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REVIEW

Pregnane X receptor- and *CYP3A4*-humanized mouse models and their applications

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Pregnane X receptor (PXR) is a pivotal nuclear receptor modulating xenobiotic metabolism primarily through its regulation of CYP3A4, the most important enzyme involved in drug metabolism in humans. Due to the marked species differences in ligand recognition by PXR, *PXR*-humanized (hPXR) mice, and mice expressing human PXR and CYP3A4 (Tg3A4/hPXR) were established. hPXR and Tg3A4/hPXR mice are valuable models for investigating the role of PXR in xenobiotic metabolism and toxicity, in lipid, bile acid and steroid hormone homeostasis, and in the control of inflammation.

Abbreviation

Alb, albumin promoter; AUC, area under the curve; CAR, constitutive androstane receptor; CYP3A4, cytochrome P450-3A4; CYP3A4-A, mice with albumin promoter-targeted liver-specific expression of CYP3A4; *CYP3A4*-humanized mice, *CYP3A4*-humanized mice lacking of mouse *Cyp3a*; CYP3A4-V, mice with villin promoter-targeted intestine-specific expression of CYP3A4; *Cyp3a*-null, *Cyp3a* knockout mice; DBD, DNA-binding domain; FABP, fatty acid-binding protein; FXR, farnesoid X receptor; hPXR mice, *PXR*-humanized mice; IBD, inflammatory bowel disease; LBD, ligand-binding domain; LCA, lithocholic acid; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; *Pxr*-null, *Pxr* knockout mice; RXR, retinoid X receptor; Tg3A4, transgenic *CYP3A4* (*CYP3A7*) mice with the mouse *Cyp3a* background; Tg3A4/hPXR, CYP3A4 and human PXR double transgenic mice lacking of mouse PXR

Human pregnane X receptor (PXR), encoded by the nuclear receptor subfamily 1, group I, member 2 (NR1I2) gene, is located on chromosome 3q12–q13.3 and consists of nine exons; exons 2 to 9 contain the coding region for a 434 amino acid protein (Ekins *et al.*, 2009). Similar to other nuclear receptors, PXR possesses the common modulator structure of a conserved *N*-terminal DNA-binding domain (DBD) and *C*-terminal ligand-binding domain (LBD). It functions as a heterodimer with the 9-cis retinoic acid receptor, also known as retinoid X receptor (RXR) to control gene transcription (Ngan *et al.*, 2009). However, unlike most other nuclear receptors, PXR has a markedly flexible pocket which can bind structurally diverse ligands (Kliewer *et al.*, 2002), including prescription drugs, natural products, dietary supplements, environmental pollutants, endogenous hormones and bile acids (Ma *et al.*, 2008b). Human and mouse PXR share nearly 80% amino acid identity across the LBD, 96% amino acid identity in the DBD, and display similar tissue-specific expression patterns. However, the differences between the LBD sequence result in species-specific responses to ligand activation by human and mouse PXR (Lehmann *et al.*, 1998). For example, rifampicin has virtually no activity on the mouse PXR at typical pharmacological doses, but is a very potent activator of human PXR. Conversely, pregnenolone-16α-carbonitrile (PCN) only weakly activates human PXR but is an efficacious activator of mouse PXR (Lehmann *et al.*, 1998). Site-directed mutagenesis studies have revealed that four-polar amino acids contribute to the specific recognition of human PXR ligands in the ligand-binding pocket (Watkins *et al.*, 2001).

The species selectivity in ligand binding of PXR inspired the demand for establishing *PXR*-humanized (hPXR) mouse

models. In one case, a human PXR transgene encoded by a bacterial artificial chromosome genomic clone was inserted into the mouse genome on the *Pxr*-null background and was stably expressed in a tissue-specific pattern that reflects expression in humans (Ma *et al.*, 2007a). This whole body hPXR mouse model serves as a valuable platform in pharmacology and toxicology to explore clinical drug–drug interactions and PXR-mediated endogenous metabolic homeostasis.

Cytochrome P450-3A4 (CYP3A4), involved in metabolism of over 50% of clinically used drugs (Isin and Guengerich, 2006), is highly sensitive to upstream modulation by PXR. CYP3A4 has a large pocket with wide substrate-binding selectivity. Many CYP3A4 substrates are also human PXR activators (Istrate *et al.*, 2010). PXR and CYP3A4 facilitate the metabolism and elimination of xenobiotics including drugs and toxicants. The CYP3A4 promoter contains a direct repeat of AG (G/T) TCA denoted as the ER6 motif that binds the human PXR/RXR heterodimer; this sequence is not found in the mouse CYP3A11 (Istrate *et al.*, 2010). Consequently, rifampicin is a robust activator of CYP3A4 in the Tg3A4/hPXR double transgenic model (Ma *et al.*, 2008a) compared with only slight induction of mouse Cyp3a11 (Cheng *et al.*, 2009). In contrast, rifampicin barely increases CYP3A4 in CYP3A4 transgenic mice containing the endogenous mouse PXR, as expected from the known species differences in ligand activation. Given the importance of CYP3A4 in drug development, the Tg3A4/hPXR mice could be a valuable model to study drug metabolism and to predict drug–drug interactions and drug safety.

Mouse models

The genetically engineered PXR and CYP3A4 mouse models available are summarized in Figure 1. The models include *Pxr*-null, hPXR, *Cyp3a*-null, *CYP3A4*-humanized and *CYP3A4*/*hPXR* double transgenic mice (Tg3A4/hPXR), as well as models in which the proteins are expressed only in liver and intestine, or constitutively activated. *Pxr*-null mice were produced either by disruption of two exons of the mouse *Pxr*, including the critical amino acid residues from 63 to 170 of the DBD (Xie *et al.*, 2000), or by removal of the first coding exon encoding the translation start site and the first zinc finger of the DBD (Staudinger *et al.*, 2001). Both *Pxr*-null mouse lines are viable and fertile with no significant differences in development, physiological homeostasis and serum biochemistry. They have been widely used for investigation of PXR-dependent signalling pathways and to determine the function of PXR in xenobiotic metabolism. Conditional hPXR or whole body hPXR mice were generated on the mouse *Pxr*-null background (Xie *et al.*, 2000). A liver-specific albumin promoter (Alb-PXR) and albumin-VP16 activation domain (Alb-VP-PXR) of the herpes simplex virus were used to construct liver-specific hPXR mice (Xie *et al.*, 2000). The fatty acid-binding protein (FABP)-activated VP16 human PXR (FABP-VP-PXR) mouse model was developed for driving human PXR expression in the liver and throughout the intestine with notable high expression in the caecum and colon (Gong *et al.*, 2006). The whole body hPXR mouse model was generated by insertion of the complete human PXR coding sequence including 5′ and 3′ flaking sequences and all exons

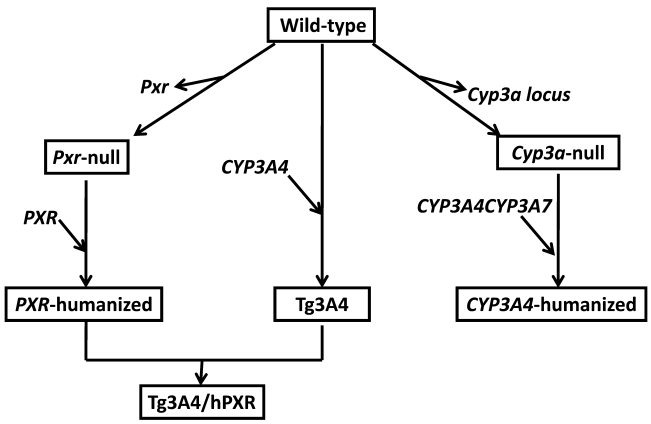

Figure 1

Flow chart of genetically engineered PXR and CYP3A4 mouse models. This figure lists the published mouse models. *PXR*-humanized (hPXR) mice can be grouped into tissue-specific conditional hPXR mice, including liver-specific (Alb)-PXR mice or liver/intestine-specific (FABP)-PXR mice, and whole body hPXR and huPXR mice. Three *CYP3A4*-humanized mice and transgenic *CYP3A4* (*CYP3A7*) mice were produced, and *CYP3A4*/*LacZ* and CYP3A4-luc mice have been established for tracking the expression of CYP3A4 *in vivo*. Tg3A4/hPXR double transgenic mice were generated by crossing hPXR mice with Tg3A4 mice, on the mouse *Pxr*-null and wild-type *Cyp3a* background. Alb, albumin promoter; CYP3A4, cytochrome P450-3A4; FABP, fatty acid-binding protein; hPXR mice, *PXR*-humanized mice; Tg3A4, transgenic *CYP3A4* (*CYP3A7*) mice with the mouse *Cyp3a* background; Tg3A4/hPXR, CYP3A4 and human PXR double transgenic mice lacking of mouse PXR.

contained within a bacterial artificial chromosome (Ma *et al.*, 2007a). Another *PXR*-humanized mouse line (huPXR) was produced by insertion of the human PXR coding region (exon 2–9) into wild-type mice by using the flipase recombinase system to specifically delete a hygromycin selection cassette fused with the human PXR gene. In contrast to the previous whole body hPXR model, expression of human PXR in this mouse line is under the control of the native mouse *Pxr* promoter (Scheer *et al.*, 2008). All hPXR mice show a normal phenotype, except for the Alb-VP-PXR mice that exhibited growth retardation, hepatomegaly and histological liver toxicity.

A *Cyp3a*-null mouse model was generated by a heroic effort through deletion of the complete mouse *Cyp3a* cluster, including the catalytically active Cyp3a13, Cyp3a57 and Cyp3a59 enzymes (van Herwaarden *et al.*, 2007). Surprisingly, there were no marked developmental or physiological abnormalities, thus revealing that these genes are dispensable in mice in the absence of dietary or chemical stress. However, the detoxification capabilities of *Cyp3a*-null mice are markedly reduced, as revealed by exposure to chemotherapeutic drugs. In contrast, *CYP3A4*-humanized mice (CYP3A4-A, mice with Alb-targeted, liver-specific expression of CYP3A4 and CYP3A4-V, mice with villin promoter-targeted intestine-specific expression of CYP3A4) established on mice lacking the *Cyp3a* gene cluster both have the ability to detoxify drugs upon challenge.

The transgenic CYP3A4 mouse (Tg3A4) was produced by direct insertion, by transgenesis, of the *CYP3A4* and *CYP3A7*

genes on the wild-type *Cyp3a* background (Cheung *et al.*, 2006); these mice exhibited gender-dependent CYP3A4 expression in liver and the gastrointestinal track (Yu *et al.*, 2005). Constitutive expression of CYP3A4 in the gut leads to a unique abnormal development of the mammary gland that is associated with a lactation deficiency suggesting a role for CYP3A4 in oestradiol homeostasis. Additionally, CYP3A4/*lacZ* (Robertson *et al.*, 2003) or CYP3A4-luc transgenic mice models (Zhang *et al.*, 2003) were also generated on the wild-type *Cyp3a* background, which were produced by integration of vectors containing the *CYP3A4* gene constructed with luminescent motifs. These two models have been applied for tracking the expression of CYP3A4. Furthermore, given the species selectivity of PXR and CYP3A, a human PXR and CYP3A4 double transgenic mouse model (Tg3A4/hPXR) (Ma *et al.*, 2008a) was established to explore transcriptional regulation and drug interaction studies without the mouse PXR background. In conclusion, all the mice models developed in different laboratories are denoted as *Pxr*-null, Alb-VP-PXR, Alb-PXR, FABP-VP-PXR, hPXR, huPXR, *Cyp3a*-null, CYP3A4-A, CYP3A4-V, Tg3A4, CYP3A4/*lacZ*, CYP3A4-luc and Tg3A4/hPXR.

Application of mouse models

Drug metabolism and toxicity

Cyp3a-null mice and CYP3A4-A (liver) and CYP3A4-V (intestine) humanized models have been largely used to study CYP3A4-mediated phase I drug metabolism transport and elimination. Docetaxel, a derivative of paclitaxel and an advanced chemotherapeutic drug, exhibits 18-fold and sevenfold higher area under the curve (AUC) after oral or intravenous administration of drug to *Cyp3a*-null mice compared with wild-type mice (van Herwaarden *et al.*, 2007). In contrast, *CYP3A4*-humanized mice rapidly metabolize docetaxel as revealed by reduced AUC compared with wild-type mice. Additionally, an efficient elimination of docetaxel was noted in CYP3A4-V mice after oral administration, while only a marginally lower elimination was observed in CYP3A4-A mice, thus indicating a predominant role for intestinal CYP3A4 in preventing docetaxel from entering the systemic circulation resulting in lower bioavailability. Similar decreases in AUC were observed with the CYP3A4 probe substrates of midazolam and cyclosporine A (van Herwaarden *et al.*, 2005) in these two humanized CYP3A4 mouse models compared with wild-type mice. Thus, CYP3A4-A and CYP3A4-V mice as well as the *Cyp3a*-null mouse can reveal the pathways of substrate metabolism by CYP3A4, and differentiate the role of intestinal versus hepatic CYP3A4 in metabolism and bioavailability. The evaluation of drug-efflux enzymes like ATP-binding cassette subfamily G member 2, multidrug resistance protein 2 and multidrug resistance 1, etc. (van Waterschoot *et al.*, 2009; 2010) will be of great value in the preclinical *in vivo* prediction of the pharmacological properties of xenobiotic compounds, including its absorption, transportation, metabolism and elimination.

Together with hPXR mice, Tg3A4/hPXR double transgenic mice present advantages in determining drug metabolism, drug–drug interactions and toxicity involving CYP3A4 induction and its metabolic activities (Figure 2). A recent study using the Tg3A4/hPXR double transgenic mouse line revealed that human PXR potentiates the hepatotoxicity of the widely used over-the-counter analgesic acetaminophen (APAP)

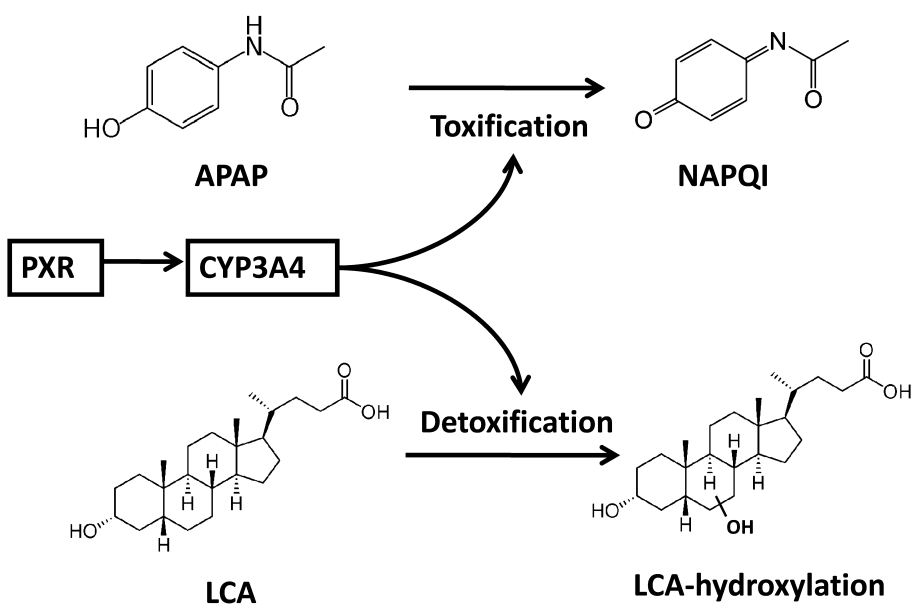

Figure 2

Application of mouse models for the study of drug metabolism and toxicity. Toxification is represented by the conversion of acetaminophen (APAP) into *N*-acetyl-*p*-benzoquinone imine (NAPQI) in Tg3A4/hPXR mice. In contrast, lithocholic acid (LCA) hydroxylation is an example of a preventive role for PXR in drug detoxification. CYP3A4, cytochrome P450-3A4; PXR, pregnane X receptor; Tg3A4/hPXR, CYP3A4 and human PXR double transgenic mice lacking of mouse PXR.

through CYP3A4 induction and increased production of *N*-acetyl-*p*-benzoquinone imine (NAPQI) resulting in elevated oxidative stress (Cheng *et al.*, 2009). A marked increase in serum enzymes diagnostic for liver toxicity alanine amino transferase and aspartate amino transferase was observed in Tg3A4/hPXR mice administered APAP compared with APAP injection to hPXR, wild-type and *Pxr*-null mice. A higher level of cysteine-APAP and 3-*N*-acetyl-cysteinyl-APAP in urine associated with the APAP dimer in serum of Tg3A4/hPXR mice, revealed increased production of the potentially toxic quinone metabolite NAPQI. This study suggests that drug–drug interactions involving APAP and other PXR activators could lead to liver damage.

Activation of human PXR may exacerbate the untoward effects of compounds through activation of downstream enzymes, elevation of oxidative stress and production of reactive intermediate as occurs with APAP metabolism or reactive free radical generated from paraquat metabolism (Gong *et al.*, 2006). However, there is much evidence indicating that PXR usually exerts protection against drug-induced toxicity (Figure 2). For example, Alb-PXR mice were used to investigate the relationship between human PXR and lithocholic acid (LCA)-induced hepatotoxicity (Staudinger *et al.*, 2001; Xie *et al.*, 2001). LCA is a secondary bile acid and known to cause intrahepatic cholestasis. LCA and its metabolite are efficacious inducers of human PXR and LCA detoxification via human PXR activation probably through repression of Cyp7a1-mediated metabolism and elevation of LCA hydroxylation of toxic bile acids through *Cyp3a* and its sulphation; an increased production of organic anion transporter polypeptides 2 (Oatp2) may also play a role. These findings reveal a potential role for human PXR in cholestasis and other hepatic diseases.

Screening of hPXR agonist and antagonist

Screening of compounds that can activate or inhibit PXR can identify PXR activators and predict drug–drug interactions. For example, rifaximin, a structural analogue of rifampicin and a semisynthetic rifamycin-derived antibiotic, has been used in the treatment of traveler's diarrhoea, inflammatory bowel disease (IBD) and hepatic encephalopathy. Orally administered rifaximin is poorly absorbed into the circulation. Rifaximin was found to be a gut-specific human PXR agonist that does not activate hepatic PXR as revealed by hPXR mice and luciferase reporter gene assays (Ma *et al.*, 2007b). It does not activate mouse PXR at pharmacological concentrations.

Compound S20, a C-cyclopropylalkylamide, is another novel agonist triggering chirally dependent and species-specific ligand binding, as determined by primary human hepatocytes, and hPXR mice (Mu *et al.*, 2005). Enantiomer (+)-S20 preferentially activates human PXR, while the enantiomer (–)-S20 is a better activator of mouse PXR. Mutagenesis of mouse PXR F305L leads to lower activation by (–)-S20, whereas mutagenesis of human PXR L308F does not alter activation by (+)-S20. This study suggests that enantiomers identified in chemical libraries might have species-specific selectivity attributed to the variable ligand-binding pocket of human PXR.

Nutrients and natural products, such as St. John's wort, have been widely studied for their activation of PXR. Compared with human primary hepatocytes and other *in vitro* systems, the hPXR mouse model yields a more comprehensive understanding of complex metabolic pathways. Vitamin K, which promotes bone formation and is used in osteoporosis therapy, was reported to activate human PXR resulting in induction of CYP3A4 (Tabb *et al.*, 2003). Other agonists like rifampicin and hyperforin have shown a similar induction of a panel of bone markers, thus indicating that a subset of PXR activators may function as effective therapeutic agents for the management of osteoporosis. Subsequent studies revealed that the osteoblastogenic transcription factor Msx2 is a PXR target gene as analysed by chromatin immunoprecipitation assays, promoter analysis and gene knock-down (Igarashi *et al.*, 2007). Traditional Chinese medicines (TCMs), or alternative medicines, have also been evaluated for their ability to activate PXR. Tian xian, a traditional Chinese herbal anticancer remedy, has the potential to interact with other conventional chemotherapeutic agents via human PXR activation and CYP3A4 metabolism (Lichti-Kaiser and Staudinger, 2008). Thus, the activation of PXR and induction of detoxifying enzymes provide a molecular mechanism for the hepatoprotective effects of certain TCMs.

Compared with the large number of human PXR agonists uncovered, only a few antagonists have been discovered and evaluated. Ketoconazole (Lim *et al.*, 2009), ecteinascidin-743 (Sparfel *et al.*, 2003) and leflunomide (Ekins *et al.*, 2008) were found to be PXR antagonists by *in vitro* cell-based assays and/or computational analysis. For example, coumestrol is an isoflavonoid-like phytooestrogen with oestrogen structure and actions. *In vivo* assays using hPXR mice revealed a significant inhibition of coumestrol on human PXR and no activity towards rodent PXR; these results were supported by additional *in vitro* binding analysis (Wang *et al.*, 2008). Coumestrol could bind PXR at a site other than the ligand-binding pocket of the receptor protein and this binding disrupts the association of PXR with the steroid receptor coactivator-1.

Suppression of inflammation

Pregnane X receptor was found to inhibit nuclear factor-κB (NF-κB) responsible for the production of pro-inflammatory cytokines (Xie and Tian, 2006). This interaction could contribute to the mechanism by which PXR suppresses IBD. PCN activation of mouse PXR was found to protect against dextran sulphate sodium (DSS)-induced IBD in wild-type mice but not in *Pxr*-null mice (Shah *et al.*, 2007). Rifaximin, a gut-specific agonist, exerts a therapeutic role in IBD as revealed by studies using the hPXR mice; no activity was found in wild-type or *Pxr*-null mice. Two classic IBD models were used in the human PXR IBD study, DSS-induced or trinitrobenzene sulphonic acid-induced IBD. Mice were treated with rifaximin either pre- or post-administration of DSS, indicating that rifaximin functions as both a protective and therapeutic drug in IBD. Amelioration of the IBD symptoms in hPXR mice was correlated with NF-κB inhibition via human PXR activation (Cheng *et al.*, 2010). NF-κB interacts with the highly conserved RXR-DBD of the PXR/RXR heterodimer (Gu *et al.*, 2006). In addition, cAMP-dependent protein kinase (PKA) signalling modulates PXR in a species-specific pattern (Lichti-Kaiser *et al.*, 2009). PKA regulates NF-κB-dependent transcription with different expression levels of endogenous A-kinase

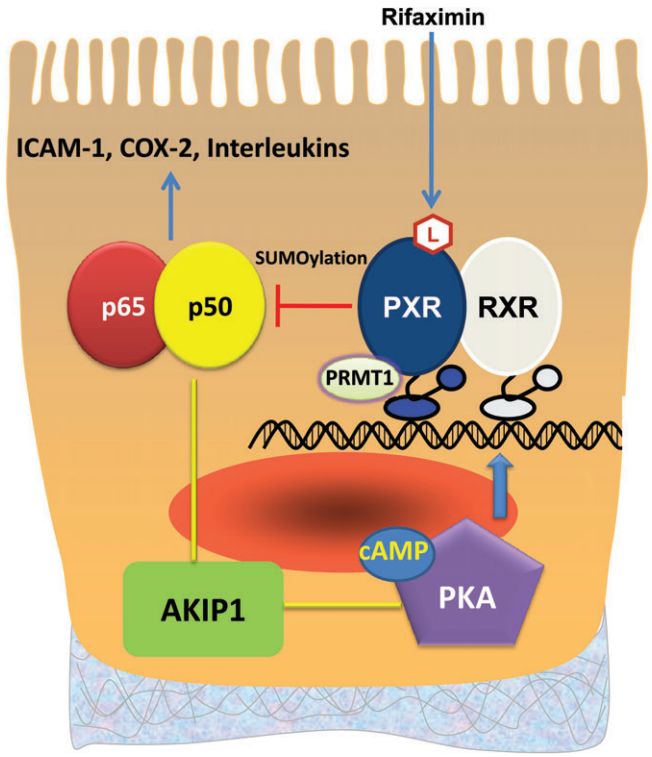

Figure 3

Prevention of inflammatory bowel disease via PXR activation. Intestinal epithelial cells are injured in inflammatory bowel disease due to increased inflammation as a result in part of NF-κB (p65 and p50 dimer) activation and subsequent release of pro-inflammatory factors like inter-cellular adhesion molecule 1 (ICAM-1), cyclooxygenase-2 (COX-2), interleukin, etc. However, following rifaximin activation of hPXR, NF-κB is inhibited, thus decreasing the liberation of pro-inflammatory cytokines. cAMP-dependent protein kinase (PKA), endogenous A-kinase interacting protein 1 (AKIP1) and protein arginine methyltransferase 1 (PRMT1), etc. might also be involved in the crosslink of PXR and NF-κB. Arrows indicate activation, and red lines indicate inhibition. hPXR mice, *PXR*-humanized mice; NF-κB, nuclear factor-κB; PXR, pregnane X receptor.

interacting protein 1 (AKIP1) in multiple cell lines (Gao *et al.*, 2010). Moreover, a recent study revealed that the histone methyltransferase, protein arginine methyltransferase 1 (PRMT1), plays a role in the transcriptional activity of PXR by controlling its cellular compartmentalization (Xie *et al.*, 2009). A recent report also revealed that rifampicin-activated human PXR SUMOylation directly represses NF-κB in liver (Hu *et al.*, 2010). The interaction of PXR with NF-κB and its role in IBD is displayed in Figure 3. While the link between PXR and NF-κB requires further investigation, the therapeutic role of rifaximin and PXR in IBD is relatively firmly established.

Coordinate regulation in human disease

Despite identification of PXR as a xenobiotic receptor, emerging evidence indicates that PXR is also an endobiotic receptor, and in some cases is involved in the coordinate regulation of endobiotics with other nuclear receptors, including constitutive androstane receptor (CAR), liver X receptor (LXR), farnesoid X receptor (FXR) and peroxisome proliferator-activated receptor (PPAR)γ (Figure 4). Hepatic lipid homeostasis is the balance of lipid formation, catabolism and secretion; lipid dysregulation in liver leads to hepatic steatosis or cholestasis. Alb-VP-PXR mice express the liver-specific constitutively activated PXR (in the absence of ligand), and present hepatic steatosis resulting from accumulation of hepatic triglycerides (Zhou *et al.*, 2008a). Cluster of differentiation 36 (CD36), stearoyl-coenzyme A desaturase 1 and fatty acid elongase 2 are up-regulated independent of sterol regulatory element-binding protein-1, through PXR. CD36, a key fatty acid transporter for lipogenesis and a known PPARγ target gene, was postulated to be a PXR target gene responsible for hepatic lipid accumulation. The aldo-keto reductase family 1, member 7 (Akr1b7) was also found to play an important role in lipid peroxidation and up-regulated by PXR and CAR in liver and small intestine. Wild-type mice treated with PCN or TCPOBOP exhibit increased Akr1b7 levels in liver and intestine, associated with elevated intestinal malondialdehyde (Liu *et al.*, 2009), However, PXR ligands cannot alter the Akr1b7 expression in *Pxr*-null mice. Unraveling the role of CD36 and Akr1b7 in lipid homestasis and hepatic injury could broaden the involvement of PXR in lipid homeostasis.

Bile acids are the terminal products of cholesterol metabolism and abnormal hepatic accumulation of bile acids is potentially toxic and can lead to cholestasis. LCA activates hepatic PXR and lack of either PXR or CAR and both PXR and CAR increased the sensitivity of mice to LCA-induced toxicity in a male-selective manner (Uppal *et al.*, 2005). Several bile acid transporters (bile salt export pump, Oatp1, Oatp4), catabolism enzymes (Cyp3a11) and upstream nuclear receptors (small heterodimer partner, FXR) appear to be significantly modified in *Pxr*-null, *Car*-null or *Pxr/Car*-null mice by LCA treatment compared with wild-type mice (Uppal *et al.*, 2005). In addition, PXR and LXR also interact in modulating the homeostasis of bile acids (Makishima, 2005). Similarly, activated LXR is sexually dimorphic in the prevention of bile acid-induced toxicity and cholestasis (Gong and Xie, 2004). As toxic bile acids are potentially promoters of colon cancer, the possibility exists that PXR may have a role in the chemoprevention of colon carcinogenesis. Indeed, activation of PXR in hPXR mice inhibits bile acid-induced colonic epithelial apoptosis and sensitizes mice to dimethylhydrazine-induced colonic carcinogenesis (Zhou *et al.*, 2008b). Multiple antiapoptotic genes, including B-cell lymphomu 2 (BCL2)-associated athanogene 3, baculoviral inhibitor of apoptosis repeat-containing protein 2 and myeloid cell leukaemia sequence 1, are increased, while in contrast, pro-apoptotic genes, such as BCL2 antagonist/killer 1 and tumour protein P53 are down-regulated after bile acid treatment (Zhou *et al.*, 2008b).

Bilirubin is a primary haem byproduct. Its accumulation results in hyperbilirubinaemia and jaundice. An unexpected increase in bilirubin clearance was observed in the *Pxr*-null mice and also observed in humanized mice that express the constitutively activated PXR or CAR. Activated PXR and CAR in humanized mice also stimulate bilirubin catabolism (Saini *et al.*, 2005). The constitutively activated PXR suppresses CAR and loss of PXR causes derepression and resultant up-regulation of bilirubin-detoxifying enzymes and transporters, thus indicating that PXR plays dual roles in

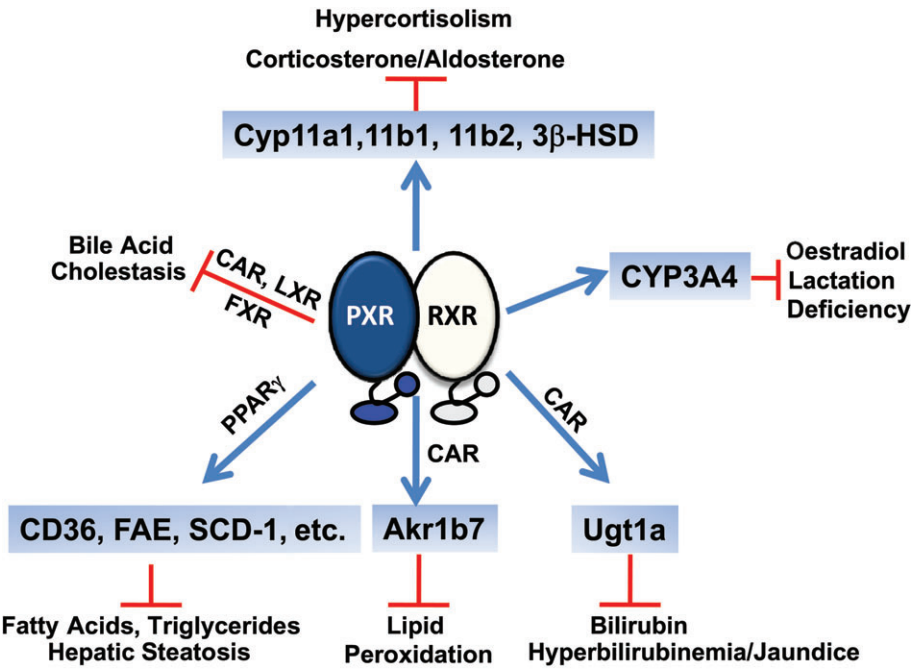

Figure 4

Coordinated regulation of PXR *in vivo* in transgenic mouse models. PXR can modulate hepatic steatosis, and lipid, bile acid and steroid homeostasis by interplay with CAR, LXR, FXR and PPARγ, etc. PXR activates target genes and attenuates the progress of hepatic steatosis, hyperbilirubinaemia or jaundice, hypercortisolism and cholestasis, while disrupting the oestradiol homeostasis by increasing CYP3A4 via human PXR activation. Arrows indicate activation, and red lines indicate inhibition. 3β-HSD, 3β-hydroxysteroid dehydrogenase; CAR, constitutive androstane receptor; CYP3A4, cytochrome P450-3A4; FXR, farnesoid X receptor; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor.

regulating bilirubin homeostasis. Furthermore, hPXR mice demonstrated a massive increase in Ugt1a in humanized mice; Ugt1a catalyses the conversion of bilirubin to bilirubin-glucuronide. Thus, regulation of Ugt1a by PXR enhances bilirubin clearance and increases the clearance of oestrogen, thyroxin and potential carcinogens (Xie *et al.*, 2003; Zhou *et al.*, 2005).

Constitutively-activated hPXR mice and ligand-activated hPXR mice also demonstrated adrenocorticotropic hormone-independent hypercortisolism (Zhai *et al.*, 2007). The glucocorticoid effect appears to be PXR-specific, as activation of CAR has little effect. Activated PXR markedly increase plasma concentrations of corticosterone and aldosterone that are associated with induction of adrenal steroidogenic enzymes, including Cyp11a1, Cyp11b1, Cyp11b2 and 3β-hydroxysteroid dehydrogenase. Independent of the PXR effect, Tg-3A4 mice also exhibit a hormone disorder modulated by growth secretion patterns. These mice display sexually dimorphic expression of CYP3A4 associated with oestradiol dysregulation in pregnant mice leading to a lactation deficiency (Yu *et al.*, 2005). Thus, PXR and CYP3A4 are the potential endocrine disrupting factors that may have broad implications in steroid homeostasis and drug–hormone interactions.

Conclusion

Human PXR and CYP3A4 mice models have been important tools in the exploration of PXR-mediated xenobiotic metabolism and toxicity, and elucidation of PXR endobiotic roles in balancing hepatic steatosis, and regulating lipid, bile acid and steroid homeostasis. Studies of PXR function in human disease needs to consider the coordinated regulation of PXR with CAR, LXR, FXR, PPARγ and retinoid-related orphan receptor α/γ, etc., as well as to determine the role of PXR in diseases such as arteriosclerosis, obesity or diabetes. hPXR and CYP3A4 mouse models could be developed for screening of drug–drug interactions, as well as pharmacological and toxicological evaluation of drug candidates.

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Conflict of interest

None declared.

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