glucose, or liver enzyme levels (Staudinger et al. 2001; Xie et al. 2000a) suggesting that PXR is not essential for normal development or adult physiology under normal conditions. Although the loss of PXR does not alter the basal expression of PXR target genes, the PXR-null mice did not respond normally to xenobiotic treatment (Staudinger et al. 2001; Xie et al. 2000a). Hence, the PXR-null mouse is a valuable and reliable tool for dissecting PXR-dependent functions *in vivo*.

One critical problem in PXR functional studies lies in ligand selectivity between human and mouse PXR because of the marked species differences in amino acid sequences in the PXR LBD (Ekins et al. 2002). To overcome differences in PXR ligand recognition across species, humanized PXR mouse models have been generated. The BAC-hPXR mouse was generated by introducing a bacterial artificial chromosome (BAC) clone containing the complete hPXR gene and its 5′- and 3′- flanking sequences, whereas the Alb-hPXR mouse was developed by introducing the hPXR gene under the control of an albumin promoter (Alb), in PXR-null mouse (Ma et al. 2007; Xie et al. 2000a). As expected in these humanized PXR mice, no significant response was found after treatment with PCN, whereas rifampicin efficiently induced Cyp3a11 expression (Ma et al. 2007; Xie et al. 2000b). Another humanized PXR mouse model was generated that expressed a constitutively active hPXR (VP-hPXR, created by fusing hPXR to VP16, a potent viral transcriptional activator) in the livers of PXR-null mice (Xie et al. 2000a). In the VP16-hPXR mice, Cyp3a11 was constitutively induced in the liver. In addition, these mice exhibited growth retardation, hepatomegaly, and liver toxicity, suggesting that sustained activation of PXR may be deleterious (Xie et al. 2000a). Recently, a double transgenic mouse model expressing human PXR and CYP3A4, designated the TgCYP3A4/hPXR mouse, was generated (Ma et al. 2008a). Treatment of TgCYP3A4/hPXR mice with PXR ligands mimicked the human response but not the mouse response (Ma et al. 2008a). This model provides a useful tool to study hPXR-mediated human CYP3A4 expression and predict drug-drug interaction in the human body (Ma et al. 2008a). Overall, these PXR-humanized mouse models are more suitable as *in vivo* tools for studying xenobiotics metabolism mediated by hPXR.

8. Conclusion

Over the past decade, an enormous body of work has been invested toward understanding the mechanism of PXR regulation and its physiologic role in health and disease. Structure-based studies revealed its ligand promiscuity and the possibility of PXR existing as oligomers, while biochemical studies revealed the intricacies and the involvement of PXR in cross-talk across multiple signaling pathways. Genetic studies with animal models further support the *in vitro* findings, demonstrating that PXR protein expression is not limited to the liver and intestinal tract and revealing its role in maintaining cellular homeostasis. Hence, PXR serves as an attractive target for the development of pharmacologic modulators for mediating a plethora of diseases and, more importantly, MDR in chemotherapeutics.

9. Acknowledgment

We thank David Galloway for editing the manuscript. This work was supported by the American Lebanese Syrian Associated Charities (ALSAC), St. Jude Children's Research Hospital, National Institute of General Medical Sciences grant GM086415 and National Cancer Institute grant P30CA027165.

10. References

Albermann, N, Schmitz-Winnenthal, FH, Z'Graggen, K, Volk, C, Hoffmann, MM, Haefeli, WE & 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*, Vol. 70(6): pp. 949-58, ISSN 0006-2952

Allen, K, Jaeschke, H & Copple, BL (2011). Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. *Am J Pathol*, Vol. 178(1): pp. 175-86, ISSN 1525-2191

Alnouti, Y & Klaassen, CD (2008). Regulation of sulfotransferase enzymes by prototypical microsomal enzyme inducers in mice. *J Pharmacol Exp Ther*, Vol. 324(2): pp. 612-21, ISSN 1521-0103

Andress, DL, Ozuna, J, Tirschwell, D, Grande, L, Johnson, M, Jacobson, AF & Spain, W (2002). Antiepileptic drug-induced bone loss in young male patients who have seizures. *Arch Neurol*, Vol. 59(5): pp. 781-6, ISSN 0003-9942

Ayrton, A & Morgan, P (2001). Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica*, Vol. 31(8-9): pp. 469-97, ISSN 0049-8254

Azuma, K, Casey, SC, Ito, M, Urano, T, Horie, K, Ouchi, Y, Kirchner, S, Blumberg, B & Inoue, S (2010). Pregnane X receptor knockout mice display osteopenia with reduced bone formation and enhanced bone resorption. *J Endocrinol*, Vol. 207(3): pp. 257-63, ISSN 1479-6805

Bailey, I, Gibson, GG, Plant, K, Graham, M & Plant, N (2011). A PXR-mediated negative feedback loop attenuates the expression of CYP3A in response to the PXR agonist pregnenalone-16alpha-carbonitrile. *PLoS One*, Vol. 6(2): pp. e16703, 1932-6203

Bartel, DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, Vol. 116(2): pp. 281-97, ISSN 0092-8674

Bauer, B, Hartz, AM, Fricker, G & Miller, DS (2004). Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. *Mol Pharmacol*, Vol. 66(3): pp. 413-9, ISSN 0026-895X

Baylin, SB, Herman, JG, Graff, JR, Vertino, PM & Issa, JP (1998). Alterations in DNA methylation: a fundamental aspect of neoplasia. *Adv Cancer Res*, Vol. 72: pp. 141-96, ISSN 0065-230X

Bertilsson, G, Heidrich, J, Svensson, K, Asman, M, Jendeberg, L, Sydow-Backman, M, Ohlsson, R, Postlind, H, Blomquist, P & Berkenstam, A (1998). Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci U S A*, Vol. 95(21): pp. 12208-13, ISSN 0027-8424

Biswas, A, Pasquel, D, Tyagi, RK & Mani, S (2011). Acetylation of pregnane X receptor protein determines selective function independent of ligand activation. *Biochem Biophys Res Commun*, Vol. 406(3): pp. 371-6, ISSN 1090-2104

Blumberg, B, Kang, H, Bolado, J, Jr., Chen, H, Craig, AG, Moreno, TA, Umesono, K, Perlmann, T, De Robertis, EM & Evans, RM (1998a). BXR, an embryonic orphan nuclear receptor activated by a novel class of endogenous benzoate metabolites. *Genes Dev*, Vol. 12(9): pp. 1269-77, ISSN 0890-9369

Blumberg, B, Sabbagh, W, Jr., Juguilon, H, Bolado, J, Jr., van Meter, CM, Ong, ES & Evans, RM (1998b). SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Genes Dev*, Vol. 12(20): pp. 3195-205, ISSN 0890-9369

Brobst, DE, Ding, X, Creech, KL, Goodwin, B, Kelley, B & Staudinger, JL (2004). Guggulsterone activates multiple nuclear receptors and induces CYP3A gene expression through the pregnane X receptor. *J Pharmacol Exp Ther*, Vol. 310(2): pp. 528-35, ISSN 0022-3565

Carr, A, Samaras, K, Chisholm, DJ & Cooper, DA (1998). Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet*, Vol. 351(9119): pp. 1881-3, ISSN 0140-6736

Chen, C, Staudinger, JL & Klaassen, CD (2003). Nuclear receptor, pregname X receptor, is required for induction of UDP-glucuronosyltranferases in mouse liver by pregnenolone-16 alpha-carbonitrile. *Drug Metab Dispos*, Vol. 31(7): pp. 908-15, ISSN 0090-9556

Chen, T (2008). Nuclear receptor drug discovery. *Curr Opin Chem Biol*, Vol. 12(4): pp. 418-26, ISSN 1367-5931

Chen, T (2010). Overcoming drug resistance by regulating nuclear receptors. *Adv Drug Deliv Rev*, Vol. 62(13): pp. 1257-64, ISSN 1872-8294

Chen, Y, Tang, Y, Wang, MT, Zeng, S & Nie, D (2007). Human pregnane X receptor and resistance to chemotherapy in prostate cancer. *Cancer Res*, Vol. 67(21): pp. 10361-7, ISSN 1538-7445

Cheng, J, Ma, X, Krausz, KW, Idle, JR & Gonzalez, FJ (2009). Rifampicin-activated human pregnane X receptor and CYP3A4 induction enhance acetaminophen-induced toxicity. *Drug Metab Dispos*, Vol. 37(8): pp. 1611-21, ISSN 1521-009X

Chrencik, JE, Orans, J, Moore, LB, Xue, Y, Peng, L, Collins, JL, Wisely, GB, Lambert, MH, Kliewer, SA & Redinbo, MR (2005). Structural disorder in the complex of human pregnane X receptor and the macrolide antibiotic rifampicin. *Mol Endocrinol*, Vol. 19(5): pp. 1125-34, ISSN 0888-8809

Cui, JY, Gunewardena, SS, Rockwell, CE & Klaassen, CD (2010). ChIPing the cistrome of PXR in mouse liver. *Nucleic Acids Res*, Vol. 38(22): pp. 7943-63, ISSN 1362-4962

Diehl, AM (2010). Genetic susceptibility to hepatic steatosis. *N Engl J Med*, Vol. 362(12): pp. 1142-3, ISSN 1533-4406

Ding, X & Staudinger, JL (2005a). Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase a signal transduction pathway. *J Pharmacol Exp Ther*, Vol. 312(2): pp. 849-56, ISSN 0022-3565

Ding, X & Staudinger, JL (2005b). Repression of PXR-mediated induction of hepatic CYP3A gene expression by protein kinase C. *Biochem Pharmacol*, Vol. 69(5): pp. 867-73, ISSN 0006-2952

Dong, H, Lin, W, Wu, J & Chen, T (2010). Flavonoids activate pregnane x receptor-mediated CYP3A4 gene expression by inhibiting cyclin-dependent kinases in HepG2 liver carcinoma cells. *BMC Biochem*, Vol. 11: pp. 23, ISSN 1471-2091

Dotzlaw, H, Leygue, E, Watson, P & Murphy, LC (1999). The human orphan receptor PXR messenger RNA is expressed in both normal and neoplastic breast tissue. *Clin Cancer Res*, Vol. 5(8): pp. 2103-7, ISSN 1078-0432

Dring, MM, Goulding, CA, Trimble, VI, Keegan, D, Ryan, AW, Brophy, KM, Smyth, CM, Keeling, PW, O'Donoghue, D, O'Sullivan, M, O'Morain, C, Mahmud, N, Wikstrom, AC, Kelleher, D & McManus, R (2006). The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology*, Vol. 130(2): pp. 341-8; quiz 592, ISSN 0016-5085

Dubrac, S, Elentner, A, Ebner, S, Horejs-Hoeck, J & Schmuth, M (2010). Modulation of T lymphocyte function by the pregnane X receptor. *J Immunol*, Vol. 184(6): pp. 2949-57, ISSN 1550-6606

Dussault, I, Lin, M, Hollister, K, Wang, EH, Synold, TW & Forman, BM (2001). Peptide mimetic HIV protease inhibitors are ligands for the orphan receptor SXR. *J Biol Chem*, Vol. 276(36): pp. 33309-12, ISSN 0021-9258

Eiris, JM, Lojo, S, Del Rio, MC, Novo, I, Bravo, M, Pavon, P & Castro-Gago, M (1995). Effects of long-term treatment with antiepileptic drugs on serum lipid levels in children with epilepsy. *Neurology*, Vol. 45(6): pp. 1155-7, ISSN 0028-3878

Ekins, S, Kholodovych, V, Ai, N, Sinz, M, Gal, J, Gera, L, Welsh, WJ, Bachmann, K & Mani, S (2008). Computational discovery of novel low micromolar human pregnane X receptor antagonists. *Mol Pharmacol*, Vol. 74(3): pp. 662-72, ISSN 1521-0111

Ekins, S, Mirny, L & Schuetz, EG (2002). A ligand-based approach to understanding selectivity of nuclear hormone receptors PXR, CAR, FXR, LXRalpha, and LXRbeta. *Pharm Res*, Vol. 19(12): pp. 1788-800, ISSN 0724-8741

El-Sheikh, AA, Masereeuw, R & Russel, FG (2008). Mechanisms of renal anionic drug transport. *Eur J Pharmacol*, Vol. 585(2-3): pp. 245-55, ISSN 0014-2999

Fang, Y, Hu, C, Tao, X, Wan, Y & Tao, F (2011). Effect of vitamin K on bone mineral density: a meta-analysis of randomized controlled trials. *J Bone Miner Metab*, Vol.: pp., 1435-5604

Ferguson, SS, Chen, Y, LeCluyse, EL, Negishi, M & Goldstein, JA (2005). Human CYP2C8 is transcriptionally regulated by the nuclear receptors constitutive androstane receptor, pregnane X receptor, glucocorticoid receptor, and hepatic nuclear factor 4alpha. *Mol Pharmacol*, Vol. 68(3): pp. 747-57, ISSN 0026-895X

Fu, M, Wang, C, Zhang, X & Pestell, RG (2004). Acetylation of nuclear receptors in cellular growth and apoptosis. *Biochem Pharmacol*, Vol. 68(6): pp. 1199-208, ISSN 0006-2952

Fuhr, U (2000). Induction of drug metabolising enzymes: pharmacokinetic and toxicological consequences in humans. *Clin Pharmacokinet*, Vol. 38(6): pp. 493-504, ISSN 0312-5963

Geick, A, Eichelbaum, M & Burk, O (2001). Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem*, Vol. 276(18): pp. 14581-7, ISSN 0021-9258

Gerbal-Chaloin, S, Pascussi, JM, Pichard-Garcia, L, Daujat, M, Waechter, F, Fabre, JM, Carrere, N & Maurel, P (2001). Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab Dispos*, Vol. 29(3): pp. 242-51, ISSN 0090-9556

Goodwin, B, Gauthier, KC, Umetani, M, Watson, MA, Lochansky, MI, Collins, JL, Leitersdorf, E, Mangelsdorf, DJ, Kliewer, SA & Repa, JJ (2003). Identification of bile acid precursors as endogenous ligands for the nuclear xenobiotic pregnane X receptor. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 100(1): pp. 223-28, ISSN 0027-8424

Goodwin, B, Moore, LB, Stoltz, CM, McKee, DD & Kliewer, SA (2001). Regulation of the human CYP2B6 gene by the nuclear pregnane X receptor. *Mol Pharmacol*, Vol. 60(3): pp. 427-31, ISSN 0026-895X

Gu, X, Ke, S, Liu, D, Sheng, T, Thomas, PE, Rabson, AB, Gallo, MA, Xie, W & Tian, Y (2006). Role of NF-kappaB in regulation of PXR-mediated gene expression: a mechanism for the suppression of cytochrome P-450 3A4 by proinflammatory agents. *J Biol Chem*, Vol. 281(26): pp. 17882-9, ISSN 0021-9258

Guengerich, FP (2001). Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol*, Vol. 14(6): pp. 611-50, ISSN 0893-228X

Guo, GL, Moffit, JS, Nicol, CJ, Ward, JM, Aleksunes, LA, Slitt, AL, Kliewer, SA, Manautou, JE & Gonzalez, FJ (2004). Enhanced acetaminophen toxicity by activation of the pregnane X receptor. *Toxicol Sci*, Vol. 82(2): pp. 374-80, ISSN 1096-6080

Gupta, D, Venkatesh, M, Wang, H, Kim, S, Sinz, M, Goldberg, GL, Whitney, K, Longley, C & Mani, S (2008). Expanding the roles for pregnane X receptor in cancer: proliferation and drug resistance in ovarian cancer. *Clin Cancer Res*, Vol. 14(17): pp. 5332-40, ISSN 1078-0432

Habano, W, Gamo, T, Terashima, J, Sugai, T, Otsuka, K, Wakabayashi, G & Ozawa, S (2011). Involvement of promoter methylation in the regulation of Pregnane X receptor in colon cancer cells. *BMC Cancer*, Vol. 11: pp. 81, ISSN 1471-2407

Hariparsad, N, Chu, X, Yabut, J, Labhart, P, Hartley, DP, Dai, X & Evers, R (2009). Identification of pregnane-X receptor target genes and coactivator and corepressor binding to promoter elements in human hepatocytes. *Nucleic Acids Res*, Vol. 37: pp. 1160-73, ISSN 1362-4962

Hayhurst, GP, Lee, YH, Lambert, G, Ward, JM & Gonzalez, FJ (2001). Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol*, Vol. 21(4): pp. 1393-403, ISSN 0270-7306

Healan-Greenberg, C, Waring, JF, Kempf, DJ, Blomme, EA, Tirona, RG & Kim, RB (2008). A human immunodeficiency virus protease inhibitor is a novel functional inhibitor of human pregnane X receptor. *Drug Metab Dispos*, Vol. 36(3): pp. 500-7, ISSN 1521-009X

Higgins, LG & Hayes, JD (2011). Mechanisms of induction of cytosolic and microsomal glutathione transferase (GST) genes by xenobiotics and pro-inflammatory agents. *Drug Metab Rev*, Vol. 43(2): pp. 92-137, ISSN 1097-9883

Huang, H, Wang, H, Sinz, M, Zoeckler, M, Staudinger, J, Redinbo, MR, Teotico, DG, Locker, J, Kalpana, GV & Mani, S (2007). Inhibition of drug metabolism by blocking the activation of nuclear receptors by ketoconazole. *Oncogene*, Vol. 26(2): pp. 258-68, ISSN 0950-9232

Ichikawa, T, Horie-Inoue, K, Ikeda, K, Blumberg, B & Inoue, S (2006). Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J Biol Chem*, Vol. 281(25): pp. 16927-34, ISSN 0021-9258

Igarashi, M, Yogiashi, Y, Mihara, M, Takada, I, Kitagawa, H & Kato, S (2007). Vitamin K induces osteoblast differentiation through pregnane X receptor-mediated transcriptional control of the Msx2 gene. *Mol Cell Biol*, Vol. 27(22): pp. 7947-54, ISSN 1098-5549

Ihunnah, CA, Jiang, M & Xie, W (2011). Nuclear receptor PXR, transcriptional circuits and metabolic relevance. *Biochim Biophys Acta*, Vol. 1812(8): pp. 956-63, 0006-3002

Ingelman-Sundberg, M (2004). Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol*, Vol. 369(1): pp. 89-104, ISSN 0028-1298

Ingraham, HA & Redinbo, MR (2005). Orphan nuclear receptors adopted by crystallography. *Curr Opin Struct Biol*, Vol. 15(6): pp. 708-15, ISSN 0959-440X

Jacobs, MN (2004). In silico tools to aid risk assessment of endocrine disrupting chemicals. *Toxicology*, Vol. 205(1-2): pp. 43-53, ISSN 0300-483X

Jecklin, MC, Schauer, S, Dumelin, CE & Zenobi, R (2009). Label-free determination of protein-ligand binding constants using mass spectrometry and validation using surface plasmon resonance and isothermal titration calorimetry. *Journal of Molecular Recognition*, Vol. 22(4): pp. 319-29, ISSN 0952-3499

Jones, SA, Moore, LB, Shenk, JL, Wisely, GB, Hamilton, GA, McKee, DD, Tomkinson, NC, LeCluyse, EL, Lambert, MH, Willson, TM, Kliewer, SA & Moore, JT (2000). The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol Endocrinol*, Vol. 14(1): pp. 27-39, ISSN 0888-8809

Jurutka, PW, Bartik, L, Whitfield, GK, Mathern, DR, Barthel, TK, Gurevich, M, Hsieh, JC, Kaczmarska, M, Haussler, CA & Haussler, MR (2007). Vitamin D receptor: key roles in bone mineral pathophysiology, molecular mechanism of action, and novel nutritional ligands. *J Bone Miner Res*, Vol. 22 Suppl 2: pp. V2-10, ISSN 1523-4681

Kamiya, A, Inoue, Y & Gonzalez, FJ (2003). Role of the hepatocyte nuclear factor 4alpha in control of the pregnane X receptor during fetal liver development. *Hepatology*, Vol. 37(6): pp. 1375-84, ISSN 0270-9139

Kast, HR, Goodwin, B, Tarr, PT, Jones, SA, Anisfeld, AM, Stoltz, CM, Tontonoz, P, Kliewer, S, Willson, TM & Edwards, PA (2002). Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. *J Biol Chem*, Vol. 277(4): pp. 2908-15, ISSN 0021-9258

Khogali, AM, Chazan, BI, Metcalf, VJ & Ramsay, JH (1974). Hyperlipidaemia as a complication of rifampicin treatment. *Tubercle*, Vol. 55(3): pp. 231-3, ISSN 0041-3879

Kliewer, SA, Goodwin, B & Willson, TM (2002). The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr Rev*, Vol. 23(5): pp. 687-702, 0163-769X

Kliewer, SA, Moore, JT, Wade, L, Staudinger, JL, Watson, MA, Jones, SA, McKee, DD, Oliver, BB, Willson, TM, Zetterstrom, RH, Perlmann, T & Lehmann, JM (1998). An

orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell*, Vol. 92(1): pp. 73-82, ISSN 0092-8674

Kodama, S, Koike, C, Negishi, M & Yamamoto, Y (2004). Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Mol Cell Biol*, Vol. 24(18): pp. 7931-40, ISSN 0270-7306

Kodama, S, Moore, R, Yamamoto, Y & Negishi, M (2007). Human nuclear pregnane X receptor cross-talk with CREB to repress cAMP activation of the glucose-6-phosphatase gene. *Biochem J*, Vol. 407(3): pp. 373-81, ISSN 1470-8728

Konno, Y, Negishi, M & Kodama, S (2008). The roles of nuclear receptors CAR and PXR in hepatic energy metabolism. *Drug Metab Pharmacokinet*, Vol. 23(1): pp. 8-13, ISSN 1880-0920

Koyano, S, Kurose, K, Ozawa, S, Saeki, M, Nakajima, Y, Hasegawa, R, Komamura, K, Ueno, K, Kamakura, S, Nakajima, T, Saito, H, Kimura, H, Goto, Y, Saitoh, O, Katoh, M, Ohnuma, T, Kawai, M, Sugai, K, Ohtsuki, T, Suzuki, C, Minami, N, Saito, Y & Sawada, J (2002). Eleven novel single nucleotide polymorphisms in the NR1I2 (PXR) gene, four of which induce non-synonymous amino acid alterations. *Drug Metab Pharmacokinet*, Vol. 17(6): pp. 561-5, ISSN 1347-4367

Lamba, V, Yasuda, K, Lamba, JK, Assem, M, Davila, J, Strom, S & Schuetz, EG (2004). PXR (NR1I2): splice variants in human tissues, including brain, and identification of neurosteroids and nicotine as PXR activators. *Toxicol Appl Pharmacol*, Vol. 199(3): pp. 251-65, ISSN 0041-008X

Langmann, T, Moehle, C, Mauerer, R, Scharl, M, Liebisch, G, Zahn, A, Stremmel, W & Schmitz, G (2004). Loss of detoxification in inflammatory bowel disease: dysregulation of pregnane X receptor target genes. *Gastroenterology*, Vol. 127(1): pp. 26-40, ISSN 0016-5085

Lehmann, JM, McKee, DD, Watson, MA, Willson, TM, Moore, JT & Kliewer, SA (1998). The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest*, Vol. 102(5): pp. 1016-23, ISSN 0021-9738

Li, J, Ning, G & Duncan, SA (2000). Mammalian hepatocyte differentiation requires the transcription factor HNF-4alpha. *Genes Dev*, Vol. 14(4): pp. 464-74, ISSN 0890-9369

Li, T, Chen, W & Chiang, JY (2007). PXR induces CYP27A1 and regulates cholesterol metabolism in the intestine. *J Lipid Res*, Vol. 48(2): pp. 373-84, ISSN 0022-2275

Lichti-Kaiser, K, Brobst, D, Xu, C & Staudinger, JL (2009a). A systematic analysis of predicted phosphorylation sites within the human pregnane X receptor protein. *J Pharmacol Exp Ther*, Vol. 331(1): pp. 65-76, ISSN 1521-0103

Lichti-Kaiser, K, Xu, C & Staudinger, JL (2009b). Cyclic AMP-dependent protein kinase signaling modulates pregnane x receptor activity in a species-specific manner. *J Biol Chem*, Vol. 284(11): pp. 6639-49, ISSN 0021-9258

Lin, W, Wu, J, Dong, H, Bouck, D, Zeng, FY & Chen, T (2008). Cyclin-dependent kinase 2 negatively regulates human pregnane X receptor-mediated CYP3A4 gene expression in HepG2 liver carcinoma cells. *J Biol Chem*, Vol. 283(45): pp. 30650-7, ISSN 0021-9258

Lonard, DM & O'Malley, BW (2009). Chapter 4 emerging roles of the ubiquitin proteasome system in nuclear hormone receptor signaling. *Prog Mol Biol Transl Sci*, Vol. 87: pp. 117-35, ISSN 1878-0814

Lutjohann, D, Hahn, C, Prange, W, Sudhop, T, Axelson, M, Sauerbruch, T, von Bergmann, K & Reichel, C (2004). Influence of rifampin on serum markers of cholesterol and bile acid synthesis in men. *Int J Clin Pharmacol Ther*, Vol. 42(6): pp. 307-13, ISSN 0946-1965

Ma, X, Cheung, C, Krausz, KW, Shah, YM, Wang, T, Idle, JR & Gonzalez, FJ (2008a). A double transgenic mouse model expressing human pregnane X receptor and cytochrome P450 3A4. *Drug Metab Dispos*, Vol. 36(12): pp. 2506-12, ISSN 1521-009X

Ma, X, Idle, JR & Gonzalez, FJ (2008b). The pregnane X receptor: from bench to bedside. *Expert Opin Drug Metab Toxicol*, Vol. 4(7): pp. 895-908, ISSN 1742-5255

Ma, X, Shah, Y, Cheung, C, Guo, GL, Feigenbaum, L, Krausz, KW, Idle, JR & Gonzalez, FJ (2007). The Pregnane X receptor gene-humanized mouse: a model for investigating drug-drug interactions mediated by cytochromes P450 3A. *Drug Metab Dispos*, Vol. 35(2): pp. 194-200, ISSN 0090-9556

Mai, I, Bauer, S, Perloff, ES, Johne, A, Uehleke, B, Frank, B, Budde, K & Roots, I (2004). Hyperforin content determines the magnitude of the St John's wort-cyclosporine drug interaction. *Clin Pharmacol Ther*, Vol. 76(4): pp. 330-40, ISSN 0009-9236

Masuyama, H, Hiramatsu, Y, Kodama, J & Kudo, T (2003). Expression and potential roles of pregnane X receptor in endometrial cancer. *J Clin Endocrinol Metab*, Vol. 88(9): pp. 4446-54, ISSN 0021-972X

Masuyama, H, Hiramatsu, Y, Mizutani, Y, Inoshita, H & Kudo, T (2001). The expression of pregnane X receptor and its target gene, cytochrome P450 3A1, in perinatal mouse. *Mol Cell Endocrinol*, Vol. 172(1-2): pp. 47-56, ISSN 0303-7207

Masuyama, H, Inoshita, H, Hiramatsu, Y & Kudo, T (2002). Ligands have various potential effects on the degradation of pregnane X receptor by proteasome. *Endocrinology*, Vol. 143(1): pp. 55-61, ISSN 0013-7227

Masuyama, H, Nakatsukasa, H, Takamoto, N & 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*, Vol. 72(4): pp. 1045-53, ISSN 0026-895X

Masuyama, H, Suwaki, N, Tateishi, Y, Nakatsukasa, H, Segawa, T & Hiramatsu, Y (2005). The pregnane X receptor regulates gene expression in a ligand- and promoter-selective fashion. *Mol Endocrinol*, Vol. 19(5): pp. 1170-80, ISSN 0888-8809

McCarver, DG & Hines, RN (2002). The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. *J Pharmacol Exp Ther*, Vol. 300(2): pp. 361-6, ISSN 0022-3565

McKenna, NJ & O'Malley, BW (2002). Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell*, Vol. 108(4): pp. 465-74, ISSN 0092-8674

Mencarelli, A, Migliorati, M, Barbanti, M, Cipriani, S, Palladino, G, Distrutti, E, Renga, B & Fiorucci, S (2010). Pregnane-X-receptor mediates the anti-inflammatory activities of rifaximin on detoxification pathways in intestinal epithelial cells. *Biochem Pharmacol*, Vol. 80(11): pp. 1700-7, ISSN 1873-2968

Mensah-Osman, EJ, Thomas, DG, Tabb, MM, Larios, JM, Hughes, DP, Giordano, TJ, Lizyness, ML, Rae, JM, Blumberg, B, Hollenberg, PF & Baker, LH (2007). Expression levels and activation of a PXR variant are directly related to drug resistance in osteosarcoma cell lines. *Cancer*, Vol. 109(5): pp. 957-65, ISSN 0008-543X

Meyer zu Schwabedissen, HE, Tirona, RG, Yip, CS, Ho, RH & Kim, RB (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*, Vol. 68(22): pp. 9338-47, ISSN 1538-7445

Miki, Y, Suzuki, T, Kitada, K, Yabuki, N, Shibuya, R, Moriya, T, Ishida, T, Ohuchi, N, Blumberg, B & Sasano, H (2006). Expression of the steroid and xenobiotic receptor and its possible target gene, organic anion transporting polypeptide-A, in human breast carcinoma. *Cancer Res*, Vol. 66(1): pp. 535-42, ISSN 0008-5472

Misawa, A, Inoue, J, Sugino, Y, Hosoi, H, Sugimoto, T, Hosoda, F, Ohki, M, Imoto, I & Inazawa, J (2005). 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*, Vol. 65(22): pp. 10233-42, ISSN 0008-5472

Moore, DD, Kato, S, Xie, W, Mangelsdorf, DJ, Schmidt, DR, Xiao, R & Kliewer, SA (2006). International Union of Pharmacology. LXII. The NR1H and NR1I receptors: constitutive androstane receptor, pregnene X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor. *Pharmacol Rev*, Vol. 58: pp. 742-59, ISSN 0031-6997

Moore, LB, Goodwin, B, Jones, SA, Wisely, GB, Serabjit-Singh, CJ, Willson, TM, Collins, JL & Kliewer, SA (2000). St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor. *Proc Natl Acad Sci U S A*, Vol. 97(13): pp. 7500-2, ISSN 0027-8424

Moreau, A, Teruel, C, Beylot, M, Albalea, V, Tamasi, V, Umbdenstock, T, Parmentier, Y, Sa-Cunha, A, Suc, B, Fabre, JM, Navarro, F, Ramos, J, Meyer, U, Maurel, P, Vilarem, MJ & Pascussi, JM (2009). A novel pregnane X receptor and S14-mediated lipogenic pathway in human hepatocyte. *Hepatology*, Vol. 49(6): pp. 2068-79, ISSN 1527-3350

Mu, Y, Zhang, J, Zhang, S, Zhou, HH, Toma, D, Ren, S, Huang, L, Yaramus, M, Baum, A, Venkataramanan, R & Xie, W (2006). Traditional Chinese medicines Wu Wei Zi (Schisandra chinensis Baill) and Gan Cao (Glycyrrhiza uralensis Fisch) activate pregnane X receptor and increase warfarin clearance in rats. *J Pharmacol Exp Ther*, Vol. 316(3): pp. 1369-77, ISSN 0022-3565

Nakajima, M & Yokoi, T (2011). MicroRNAs from biology to future pharmacotherapy: Regulation of cytochrome P450s and nuclear receptors. *Pharmacol Ther*, Vol.: pp., ISSN 1879-016X

Nakamura, K, Moore, R, Negishi, M & Sueyoshi, T (2007). Nuclear pregnane X receptor cross-talk with FoxA2 to mediate drug-induced regulation of lipid metabolism in fasting mouse liver. *J Biol Chem*, Vol. 282(13): pp. 9768-76, ISSN 0021-9258

Nebert, DW & Gonzalez, FJ (1987). P450 genes: structure, evolution, and regulation. *Annu Rev Biochem*, Vol. 56: pp. 945-93, ISSN 0066-4154

Ngan, CH, Beglov, D, Rudnitskaya, AN, Kozakov, D, Waxman, DJ & Vajda, S (2009). The Structural Basis of Pregnane X Receptor Binding Promiscuity. *Biochemistry*, Vol. 48(48): pp. 11572-81, ISSN 0006-2960

Niemi, M, Backman, JT, Fromm, MF, Neuvonen, PJ & Kivisto, KT (2003). Pharmacokinetic interactions with rifampicin : clinical relevance. *Clin Pharmacokinet*, Vol. 42(9): pp. 819-50, ISSN 0312-5963

Noble, SM, Carnahan, VE, Moore, LB, Luntz, T, Wang, H, Ittoop, OR, Stimmel, JB, Davis-Searles, PR, Watkins, RE, Wisely, GB, LeCluyse, E, Tripathy, A, McDonnell, DP & Redinbo, MR (2006). Human PXR forms a tryptophan zipper-mediated homodimer. *Biochemistry*, Vol. 45(28): pp. 8579-89, ISSN 0006-2960

Orans, J, Teotico, DG & Redinbo, MR (2005). The nuclear xenobiotic receptor pregnane X receptor: recent insights and new challenges. *Mol Endocrinol*, Vol. 19(12): pp. 2891-900, ISSN 0888-8809

Ourlin, JC, Lasserre, F, Pineau, T, Fabre, JM, Sa-Cunha, A, Maurel, P, Vilarem, MJ & Pascussi, JM (2003). The small heterodimer partner interacts with the pregnane X receptor and represses its transcriptional activity. *Mol Endocrinol*, Vol. 17: pp. 1693-703, ISSN 0888-8809

Ouyang, N, Ke, S, Eagleton, N, Xie, Y, Chen, G, Laffins, B, Yao, H, Zhou, B & Tian, Y (2010). Pregnane X receptor suppresses proliferation and tumourigenicity of colon cancer cells. *Br J Cancer*, Vol. 102(12): pp. 1753-61, ISSN 1532-1827

Pascussi, JM, Drocourt, L, Fabre, JM, Maurel, P & Vilarem, MJ (2000). Dexamethasone induces pregnane X receptor and retinoid X receptor-alpha expression in human hepatocytes: synergistic increase of CYP3A4 induction by pregnane X receptor activators. *Mol Pharmacol*, Vol. 58(2): pp. 361-72, ISSN 0026-895X

Pascussi, JM, Robert, A, Nguyen, M, Walrant-Debray, O, Garabedian, M, Martin, P, Pineau, T, Saric, J, Navarro, F, Maurel, P & Vilarem, MJ (2005). Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. *J Clin Invest*, Vol. 115(1): pp. 177-86, ISSN 0021-9738

Paunescu, E (1970). In vivo and in vitro suppression of humoral and cellular immunological response by rifampicin. *Nature*, Vol. 228(5277): pp. 1188-90, ISSN 0028-0836

Pondugula, SR, Brimer-Cline, C, Wu, J, Schuetz, EG, Tyagi, RK & Chen, T (2009a). A phosphomimetic mutation at threonine-57 abolishes transactivation activity and alters nuclear localization pattern of human pregnane x receptor. *Drug Metab Dispos*, Vol. 37(4): pp. 719-30, ISSN 1521-009X

Pondugula, SR, Dong, H & Chen, T (2009b). Phosphorylation and protein-protein interactions in PXR-mediated CYP3A repression. *Expert Opin Drug Metab Toxicol*, Vol. 5(8): pp. 861-73, ISSN 1744-7607

Poso, A & Honkakoski, P (2006). Ligand recognition by drug-activated nuclear receptors PXR and CAR: structural, site-directed mutagenesis and molecular modeling studies. *Mini Rev Med Chem*, Vol. 6(8): pp. 937-47, ISSN 1389-5575

Quattrochi, LC, Mills, AS, Barwick, JL, Yockey, CB & Guzelian, PS (1995). A novel cis-acting element in a liver cytochrome P450 3A gene confers synergistic induction by glucocorticoids plus antiglucocorticoids. *J Biol Chem*, Vol. 270: pp. 28917-23, ISSN 0021-9258

Raucy, J, Warfe, L, Yueh, MF & Allen, SW (2002). A cell-based reporter gene assay for determining induction of CYP3A4 in a high-volume system. *J Pharmacol Exp Ther*, Vol. 303: pp. 412-23, ISSN 0022-3565

Raynal, C, Pascussi, JM, Leguelinel, G, Breuker, C, Kantar, J, Lallemant, B, Poujol, S, Bonnans, C, Joubert, D, Hollande, F, Lumbroso, S, Brouillet, JP & Evrard, A (2010). Pregnane X Receptor (PXR) expression in colorectal cancer cells restricts irinotecan chemosensitivity through enhanced SN-38 glucuronidation. *Mol Cancer*, Vol. 9: pp. 46, ISSN 1476-4598

Repa, JJ, Turley, SD, Lobaccaro, JA, Medina, J, Li, L, Lustig, K, Shan, B, Heyman, RA, Dietschy, JM & Mangelsdorf, DJ (2000). Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science*, Vol. 289(5484): pp. 1524-9, ISSN 0036-8075

Ross, R (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med*, Vol. 340(2): pp. 115-26, ISSN 0028-4793

Saini, SP, Sonoda, J, Xu, L, Toma, D, Uppal, H, Mu, Y, Ren, S, Moore, DD, Evans, RM & Xie, W (2004). A novel constitutive androstane receptor-mediated and CYP3A-independent pathway of bile acid detoxification. *Mol Pharmacol*, Vol. 65(2): pp. 292-300, ISSN 0026-895X

Saradhi, M, Krishna, B, Mukhopadhyay, G & Tyagi, RK (2005). Purification of full-length human pregnane and xenobiotic receptor: polyclonal antibody preparation for immunological characterization. *Cell Res*, Vol. 15(10): pp. 785-95, ISSN 1001-0602

Shah, YM, Ma, X, Morimura, K, Kim, I & Gonzalez, FJ (2007). Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-kappaB target gene expression. *Am J Physiol Gastrointest Liver Physiol*, Vol. 292(4): pp. G1114-22, ISSN 0193-1857

Smith, NF, Mani, S, Schuetz, EG, Yasuda, K, Sissung, TM, Bates, SE, Figg, WD & Sparreboom, A (2010). Induction of CYP3A4 by vinblastine: Role of the nuclear receptor NR1I2. *Ann Pharmacother*, Vol. 44(11): pp. 1709-17, ISSN 1542-6270

Sonoda, J, Xie, W, Rosenfeld, JM, Barwick, JL, Guzelian, PS & Evans, RM (2002). Regulation of a xenobiotic sulfonation cascade by nuclear pregnane X receptor (PXR). *Proc Natl Acad Sci U S A*, Vol. 99(21): pp. 13801-6, ISSN 0027-8424

Staudinger, JL, Goodwin, B, Jones, SA, Hawkins-Brown, D, MacKenzie, KI, LaTour, A, Liu, Y, Klaassen, CD, Brown, KK, Reinhard, J, Willson, TM, Koller, BH & Kliewer, SA (2001). The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A*, Vol. 98(6): pp. 3369-74, ISSN 0027-8424

Staudinger, JL, Xu, C, Biswas, A & Mani, S (2011). Post-translational modification of pregnane x receptor. *Pharmacol Res*, Vol. 64(1): pp. 4-10, ISSN 1096-1186

Synold, TW, Dussault, I & Forman, BM (2001). The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux. *Nat Med*, Vol. 7(5): pp. 584-90, ISSN 1078-8956

Takagi, S, Nakajima, M, Mohri, T & Yokoi, T (2008). Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. *J Biol Chem*, Vol. 283(15): pp. 9674-80, 0021-9258

Takeshita, A, Taguchi, M, Koibuchi, N & Ozawa, Y (2002). Putative role of the orphan nuclear receptor SXR (steroid and xenobiotic receptor) in the mechanism of CYP3A4 inhibition by xenobiotics. *J Biol Chem*, Vol. 277(36): pp. 32453-8, 0021-9258

Teng, S, Jekerle, V & Piquette-Miller, M (2003). Induction of ABCC3 (MRP3) by pregnane X receptor activators. *Drug Metab Dispos*, Vol. 31(11): pp. 1296-9, ISSN 0090-9556

Teng, S & Piquette-Miller, M (2007). Hepatoprotective role of PXR activation and MRP3 in cholic acid-induced cholestasis. *Br J Pharmacol*, Vol. 151(3): pp. 367-76, 0007-1188

Teotico, DG, Bischof, JJ, Peng, L, Kliewer, SA & Redinbo, MR (2008). Structural Basis of Human Pregnane X Receptor Activation by the Hops Constituent Colupulone. *Molecular Pharmacology*, Vol. 74(6): pp. 1512-20, ISSN 0026-895X

Ung, CY, Li, H, Yap, CW & Chen, YZ (2007). In silico prediction of pregnane X receptor activators by machine learning approaches. *Mol Pharmacol*, Vol. 71(1): pp. 158-68, ISSN 0026-895X

Velaparthi, U, Wittman, M, Liu, P, Carboni, JM, Lee, FY, Attar, R, Balimane, P, Clarke, W, Sinz, MW, Hurlburt, W, Patel, K, Discenza, L, Kim, S, Gottardis, M, Greer, A, Li, A, Saulnier, M, Yang, Z, Zimmermann, K, Trainor, G & Vyas, D (2008). Discovery and evaluation of

4-(2-(4-chloro-1H-pyrazol-1-yl)ethylamino)-3-(6-(1-(3-fluoropropyl)piperid in-4-yl)-4-methyl-1H-benzo[d]imidazol-2-yl)pyridin-2(1H)-one (BMS-695735), an orally efficacious inhibitor of insulin-like growth factor-1 receptor kinase with broad spectrum in vivo antitumor activity. *J Med Chem*, Vol. 51(19): pp. 5897-900, ISSN 1520-4804

Venkatesh, M, Wang, H, Cayer, J, Leroux, M, Salvail, D, Das, B, Wrobel, JE & Mani, S (2011). In Vivo and In Vitro Characterization of a First-in-Class Novel Azole Analog That Targets Pregnane X Receptor Activation. *Mol Pharmacol*, Vol. 80(1): pp. 124-35, ISSN 1521-0111

Wagner, M, Halilbasic, E, Marschall, HU, Zollner, G, Fickert, P, Langner, C, Zatloukal, K, Denk, H & Trauner, M (2005). CAR and PXR agonists stimulate hepatic bile acid and bilirubin detoxification and elimination pathways in mice. *Hepatology*, Vol. 42(2): pp. 420-30, ISSN 0270-9139

Wang, H, Li, H, Moore, LB, Johnson, MD, Maglich, JM, Goodwin, B, Ittoop, OR, Wisely, B, Creech, K, Parks, DJ, Collins, JL, Willson, TM, Kalpana, GV, Venkatesh, M, Xie, W, Cho, SY, Roboz, J, Redinbo, M, Moore, JT & Mani, S (2008a). The phytoestrogen coumestrol is a naturally occurring antagonist of the human pregnane X receptor. *Mol Endocrinol*, Vol. 22(4): pp. 838-57, ISSN 0888-8809

Wang, W, Prosise, WW, Chen, J, Taremi, SS, Le, HV, Madison, V, Cui, X, Thomas, A, Cheng, KC & Lesburg, CA (2008b). Construction and characterization of a fully active PXR/SRC-1 tethered protein with increased stability. *Protein Eng Des Sel*, Vol. 21(7): pp. 425-33, ISSN 1741-0126

Watkins, RE, Davis-Searles, PR, Lambert, MH & Redinbo, MR (2003a). Coactivator binding promotes the specific interaction between ligand and the pregnane X receptor. *J Mol Biol*, Vol. 331(4): pp. 815-28, ISSN 0022-2836

Watkins, RE, Maglich, JM, Moore, LB, Wisely, GB, Noble, SM, Davis-Searles, PR, Lambert, MH, Kliewer, SA & Redinbo, MR (2003b). 2.1 A crystal structure of human PXR in complex with the St. John's wort compound hyperforin. *Biochemistry*, Vol. 42(6): pp. 1430-8, ISSN 0006-2960

Watkins, RE, Wisely, GB, Moore, LB, Collins, JL, Lambert, MH, Williams, SP, Willson, TM, Kliewer, SA & Redinbo, MR (2001). The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity. *Science*, Vol. 292(5525): pp. 2329-33, ISSN 0036-8075

Wilke, RA, Lin, DW, Roden, DM, Watkins, PB, Flockhart, D, Zineh, I, Giacomini, KM & Krauss, RM (2007). Identifying genetic risk factors for serious adverse drug reactions: current progress and challenges. *Nat Rev Drug Discov*, Vol. 6(11): pp. 904-16, ISSN 1474-1784

Willson, TM & Kliewer, SA (2002). PXR, CAR and drug metabolism. *Nat Rev Drug Discov*, Vol. 1(4): pp. 259-66, ISSN 1474-1776

Xiao, L, Nickbarg, E, Wang, W, Thomas, A, Ziebell, M, Prosise, WW, Lesburg, CA, Taremi, SS, Gerlach, VL, Le, HV & Cheng, KC (2011). Evaluation of in vitro PXR-based assays and in silico modeling approaches for understanding the binding of a structurally diverse set of drugs to PXR. *Biochem Pharmacol*, Vol. 81(5): pp. 669-79, ISSN 1873-2968

Xie, W, Barwick, JL, Downes, M, Blumberg, B, Simon, CM, Nelson, MC, Neuschwander-Tetri, BA, Brunt, EM, Guzelian, PS & Evans, RM (2000a). Humanized xenobiotic response in mice expressing nuclear receptor SXR. *Nature*, Vol. 406(6794): pp. 435-9, ISSN 0028-0836

Xie, W, Barwick, JL, Simon, CM, Pierce, AM, Safe, S, Blumberg, B, Guzelian, PS & Evans, RM (2000b). Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. *Genes Dev*, Vol. 14(23): pp. 3014-23, ISSN 0890-9369

Xie, W, Radominska-Pandya, A, Shi, Y, Simon, CM, Nelson, MC, Ong, ES, Waxman, DJ & Evans, RM (2001). An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc Natl Acad Sci U S A*, Vol. 98(6): pp. 3375-80, ISSN 0027-8424

Xu, Y, Hashizume, T, Shuhart, MC, Davis, CL, Nelson, WL, Sakaki, T, Kalhorn, TF, Watkins, PB, Schuetz, EG & Thummel, KE (2006). Intestinal and hepatic CYP3A4 catalyze hydroxylation of 1alpha,25-dihydroxyvitamin D(3): implications for drug-induced osteomalacia. *Mol Pharmacol*, Vol. 69(1): pp. 56-65, ISSN 0026-895X

Xue, Y, Chao, E, Zuercher, WJ, Willson, TM, Collins, JL & Redinbo, MR (2007). Crystal structure of the PXR-T1317 complex provides a scaffold to examine the potential for receptor antagonism. *Bioorg Med Chem*, Vol. 15(5): pp. 2156-66, ISSN 0968-0896

Yang, XJ & Seto, E (2008). Lysine acetylation: codified crosstalk with other posttranslational modifications. *Mol Cell*, Vol. 31(4): pp. 449-61, ISSN 1097-4164

Yeung, EY, Sueyoshi, T, Negishi, M & Chang, TK (2008). Identification of Ginkgo biloba as a novel activator of pregnane X receptor. *Drug Metab Dispos*, Vol. 36(11): pp. 2270-6, ISSN 1521-009X

Zhai, Y, Pai, HV, Zhou, J, Amico, JA, Vollmer, RR & Xie, W (2007). Activation of pregnane X receptor disrupts glucocorticoid and mineralocorticoid homeostasis. *Mol Endocrinol*, Vol. 21(1): pp. 138-47, ISSN 0888-8809

Zhou, C, King, N, Chen, KY & Breslow, JL (2009). Activation of PXR induces hypercholesterolemia in wild-type and accelerates atherosclerosis in apoE deficient mice. *J Lipid Res*, Vol. 50(10): pp. 2004-13, ISSN 0022-2275

Zhou, C, Poulton, EJ, Grun, F, Bammler, TK, Blumberg, B, Thummel, KE & Eaton, DL (2007). The dietary isothiocyanate sulforaphane is an antagonist of the human steroid and xenobiotic nuclear receptor. *Mol Pharmacol*, Vol. 71(1): pp. 220-9, ISSN 0026-895X

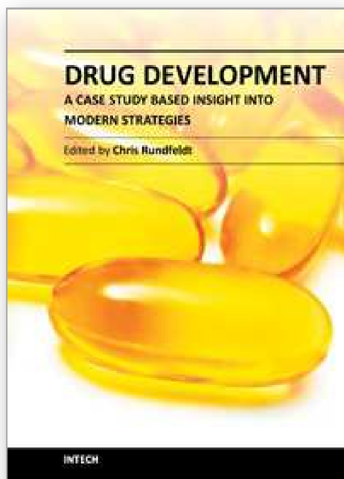
Zhou, J, Febbraio, M, Wada, T, Zhai, Y, Kuruba, R, He, J, Lee, JH, Khadem, S, Ren, S, Li, S, Silverstein, RL & Xie, W (2008a). Hepatic fatty acid transporter Cd36 is a common target of LXR, PXR, and PPARgamma in promoting steatosis. *Gastroenterology*, Vol. 134(2): pp. 556-67, ISSN 1528-0012

Zhou, J, Liu, M, Zhai, Y & Xie, W (2008b). The antiapoptotic role of pregnane X receptor in human colon cancer cells. *Mol Endocrinol*, Vol. 22(4): pp. 868-80, ISSN 0888-8809

Zhou, J, Zhai, Y, Mu, Y, Gong, H, Uppal, H, Toma, D, Ren, S, Evans, RM & Xie, W (2006). A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J Biol Chem*, Vol. 281(21): pp. 15013-20, ISSN 0021-9258

Zhu, Z, Kim, S, Chen, T, Lin, JH, Bell, A, Bryson, J, Dubaquie, Y, Yan, N, Yanchunas, J, Xie, D, Stoffel, R, Sinz, M & Dickinson, K (2004). Correlation of high-throughput pregnane X receptor (PXR) transactivation and binding assays. *J Biomol Screen*, Vol. 9(6): pp. 533-40, ISSN 1087-0571

Zimmermann, K, Wittman, MD, Saulnier, MG, Velaparthi, U, Sang, X, Frennesson, DB, Struzynski, C, Seitz, SP, He, L, Carboni, JM, Li, A, Greer, AF, Gottardis, M, Attar, RM, Yang, Z, Balimane, P, Discenza, LN, Lee, FY, Sinz, M, Kim, S & Vyas, D (2010). SAR of PXR transactivation in benzimidazole-based IGF-1R kinase inhibitors. *Bioorg Med Chem Lett*, Vol. 20(5): pp. 1744-8, ISSN 1464-3405



Drug Development - A Case Study Based Insight into Modern Strategies
Edited by Dr. Chris Rundfeldt

ISBN 978-953-307-257-9
Hard cover, 654 pages
Publisher InTech
Published online 07, December, 2011
Published in print edition December, 2011

This book represents a case study based overview of many different aspects of drug development, ranging from target identification and characterization to chemical optimization for efficacy and safety, as well as bioproduction of natural products utilizing for example lichen. In the last section, special aspects of the formal drug development process are discussed. Since drug development is a highly complex multidisciplinary process, case studies are an excellent tool to obtain insight in this field. While each chapter gives specific insight and may be read as an independent source of information, the whole book represents a unique collection of different facets giving insight in the complexity of drug development.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Su Sien Ong, Yue-Ming Wang, Sergio C. Chai and Taosheng Chen (2011). Pregnane X Receptor in Drug Development, Drug Development - A Case Study Based Insight into Modern Strategies, Dr. Chris Rundfeldt (Ed.), ISBN: 978-953-307-257-9, InTech, Available from: http://www.intechopen.com/books/drug-development-a-case-study-based-insight-into-modern-strategies/pregnane-x-receptor-in-drug-development

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.