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## Review

# Nuclear receptor PXR, transcriptional circuits and metabolic relevance☆☆☆

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## ARTICLE INFO

### Article history:

Received 29 October 2010

Received in revised form 25 January 2011

Accepted 26 January 2011

Available online 2 February 2011

### Keywords:

Nuclear receptor  
Gene regulation  
Xenobiotic receptor  
Xenobiotics  
Endobiotics

## ABSTRACT

The pregnane X receptor (PXR, NR1I2) is a ligand activated transcription factor that belongs to the nuclear hormone receptor (NR) superfamily. PXR is highly expressed in the liver and intestine, but low levels of expression have also been found in many other tissues. PXR plays an integral role in xenobiotic and endobiotic metabolism by regulating the expression of drug-metabolizing enzymes and transporters, as well as genes implicated in the metabolism of endobiotics. PXR exerts its transcriptional regulation by binding to its DNA response elements as a heterodimer with the retinoid X receptor (RXR) and recruitment of a host of coactivators. The biological and physiological implications of PXR activation are broad, ranging from drug metabolism and drug–drug interactions to the homeostasis of numerous endobiotics, such as glucose, lipids, steroids, bile acids, bilirubin, retinoic acid, and bone minerals. The purpose of this article is to provide an overview on the transcriptional circuits and metabolic relevance controlled by PXR. This article is part of a Special Issue entitled: Translating Nuclear Receptors from Health to Disease.

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## 1. Introduction

### 1.1. Discovery of PXR

The pregnane X receptor (PXR, NR1I2) belongs to the nuclear hormone receptor (NR) superfamily of ligand activated transcription factors [1]. PXR has been shown to play an essential role in xenobiotic metabolism in humans, mice, rats, and rabbits [2–5]. Subsequent studies have strongly suggested that PXR also plays an important role in endobiotic metabolism in humans, mice, and rats [6–16]. The mouse PXR (mPXR) was first discovered and cloned in 1998 based on sequence homology with other NRs and was found to be activated by a variety of compounds, including natural and synthetic glucocorticoids, steroids, pregnane derivatives, antiglucocorticoids, macrocyclic antibiotics, antifungals, and herbal extracts [1,3,17–21]. The human PXR (hPXR) ortholog was subsequently reported as the steroid and xenobiotic receptor (SXR) and pregnane activated receptor (PAR), both exhibiting structural features and activation patterns similar to

mPXR [19,20]. SXR/PAR was later confirmed to be orthologous to mPXR by Xie and colleagues via the gene replacement experiment with the PXR knockout mice [22]. PXR has since been cloned from a wide array of species, including mammals, birds, and fish [3,18–21,23–25].

The structural organization of PXR follows that of a typical NR which includes an NH<sub>2</sub> - terminal ligand independent activation function domain (AF-1, A/B region), a highly conserved DNA binding domain (DBD, C region), a less conserved hinge domain (D region), followed by a C-terminal ligand binding domain (LBD, E region) and an activation function 2 domain (AF-2, F region) [26–30].

### 1.2. PXR's mode of action

When bound to and activated by ligands, PXR translocates from the cytoplasm to the nucleus of the cells [31]. PXR then binds to its DNA response elements as a heterodimer with the retinoid X receptor (RXR). PXR is also capable of recruiting a host of coactivators which includes members of the p160 family of coactivators such as steroid receptor coactivators 1 (SRC-1), TIF/GRIP (SRC-2), and peroxisome proliferator activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) [32–34]. The DBD of PXR facilitates DNA binding specificity via two highly conserved zinc finger motifs as well as a P-Box motif and D-Box motif which allow the receptor to target and bind its xenobiotic response elements (XREs) located in the 5' promoter region of PXR target genes [35]. PXR can bind to a variety of DNA response elements containing two copies of the half site consensus sequence AG(G/T)TCA with various spacing, which includes direct repeats DR-3, DR-4, and DR-5, and everted repeats ER-6 and ER-8 [28].

**Abbreviations:** CYP, cytochrome P450; DDI, drug–drug interaction; G6Pase, glucose-6-phosphatase; GST, glutathione S-transferase; NR, nuclear receptor; PCN, pnenolone-16 $\alpha$ -carbonitrile; PEPCK, phosphoenolpyruvate carboxykinase; PXR, pregnane X receptor; RIF, rifampicin; SULT, sulfotransferase; UGT, UDP-glucuronosyl transferase; XRE, xenobiotic response element

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☆☆ This article is part of a Special Issue entitled: Translating nuclear receptors from health to disease.

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## 2. PXR in xenobiotic metabolism

### 2.1. Regulation of Phase I enzymes

Functional characterization of PXR has shown that this receptor acts as a xenosensor, playing a major role in protecting organisms from exogenous chemical insults. PXR is highly expressed in the liver, intestine, and kidneys, but low levels have also been found in the peripheral blood monocytes, blood brain barrier, uterus, ovary, placenta, breast, osteoclasts, heart, adrenal glands, bone marrow, and specific brain regions of various species [23,36–40]. Given such a broad range of expression pattern, PXR is well suited to accommodate its metabolic role through the induction of metabolizing/detoxifying enzymes and transporters.

The metabolism of exogenous and endogenous compounds is quintessential for normal physiological functioning of any living organism. PXR is capable of modulating this process through induction of the major Phase I cytochromes P450 enzymes (CYPs). CYPs are a superfamily of heme-dependent monooxygenases, which catalyze the first step of detoxification of aliphatic or lipophilic compounds [41,42]. Highly expressed in the liver and intestine [41], CYPs use hydroxylation and/or oxidation reactions to convert target compounds into more soluble derivatives that are easier to excrete from the body [42]. Activation of PXR has been shown to lead to the transcription of a host of CYP genes in humans and rodents, including CYP3A4, CYP3A23, CYP3A11, CYP2B6, Cyp2b9, Cyp2c55, CYP2C8, CYP2C9, CYP2C19, and CYP1A [18,37,43–47].

It is apparent that since PXR controls the transcription of an array of CYPs, this receptor must be activated by a commensurate number of xenobiotic compounds. This is in fact the case: hPXR has been shown to be activated by a plethora of pharmaceutical drugs that include rifampicin (RIF), rifaximin [48], clotrimazole [3], dexamethasone [18], lovastatin [18] and metyrapone [49] to name a few. PXR is also activated by a variety of environmental pollutants such as 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), di-*n*-butyl phthalate (DBP), chlordane, dieldrin, and endosulfan [50–52]. Finally, PXR can be activated by a variety of medicinal compounds derived from herbal sources including *Schisandra chinensis* (anti-perspiration), *Piper methysticum* (chloraseptic), and *Agauria salicifolia* (arrhythmia) [53].

The ligand-dependent PXR activation has been shown to be species specific at times. For example, in humans and rabbits the antibiotic RIF is a potent PXR activator. However, the same drug has little effect on the mouse or rat PXR. In contrast, the synthetic antigluocorticoid pregnenolone-16 $\alpha$ -carbonitrile (PCN) can activate the mouse and rat PXR but has no effect on hPXR. These species–species differences represent a challenge for pharmaceutical companies attempting to select appropriate animal models to evaluate candidate drugs. The same notion has also led to the initial creation and characterization of the hPXR humanized mice [22].

### 2.2. Regulation of Phase II enzymes

PXR also can regulate the expression of Phase II drug-metabolizing enzymes, including UDP-glucuronosyl transferase (UGT), sulfotransferase (SULT) and glutathione S-transferase (GST) enzymes [54]. Phase II metabolic transformations are often, but do not have to be, preceded by Phase I oxidation reactions which expose or add sites that are ideal for Phase II conjugates. The Phase II metabolic enzymes add polar molecules onto xenobiotics and endobiotics, producing water-soluble, non-toxic metabolites amenable to biliary and/or urinary excretion [55]. Indeed, a major consequence of PXR-mediated Phase II metabolic enzyme regulation is the metabolism and detoxification of bile acids, estrogens, thyroxine, xenobiotics, and carcinogens [56].

UGTs are central Phase II metabolic enzymes which often have distinct as well as overlapping substrates [57]. In humans, 19 enzymes exist which contribute extensively to metabolism by catalyzing the

addition of a UDP-glucuronic acid to endo- and xenobiotics, enhancing their water solubility and elimination [57,58]. PXR activation by carbamazepine, RIF, dexamethasone and phenytoin has been linked to the transcriptional activation of several UGTs, including UGT1A1, UGT1A6, UGT1A3 and UGT1A4 [56,57,59,60]. These UGT isoforms are also responsible for the metabolism of a plethora of other drugs such as lamotrigine, olanzapine, retigabine, irinotecan/SN38, acetaminophen, cyproheptadine, nicotine and imipramine, as well as carcinogens such as 4-nitrophenol and 4-OH-PhIP, benzo[a]pyrene [56,57,61,62].

SULT enzyme activities represent another important Phase II pathway of metabolism. SULTs facilitate xenobiotic metabolism by catalyzing the addition of sulfate conjugates on drug molecules leading to more water soluble compounds [63]. In mice, PXR activation by PCN has been shown to upregulate the transcription of several SULT isoforms including Sult1a1, Sult2a1, and Sult5a1 [64]. The role of PXR in human regulation of SULTs in response to xenobiotics is loosely established. Treatment with dexamethasone has been shown to upregulate SULT2A1 in human liver cells, but rifampicin treatment has been shown to have both inductive and suppressive effects [65–67].

GSTs are also major enzymes in Phase II metabolism, as well as many other cytoprotective pathways. GSTs protect cells, organelles, and macromolecules from chemical and oxidative stress, and electrophiles. GSTs catalyze nucleophilic attack via reduced glutathione (GSH) on non-polar compounds containing an electrophilic carbon, rendering them less reactive and more hydrophilic [68,69]. In mice, PXR activation by spironolactone, dexamethasone, and PCN has been shown to induce several GSTs including Gsta3, Gstm1, Gstm2, Gstm3, Gstm4, and MGst1 [69]. The effect of genetic activation of PXR on GST expression in transgenic mice has been shown to be GST isoform-, gender-, and tissue-specific. Human PXR has not been extensively shown to induce GSTs; however, a recent report by Naspinski and colleagues correlated PXR activation by benzo[a]pyrene with subsequent upregulation of several GSTs including GSTA1, GSTA2 and GSTM1 [70].

### 2.3. Regulation of drug transporters

Drug disposition and metabolism are also regulated by an array of cellular uptake and efflux transporters that control intestinal and hepatic absorption, renal re-absorption, and biliary/urinary elimination. These transporters work in concert with Phase I and II enzymes. The major xenobiotic transporters subject to PXR regulation include the ATP binding cassette family (ABC) proteins expressed in hepatocytes, enterocytes, kidney, and blood brain barrier that regulate cellular export of drugs. Examples of PXR target ABC transporters include the multidrug resistance 1 or P-glycoprotein (MDR1/P-gp), multidrug resistance associated proteins (MRP2, MRP3, MRP4, and MRP5), and breast cancer resistance protein (BCRP) [71–74]. The organic anion transporting polypeptide family (SLC/OATP), which regulates drug and endobiotic influx/uptake into the liver, is also regulated by PXR [75]. The known PXR target SLC/OATP genes include SLC01A2/OATP1A2, SLC01B1/OATP1B1, and SLC01B3/OATP1B3. Finally, the organic ion transporter family, particularly the organic cation transporter SLC22A5/OCTN2, is proposed to have moderate PXR related regulation [76].

### 2.4. Implication of PXR in drug–drug interactions (DDIs)

As previously discussed, CYPs play an integral role in Phase I metabolism. Among CYP isoforms, the CYP3A subfamily is the most abundant in the liver and also conveys broad substrate specificity [77]. In fact, CYP3As have been shown to be responsible for the metabolism of over 50% of pharmaceuticals on the market today [77]. PXR has been shown to be a major transcriptional regulator of CYP3As, and because of this, it became increasingly apparent that the PXR-mediated regulation of drug-metabolizing enzymes could be involved in clinical DDIs. Such interactions occur when one drug accelerates the

metabolism of a second, potentially leading to adverse consequences [78]. In addition, DDIs have been shown to cause decreased or absent bioavailability for orally administered drugs and increased hepatic clearance or accelerated formation of reactive metabolites, which can lead to local or systemic toxicity [78]. The human CYP3A4 is induced by PXR and has been shown clinically to be involved in possible drug–drug interactions. An example is the effect of RIF, a hPXR agonist, on the metabolism of the antihypertensive drug Verapamil [78]. Long-term treatment with RIF caused increased hepatic and gastrointestinal levels of CYP3A4, which led to the reduced oral bioavailability of (S)-verapamil by 96%, and abolished the anti-hypertensive effect in patients [78]. Another example was reported by Maglich and colleagues when they demonstrated that the anxiolytic herb St. John's Wort could induce CYP3A4 at low concentrations. This discovery explained prevalent clinical data which showed increased metabolism and reduced efficacy of oral contraceptives, cyclosporin, and indinavir when taken with St. John's Wort [79]. Unfortunately, these are not anecdotal occurrences, as they have been demonstrated clinically in an array of situations [80–85]. In the future, it is imperative to continue to use relevant animal models, such as the hPXR humanized mice [86], in order to fully understand the pharmacokinetic profile of PXR ligands and CYP substrates.

### 3. PXR in endobiotic metabolism

Although PXR was originally characterized as a xenobiotic receptor, it has been demonstrated that PXR has equal importance as an endobiotic receptor. Many studies have revealed essential roles of PXR in glucose and lipid metabolism, steroid hormone homeostasis, bile acid and bilirubin detoxification, vitamin metabolism and inflammation. As a result, PXR activation has great implications in many patho-physiological conditions.

#### 3.1. PXR in glucose metabolism

Blood glucose is tightly controlled by insulin and glucagon through gluconeogenesis, glycogenolysis, and glycogenesis. Glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase1 (PEPCK1) are rate-limiting enzymes in gluconeogenesis and glycogenolysis [87,88]. These genes are upregulated by glucagon and glucocorticoids. Glucagon increases the formation of intracellular cAMP, which activates protein kinase A (PKA) to stimulate cAMP-response element-binding protein (CREB) that binds to and regulates the transcription of PEPCK1 and G6Pase [89,90]. Similarly, glucocorticoids induce PEPCK1 expression through a glucocorticoid response element [91]. On the other hand, insulin suppresses gluconeogenesis by downregulating the transcription of G6Pase and PEPCK1 [92]. The forkhead transcription factor 1 (FoxO1) functions as an activator of G6Pase and PEPCK1 in the absence of insulin [92,93]. Upon binding to an insulin response sequence (IRS), insulin excludes FoxO1 from the nucleus through phosphatidylinositol3-kinase (PI3K)-Akt pathway [94], resulting in a repressed expression of G6Pase and PEPCK1 and decreased glucose production.

PXR plays a role in hepatic gluconeogenesis. The expression of PEPCK and G6Pase was reduced in VP-hPXR transgenic mice in which the expression of an activated PXR (VP-PXR) was directed to the liver [95]. The PXR agonist PCN downregulated G6Pase gene expression in wild type but not PXR<sup>−/−</sup> mice [93]. Studies by Kodama and colleagues [93] suggested cross-talk between PXR and CREB and FoxO1 in regulating gluconeogenesis. They used a gel shift assay to show that PXR directly interacted with CREB and prevented its binding to the G6Pase gene promoter. Next, through a chromatin immunoprecipitation (ChIP) assay they showed that treatment with PCN decreased CREB binding to the promoter of G6Pase only in wild-type but not in PXR<sup>−/−</sup> mice. Thus, by forming a complex with phosphorylated CREB, ligand activated PXR repressed CREB-mediated gene transcription and gluconeogenesis. Finally, PXR inhibited the binding of FoxO1 to IRS by direct interaction with FoxO1, as demonstrated by both gel shift and

GST pull-down assays. A reporter gene assay also showed that FoxO1-IRS activity was repressed by PXR activation [96]. Therefore, it seems that ligand activated PXR directly interacts with FoxO1, which prevents FoxO1 from binding to IRS, leading to the suppression of G6Pase and PEPCK1 gene expression and gluconeogenesis [97]. The hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ) also positively regulates gluconeogenesis with the nuclear receptor coactivator PGC-1 $\alpha$ . Bhalla and colleagues showed that PXR could compete with HNF4 $\alpha$  for PGC-1 $\alpha$  and thus suppress gluconeogenesis [9].

#### 3.2. PXR in lipid metabolism

Triglycerides and fatty acids are also vital metabolic fuels. Lipid homeostasis centers on balancing lipid uptake and synthesis with lipid catabolism and secretion. When glucose and fatty acids exceed the body's energy needs, they are converted to triglycerides in the liver for storage. During fasting or exercise, fatty acid  $\beta$ -oxidation and ketogenesis are increased in adipocytes to enhance ketone body synthesis and to provide energy. The sterol regulatory element-binding protein 1c (SREBP-1c) is a master regulator of lipogenesis. Several nuclear receptors, such as LXR [98], HNF-4 [99] and LRH-1 [100], orchestrate lipid homeostasis through regulating the transcriptional activity of SREBP.

Interestingly, Zhou and colleagues showed that PXR induced lipogenesis in an SREBP-independent manner [95]. VP-PXR transgenic mice showed increased triglyceride accumulation in the liver, which was linked to upregulation of fatty acid translocase CD36 and several other accessory lipogenic enzymes, including SCD-1 and long-chain free fatty acid elongase. CD36 is a scavenger receptor with broad ligand specificity. Activation of CD36 facilitates free fatty acid uptake from the circulation [101] and might contribute to hepatic steatosis. Correlation between CD36 levels and triglyceride storage and secretion suggests the causative role of CD36 in hepatic steatosis [102]. In fact, PXR is both necessary and sufficient for activation of CD36 transcription. Further studies established CD36 as a direct PXR transcriptional target [95]. CD36 expression can also be positively regulated by LXR and PPAR $\gamma$ . Therefore, CD36 appears to be a shared transcriptional target of LXR, PXR and PPAR $\gamma$  in their regulation of lipid homeostasis [103].

Two key enzymes involved in  $\beta$ -oxidation and ketogenesis are carnitine palmitoyltransferase 1A (CPT1A) and mitochondrial 3-hydroxy-3-methyl-glutarate-CoA synthase 2 (HMGCS2) [104,105]. In the absence of insulin, a winged-helix/forkhead transcription factor FoxA2 stimulates the transcription of CPT1A and HMGCS2 [106]. Insulin induces the phosphorylation and nuclear exclusion of FoxA2, resulting in inactivation of FoxA2 and suppression of CPT1A and HMGCS2 transcription [107]. Nakamura and colleagues showed that PCN decreased transcription of Cpt1a and Hmgcs2 in wild-type, but not in PXR knockout mice. The underlying mechanism seemed to be direct binding of PXR to FoxA2 and suppression of Cpt1a and Hmgcs2 gene activation [108].

Cholesterol is essential to form cell membranes, bile acids and steroid hormones. On the other hand, oxidized cholesterol metabolites are cytotoxic and represent risk factors for atherosclerosis [109]. Therefore, cholesterol detoxification is crucial to protect the body from excess cholesterol. The mitochondrial sterol 27-hydroxylase (CYP27A1) is required for the cleavage and hydroxylation of cholesterol in most tissues [110,111]. Li et al. [112] showed that PXR activates CYP27A1, as well as cholesterol efflux transporters ABCA1 and ABCG1 in intestinal cells. The “good cholesterol” HDL and its major constituent apolipoprotein A-I (ApoA-I) are involved in reverse cholesterol transport and have been associated with a reduced risk of atherosclerosis. ApoA-I and HDL cholesterol levels were elevated by PXR agonist in wild-type, but not in PXR<sup>−/−</sup> mice [10]. Cholic acid mediated down-regulation of HDL cholesterol and plasma ApoA-1 was abolished in human PXR transgenic mice [113].



On the other hand, there were also studies supporting the pro-atherogenic role of PXR. Activation of PXR decreased the expression of ABCA1 in hepatocytes [114]. Clinically used PXR activating drugs caused hyperlipidemia in some patient populations [11,115,116]. Future studies are necessary to further define the role of PXR in the pathogenesis of hyperlipidemia.

### 3.3. PXR in glucocorticoid and mineralocorticoid homeostasis

Studies by Zhai et al. [117] showed the importance of PXR in adrenal steroidhomeostasis. Both genetic and pharmacological activation of PXR increased plasma levels of corticosterone and aldosterone. This increase was accompanied by activation of adrenal steroidogenic enzymes, such as CYP11a1, CYP11b1, CYP11b2, and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD). Interestingly, the PXR transgenic mice exhibited normal ACTH secretion in pituitary, and intact suppression of dexamethasone by corticosterone, indicating a functional hypothalamus–pituitary–adrenal axis in spite of severely disrupted adrenal steroidhomeostasis. Consistent with these observations, several clinical studies reported that rifampicin increased steroid secretion in urine and may have resulted in misdiagnosis of Cushing's syndrome [118,119]. Therefore, PXR has a potential to disrupt endocrine homeostasis, and it may be broadly implicated in drug–hormone interactions.

### 3.4. PXR in androgen metabolism

The androgen–androgen receptor signaling pathway plays an important role in the initiation and progression of prostate cancer. Accordingly, androgen deprivation has been the most effective endocrine therapy for hormone-dependent prostate cancer. There are at least two major PXR target genes, CYP3A and SULT2A1, which are known to play a role in the metabolic deactivation of androgens. CYP3A is the key enzyme in catalyzing hydroxylation of testosterone and progesterone, leading to inactive hormones [120]. Dehydroepiandrosterone (DHEA) sulfotransferase (SULT2A1) is the primary SULT isoform responsible for androgen sulfonation [121]. Based on these notions, Zhang and colleagues recently reported a novel PXR-mediated and metabolism-based androgen deprivation [122]. In this study, the authors showed that activation of PXR lowered androgenic activity and inhibited androgen-dependent prostate regeneration in castrated male mice that received daily injections of testosterone by inducing the expression of CYP3As and SULT2A1. In human prostate cancer cells, treatment with the PXR agonist RIF inhibited androgen-dependent proliferation of LAPC-4 cells, but had little effect on the growth of the androgen-independent isogenic LA99 cells. Down-regulation of PXR or SULT2A1 in LAPC-4 cells by shRNA or siRNA abolished the RIF effect, indicating that the inhibitory effect of RIF on androgens was PXR- and SULT2A1-dependent. PXR may represent a novel therapeutic target to lower androgen activity and may aid in the treatment and prevention of hormone-dependent prostate cancer.

### 3.5. PXR in bile acid and bilirubin detoxification

Bile acids, synthesized in the liver, are end products of cholesterol catabolism and represent the primary pathway for cholesterol elimination from our bodies [123]. When excreted into the intestine, bile acids promote the absorption of cholesterol and fat-soluble vitamins. However, excess bile acids are cytotoxic and can lead to pathological cholestasis [124]. Therefore, bile acid levels need to be tightly regulated to protect the human body from their toxic effects.

PXR plays a critical role in bile acid detoxification [12,14]. PXR agonist PCN reduced lithocholic acid (LCA) induced toxicity in wild-type, but not in PXR knockout mice. PXR transgenic mice were also resistant to LCA toxicity [12]. The protective effect of PXR can be explained by its regulation of genes involved in bile acid metabolism. PXR induced the expression of CYP3A that is essential for bile acid

hydroxylation and excretion [14]. The Phase II enzyme SULT2A is also a PXR target gene that contributes to bile acid detoxification [47]. In addition to bile acid synthesis and metabolism, PXR also regulates the expression of bile acid transporters, such as MRP2 [125] and OATP2 [14].

There were also reports suggesting that PXR may play a role in the regulation of CYP7A1. It was reported that ligand activated PXR reduced the expression of CYP7A1 without affecting SHP expression in mice [14]. However, hPXR can regulate SHP expression directly in HepG2 cells [126]. PXR has also been reported as a FXR target gene [127]. Together, these results suggested a close evolutionary relationship between PXR and FXR in protecting the human body from bile acid toxicity.

Bilirubin is the breakdown product of heme proteins. Conjugation of bilirubin by UGT converts the neurotoxic unconjugated bilirubin to nontoxic bilirubin glucuronide. It has been reported that activation of PXR prevented experimental hyperbilirubinemia in mice [56]. PXR activates the transcription of UGT1A1 [13] and several other genes critically involved in bilirubin detoxification, such as OATP2 [14] and MRP2 [125]. OATP2 mediates bilirubin uptake from blood into liver, whereas MRP2 facilitates the excretion of conjugated bilirubin to bile canaliculus. As a result, PXR ligands may represent potential therapeutic agents in treating hyperbilirubinemia.

### 3.6. PXR in vitamin metabolism and bone mineral homeostasis

Vitamin K2 is critical for bone formation and has been clinically used to treat osteoporosis. It has been reported that vitamin K2 can activate PXR and stimulate PXR target gene expression. Treatment of osteosarcoma cells with vitamin K2 increased the mRNA expression of osteoblast markers bone alkaline phosphatase, osteoprotegerin, osteopontin, and matrix Gla protein [128]. Vitamin K2 induced the expression of bone markers in primary osteocytes from wild-type, but not PXR<sup>−/−</sup> mice [128]. Ichikawa et al. [129] identified several PXR target genes with bone related functions in osteoblastic cells. Igarashi et al. [130] showed that activation of PXR by vitamin K2 induced the expression of osteoblastogenic transcription factor Msh homeobox 2, which is involved in osteoblast differentiation.

Calcium is a major component in bone development and maintenance. Calcium absorption and excretion are regulated by vitamin D, whose active metabolite 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) binds to the vitamin D receptor (VDR) [131]. VDR activates 25-hydroxyvitamin D (3)-24-hydroxylase (CYP24)-mediated 24-hydroxylation, which is critical in 1,25(OH)2D3 metabolism. Pascucci et al. [132] reported that activation of PXR upregulated CYP24 gene expression. However, Zhou et al. [133] showed that PXR activation inhibited CYP24 promoter activity. Although controversial, these results suggested a potential role of PXR in bone homeostasis, which warrants further investigation. Ligand activated PXR also suppresses the transcription of CYP2D25, an important hydroxylase in 1,25(OH)2D3 biosynthesis. It has been known for decades that prolonged treatment with anticonvulsant drugs may result in vitamin D deficiency or osteomalacia in patients [134,135]. Since many anticonvulsant drugs, such as carbamazepine and phenobarbital, are PXR ligands, these results urge caution in preventing drug-induced osteomalacia in patients.

Vitamin E is often taken as a dietary supplement for antioxidation purpose. Vitamin E is metabolized by CYPs mediated  $\omega$ -oxidation [136], followed by  $\beta$ -oxidation, conjugation including glucuronidation and sulfation [137,138], and then excretion [139,140]. These processes are facilitated by an array of enzymes and transporters that happen to be PXR target genes. It was reported that vitamin E can activate PXR [141,142], and therefore may regulate xenobiotic detoxifying genes involved in its own metabolism. The study by Landes et al. [141] showed that PXR can be activated by several forms of vitamin E in a reporter gene assay. The most potent PXR activator  $\gamma$ -tocotrienol can induce the expression of endogenous CYP3A4 as efficacious as

rifampicin. However, urinary vitamin E metabolite was significantly decreased upon PCN treatment in wild type but not PXR<sup>-/-</sup> mice, which was suggested due to PXR-mediated downregulation of hepatic sterol carrier protein 2 and attenuated  $\beta$ -oxidation [8]. These findings raise the concern of potential drug–drug interactions between vitamin E and PXR regulators, which requires further studies.

### 3.7. PXR in retinoic acid metabolism

Retinoic acid (RA) is the metabolite of vitamin A that binds to and activates the retinoic acid receptor (RAR). RAR forms a heterodimer with RXR and activates the transcription of genes associated with cell differentiation [143] and apoptosis [144], leading to inhibition of cell growth. Therefore, RAs have been used or tested as anti-cancer agents in several human cancer types [145]. However, RA resistance represents a major limit to its clinical use, which might be explained at least in part by the co-administration of a PXR agonist. Ligand activated PXR can induce expression of CYP3A and transporters such as MDR1A, MRP3 and OATP2, which accelerate RA metabolism [146]. It has been suggested that PXR antagonists might be useful in preventing RA resistance.

### 3.8. PXR in inflammation

A negative correlation between infectious disease/inflammation and drug metabolism capacity has been long suggested [147]. In understanding the molecular mechanisms behind this correlation, Teng et al. showed that treatment of wild type mice with IL-6 caused a marked decrease in PXR protein level, as well the mRNA expression of PXR and its target genes Mrp2, Bsep, and Cyp3a11. This reduction was not seen in PXR<sup>-/-</sup> mice subjected to the same treatment, suggesting that this reduction was mediated by PXR. The same group showed that IL-6 could attenuate the upregulation of PXR and its target genes after PCN treatment in wild-type mice [148]. Additionally, Beigneux et al. showed that induction of a specific type of inflammation called acute phase response by lipopolysaccharide (LPS) caused a marked decrease in the mRNA expression of PXR and its target genes in the liver of wild-type mice [149].

Reciprocally, treatment of human patients with rifampicin is known to activate PXR, leading to increased expression of PXR target genes. It is also known that patients treated with rifampicin showed marked immunosuppression. Zhou et al. showed *in vivo* and *in vitro* that activation of PXR via rifampicin can attenuate NF- $\kappa$ B proteins, which are important in facilitating immune response and inflammation. Moreover, activation of NF- $\kappa$ B was shown to inhibit PXR, and inhibition of NF- $\kappa$ B enhanced PXR activity. The same group also showed that PXR<sup>-/-</sup> mice had increased small bowel inflammation and expression of NF- $\kappa$ B target genes [150]. Taken together, inhibition of PXR may be one mechanism of inflammation responsive repression of drug metabolism, whereas activation of PXR may be one mechanism for the drug-responsive immunosuppression.

## 4. Conclusions and perspectives

As summarized in Fig. 1, many xenobiotics and endobiotics, as well as their metabolites can activate PXR. Subsequently, the activated PXR regulates the transcription of key enzymes involved in the metabolism of xenobiotics and endobiotics. Although the endobiotic functions of PXR have been appreciated, identification of physiologically relevant endogenous ligands for PXR will be beneficial in understanding the role of PXR as an endobiotic sensor. As we learn more about the roles of PXR in xenobiotic and endobiotic gene regulation, it remains to be determined whether the regulatory functions of PXR can be taken advantage of in preventing and treating human diseases.

The role of PXR in xenobiotic metabolism is vast, nuanced, and extremely important. With the burgeoning number of pharmaceuticals on the market that are PXR ligands, more research must be done

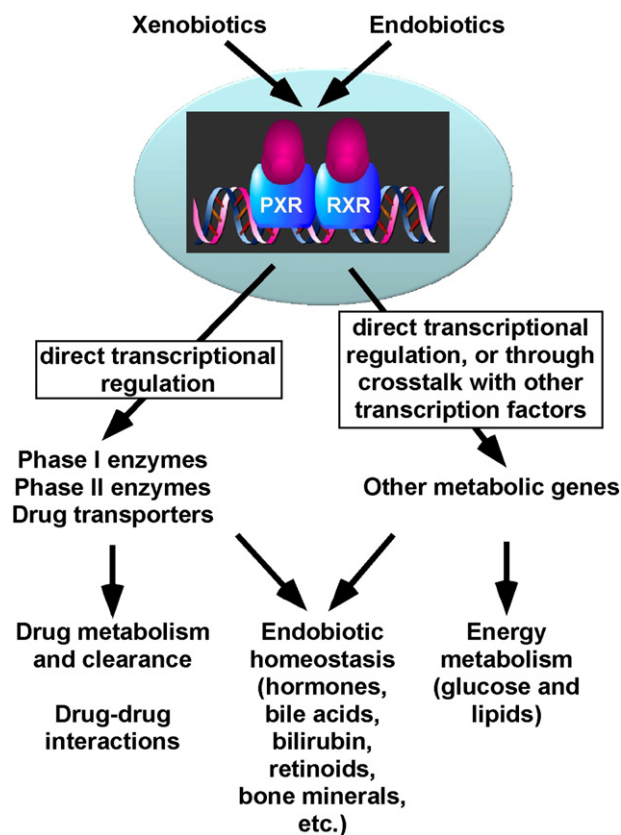


Fig. 1. Summary of the transcriptional circuits and metabolic relevance controlled by PXR. PXR, pregnane X receptor; RXR, retinoid X receptor.

toward investigating receptor-mediated drug–drug interactions. The development of humanized mice offers a promise in using the *in vivo* model to predict PXR-mediated drug–drug interactions.

In addition, polymorphisms of PXR and its major transcriptional targets such as CYP3A4 and MDR1 can affect xenobiotic and endobiotic metabolism. Considering the broad and complex transcription network controlled by PXR, the genetic variation of PXR might have broad implications in physiology and diseases [30]. To date, many splice variants of PXR have been identified and even more single nucleotide polymorphisms (SNPs) have been reported [151]. These polymorphisms exist in the coding or non-coding regions of the PXR gene, and several polymorphisms are associated with functional changes of PXR [30]. Future studies are necessary to assess metabolic differences in populations of different PXR genotypes [151].

Today's medicine and biomedical research are at the interface of the old standardized approach and the new pharmacogenetically personalized future. In order for scientists and doctors to make headway into the future, it will be important to characterize genetics-based metabolic differences. Since its inception, the importance of PXR in xenobiotic and endobiotic metabolism has been repeatedly redefined. We have discussed many of the metabolic circuits that can be controlled by PXR and described how alterations in these pathways could have substantial physiological consequences. As we move into the future, a full genetic assessment of the metabolic characteristics of PXR is imperative in order to further research that may lead to development of novel drugs or improvements on current drug therapies.

## Acknowledgments

The original research of ours described in this article was supported in part by National Institutes of Health grants ES012479, CA107011, ES014626, and DK076962.

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