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FXR signaling in the enterohepatic system

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Abstract

Enterohepatic circulation serves to capture bile acids and other steroid metabolites produced in the liver and secreted to the intestine, for reabsorption back into the circulation and reuptake to the liver. This process is under tight regulation by nuclear receptor signaling. Bile acids, produced from cholesterol, can alter gene expression in the liver and small intestine via activating the nuclear receptors farnesoid X receptor (FXR; NR1H4), pregnane X receptor (PXR; NR1I2), vitamin D receptor (VDR; NR1I1), G protein coupled receptor TGR5, and other cell signaling pathways (JNK1/2, AKT and ERK1/2). Among these controls, FXR is known to be a major bile acid-responsive ligand-activated transcription factor and a crucial control element for maintaining bile acid homeostasis. FXR has a high affinity for several major endogenous bile acids, notably cholic acid, deoxycholic acid, chenodeoxycholic acid, and lithocholic acid. By responding to excess bile acids, FXR is a bridge between the liver and small intestine to control bile acid levels and regulate bile acid synthesis and enterohepatic flow. FXR is highly expressed in the liver and gut, relative to other tissues, and contributes to the maintenance of cholesterol/bile acid homeostasis by regulating a variety of metabolic enzymes and transporters. FXR activation also affects lipid and glucose metabolism, and can influence drug metabolism.

Introduction

In 1995, the farnesoid X receptor (FXR; NR1H4) was identified as an orphan nuclear receptor from mouse [1] and rat [2]. In the early studies, farnesol and related metabolites were proposed as possible ligands for the rat homolog, thus accounting for the original name [2]. However, subsequently, bile acids were found to be the true endogenous ligands for FXR [3–5], so more accurately, this receptor should have been designated the bile acid receptor. To date, more than 80 compounds have been identified as potential FXR ligands with varying degrees of affinity; these include the endogenous bile acids, and synthetic ligands (Table 1). Several structural structurally diverse compounds show high-affinity binding and agonist activity toward FXR, including steroids, aromatics, terpenoids, alkaloids, and fatty acids (Figure 1).

Steroids are the major and most important ligands for FXR. Numerous endogenous compounds and their metabolites with important physiological functions encompass bile acids, cholesterol and hormones. Endogenous bile acids, including the primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA), and the secondary bile acids

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deoxycholic acid (DCA) and lithocholic acid (LCA), can activate FXR *in vivo* and in cultured cells, and bind the receptor *in vitro*. Bile acids have the general properties of a concave hydrophilic face and a convex hydrophobic face. They combine with the hydrophobic pocket of the FXR ligand-binding domain mainly through the hydrophobic face, while the hydroxyl groups in the hydrophilic phase also can greatly affect the affinity of bile acids with FXR. The potency of bile acids to activate FXR is CDCA>DCA>LCA>CA [5]. Many compounds unrelated to bile acids, can also act as FXR ligands, such as androsterone [6], and the exogenous natural product plant sterols forskolin [7], stigmasterol [8], and guggulsterone [9]. In addition, a series of synthetic bile acid derivatives have been developed as FXR ligands, such as 6α-ethyl-chenodeoxycholic acid (6-ECDCA) and bile alcohols, showing an even higher affinity with FXR than bile acids [10].

FXR is the chief sensor of intracellular levels of bile acids, controlling their synthesis and transport. Along with the regulation of bile acid metabolism, FXR is also involved, directly and indirectly, in several important metabolic pathways *in vivo*, such as modulation of glucose and lipid metabolism. Thus, activation or repression of FXR can have major influences on metabolic homeostasis. In addition, FXR genetic variants are associated with metabolic diseases, including intrahepatic cholestasis of pregnancy [11], and cholesterol cholelithiasis [12]. FXR ligands have been proposed for possible treatment of metabolic diseases, such as cholestasis [13], liver fibrosis [14], inflammatory bowel disease [15], type 2 diabetes [16], atherosclerosis [17], and erectile dysfunction [18]. Ursodeoxycholic acid (UDCA), an FXR agonist [19], was approved by the FDA as a drug for the treatment of primary biliary cirrhosis (PBC), and is widely used for the treatment of a variety of chronic cholestatic diseases. Thus, FXR provides a framework for developing novel therapies for several liver diseases that are due to altered bile acid homeostasis. There are more than 40 target genes for FXR, most of which are positively regulated, although some genes are indirectly down-regulated by FXR [17]. Some of the FXR target genes are involved in hepatic-intestinal bile acid synthesis, transport and homeostasis, while others are functional in other metabolic pathways.

FXR function in small intestine

In the intestine, FXR controls the absorption of bile acids, lipids, vitamins, certain drugs, and other xenobiotics through the regulation of expression of four important transporters, apical sodium dependent transporter (ASBT, also called solute carrier 10A2; SLC10A2), fatty acid-binding protein subclass 6 (FABP6), also known as intestinal bile acid-binding protein (I-BABP), and organic solute transporters α (OSTα) and β (OSTβ), which are responsible for the transport of bile acids from the intestine to the portal system. ASBT, the major bile acid transport system in ileal enterocytes, transports bile acids into the ileal enterocyte brush border (apical) membrane [20]. In humans, ASBT deficiency causes significant bile acid malabsorption [21,22]. FXR is a negative regulator of intestinal ASBT expression in mice but not in rats thus indicating a significant species difference in regulation of the *Asbt* gene by bile acids [23,24]. In mice, ASBT protein and mRNA are decreased when the animals are fed FXR ligands such as CA and TCA. Mechanistic studies revealed that bile acids exert a negative feedback on ASBT expression by FXR activation of the small heterodimer partner (SHP; NR0B2)-dependent repression of liver related homolog-1 (LRH-1; NR5A2) activity in mice. The negative regulation of ASBT expression was not, however, observed in rats, due to the absence of an LRH-1 responsive element within the rat *ASBT* promoter [24]. In addition, intestinal ASBT expression is not induced in *Fxr*-null mice in response to bile acids, thus suggesting that down-regulation of ASBT is associated with ligand-activated FXR. The repression of ASBT is also found in rabbits through a similar FXR/LRH-1/SHP-dependent mechanism [25]. In the human enterocyte-

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like Caco-2 cells, the mouse *Asbt* promoter activity is repressed by CDCA, while the rat *ASBT* promoter was not, indicating that humans respond to bile acids similar to the mouse and rabbit [26]. However, in contrast to the mouse, SHP inhibits positive regulation of the human ASBT gene through interfering with the retinoic acid receptor (RAR;NR2B2)/ retinoid X receptor (RXR;NR2B2) heterodimer complex, in contrast to mice in which SHP interferes with LRH-1 [24]. In humans, the *ASBT* gene is activated by retinoic acid, a finding that has implications for the treatment of patients with cholestasis or chronic diseases of the gastrointestinal system with vitamin A and retinoic acid-based drugs [26]. However, in contrast to mice that offer a model for pharmacological and genetic manipulation, the precise mechanism of bile acids suppression of ASBT in humans is difficult to determine. The biological significance of the species differences in ASBT suppression is also not completely understood, in particular the roles of the positive regulators LRH-1 and RAR/RXR. Finally, other studies have revealed that the membrane protein β-Klotho, involved in fibroblast growth factor (FGF) 15 (FGF-19 in humans) signaling, suppresses basal ASBT activity through the LRH-1 *cis*-element, presumably affecting the FXR/SHP pathway [27]. However, the signaling pathway linking β-Klotho and FXR is not known.

FABP6 also known as I-BABP, is expressed in the ilium and shuttles bile acids from the apical to basolateral membrane in the enterocyte [28]. It was suggested that FABP6 plays an important role in enterohepatic circulation through the regulation of bile acid trafficking [29]. In Caco-2 cells, bile acid-activated FXR can induce *FABP6* gene expression through binding to the promoter [30]. *In vivo* studies in mice further demonstrated that cholestyramine treatment dramatically decreased FABP6 mRNA levels, whereas TCA treatment increased mRNA levels. Furthermore FABP6 mRNA levels are significantly decreased in *Fxr*-null mice, thus suggesting that FXR positively regulates the *Fabp6* gene in mice [31]. Finally, the heteromeric organic solute transporters OSTα and OSTβ move bile salts to blood vessels, in accordance with their location at the basolateral membrane [32]. OSTα and OSTβ are expressed not only in the ileum, but also in the liver and kidney [32]. Ileum expression of both the genes is induced in wild-type mice after CA exposure; induction was not observed in *Fxr*-null mice [33]. Treatment with GW4064, a synthetic FXR ligand, also induces OSTα and OSTβ mRNAs in the intestine. Another study identified FXR regulatory elements (FXRE) in the promoters of the genes encoding OSTα and OSTβ in both humans and mice [34]. Introduction of human OSTα and OSTβ into HepG2 cells facilitates the uptake of conjugated-CDCA and the activation of FXR target genes. Taken together, these studies demonstrated that FXR controls transport of bile acids from the intestine to blood vessel to liver.

Intestinal FXR activation also affects hepatic events. Fibroblast growth factor 19 (FGF19 in humans, and its mouse homolog FGF15, referred to as FGF15/19) is highly expressed in the small intestine [35,36]. FGF15/19, secreted from the intestine, circulates to the liver and suppresses bile acid through binding and activation of the FGF receptor 4 (FGFR4) complexed with β-Klotho located on the surface of hepatocytes and other epithelial cells [37,38]. Activation of the FGFR4/β-Klotho complex stimulates the c-Jun N-terminal kinase (JNK) pathway, eventually suppressing the gene encoding CYP7A1, the cholesterol 7α-hydroxylase and the rate-limiting bile acid synthetic enzyme [39,40]; these effects were not observed in *Shp*-null mice thus suggesting that SHP is required for the suppressive effects of FGF15/19 [40]. Extracellular signal-regulated kinase (ERK) was markedly elevated by FGF15 administration to mice and deficiency of both JNK and ERK pathways prevented FGF15-mediated suppression of *Cyp7a1* and *Cyp8b1* expression; deficiency of either pathway alone and minimal effect on FGF15 suppression of these genes [41].

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FXR is a major regulator of the gene encoding FGF15/19 in the intestine and thus bile acid-activated intestinal FXR can down-regulate CYP7A1 expression through direct activation of intestinal FGF15/19. In addition, FGF15/19 was reported to work as a hormone for gallbladder filling via its interaction with FGFR3, a receptor that is highly expressed in the gallbladder [42]. These studies indicate that FXR-FGF15/19 signaling contributes to the control of intestinal bile acid levels. FGF15/19 was also recently reported to activate hepatic glycogen synthesis by activating glycogen synthase kinase 3 (GSK3) [43], and to inhibit hepatic gluconeogenesis [44] by inhibiting the cAMP regulatory element-binding protein (CREB)-peroxisome proliferators-activated receptor γ coactivator protein-1α (PGC-1α) pathway. Stimulation of hepatic glycogen synthesis and inhibition of glucose metabolism is mediated via extracellular signal-regulated protein (E) activated by the FGF15/19-stimulated FGFR4/β-Klotho complex, independent of insulin signaling. Thus, bile acids are involved in maintaining hepatic glucose homeostasis though FGF15/19, independent of FXR, and that FGF15/19 is postprandial regulator of hepatic carbohydrate homeostasis [44].

FXR is also expressed in pancreatic β-cells and regulates insulin signaling. In βTC-6 cells, an insulin-secreting cell line derived from transgenic mice expressing the large T-antigen of simian virus 40 (SV40) in pancreatic β-cells cells, FXR induces expression of the glucose regulated transcription factor KLF11 [45]. KLF11 may account of the effect of FXR on glucose-induced insulin gene transcription. In addition FXR regulates insulin secretion by non-genomic effects though increasing Akt phosphorylation and GLUT2 translocation at the plasma membrane, resulting in increasing the glucose uptake by these cells. The mechanisms for these effects are not known. Recent studies also revealed that bile acids can affect insulin secretion in pancreatic β-cells by altering cytosolic calcium concentrations [46]. Treatment of primary β-cell cultures with the FXR ligands taurochenodeoxycholate (TCDC) and the synthetic FXR agonist GW4064, stimulated the electrical activity and increased calcium concentration through FXR inhibition of K(ATP) channel activity; these effects were not found in *Fxr*-null mouse β-cells. The mechanism by which FXR effects K(ATP) channel activity is not through altering gene expression and thus represent a novel non-genomic mechanism whereby FXR affects insulin secretion. The role of bile acids and FXR in regulating insulin secretion and glucose transport in the intact pancreas has not been determined.

In addition to being a major regulator of bile acid homeostasis, FXR plays an important role in the intestinal defense against inflammation, interacting with nuclear factor-kappaB (NF-kB) signaling. Exposure of LPS-activated macrophages to an FXR ligand leads to the reciprocal regulation of NF-kB-dependent genes such as TNFα and IL-1β [47]. Intestinal FXR activation, responding to bile acids, controls bacterial growth and maintains mucosal integrity, regulating the expression of a variety of genes involved in defense against inflammation and mucosa protection. Therefore, FXR might be a critical factor regulating intestinal innate immunity and homeostasis.

Role of hepatic FXR in bile acid homeostasis

FXR functions as the chief sensor of intracellular bile acid levels. Hepatic bile acid levels are maintained by the control of uptake, synthesis, metabolism and export. Na^+-taurocholate cotransporting polypeptide (NTCP, also termed solute carrier 10A1; SLC10A1) and organic anion-transporting peptides (OATPs, also named SLCO family) are the major bile acid transporters in the hepatocellular basolateral membrane for the uptake of bile acids and organic solutes from portal vein to liver [48,49]. NTCP is responsible for the uptake of conjugated bile acids, whereas the OATPs are largely involved in the uptake of unconjugated bile acids. In the cholestasis mouse model of liver disease, the expression of NTCP and OATPs are significantly reduced to avoid excess accumulation of bile acids in

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the liver [50]. Hepatic NTCP expression is regulated by SHP that is induced by FXR upon bile acid activation [51]. The glucocorticoid receptor (GR; NR3C1) is also a transcriptional activator of the *NTCP* gene in humans [52]. GR induction of endogenous *NTCP* expression is suppressed by CDCA or GW4046, through FXR by its induction of SHP. Hepatocyte nuclear factor 4α (HNF4α) also binds the *NTCP* gene promoter in the rat at a cite overlapping with the retinoic acid receptor (RAR)/RXR response element, and HNF4α-mediated *NTCP* expression was also reported to be inhibited by SHP [53]. However, there are marked species differences in constitutive control of the gene encoding NTCP. HNF4α and HNF1α constitutively regulate the rat *NTCP* gene promoter and not the mouse or human promoters, while the mouse and human genes are controlled by the CCAAT/enhancer binding protein β (C/EBP β) [53]. Thus, regulation of *NTCP* is accomplished by several transcription factors, both positively and negatively, although species differences exist.

Certain members of the OATP family facilitate Na^+-independent transmembrane transport of various endogenous and xenobiotic compounds, such as bile acids, bilirubin, steroid hormone conjugates, thyroid hormones, prostaglandins, clinically used drugs, and toxins [54]. Altered FXR function can directly affect hepatic expression of the OATPs family members. CA treatment significantly decreases OATP1 (SLCO1A1) expression and increases OATP2 (SLCO1A4) expression in wild-type mouse liver; OATP1, OATP2, and OATP3 (SLCO1A5) mRNA levels in the livers of *Fxr*-null mice were similar thus indicating the involvement of FXR [55]. In human liver, bile acids repress expression of the *OATP1B1* gene but the mechanistic role of FXR was not determined in this study [56]. Taken together, FXR can negatively regulate bile acid uptake in a feedback fashion through SHP-alteration of hepatic GR, RAR, HNF4α, and HNF1α activity.

In addition to the negative regulation of bile acid transport, FXR also represses some important cytochromes P450 (CYP) that are involved in bile acid synthesis. This was firmly established *in vivo* by analysis of *Fxr*-null [31] and intestine-specific and liver-specific *Fxr*-null mice [57]. Notably, CYP7A1 and CYP8B1 are two hepatic enzymes catalyzing formation of the primary bile acids CA and CDCA from cholesterol [58]. CYP7A1 expression is negatively regulated via the hepatic FXR-SHP and intestinal FXR-FGF15/19 pathways. SHP inhibits the *CYP7A1* gene by interaction with the positive regulator LRH-1 resulting in a non-productive transcription factor [59]. Study of the *CYP7A1* gene revealed a negative bile acid response element in the promoter that can bind LRH-1 and HNF4α [60,61]. LRH-1-responsive elements are also located in the regulatory regions of *SHP* gene, and overexpression of LRH-1 can activate the *SHP* promoter in mice and tissue culture cells [62,63]. SHP-mediated inhibition of LRH1-dependent suppression of transcription is due in part to recruitment of SIRT1 histone deacetylase protein; LRH-1 activation of CYP7A1 and SHP gene transcription was significantly repressed by both SHP and SIRT1 while the inhibition of SIRT1 activity by inhibitors or a dominant negative SIRT1, or direct knockdown of SHP, released the inhibitory effect [64]. Others found that Brahma chromatin remodeling protein, Sin3a scaffold co-repressor, and histone deacetylase-1, increased the occupancy of SHP at the *CYP7A1* promoter [65]. Finally, SHP can be modified by ligand binding as revealed by use of a retinoid-like compound, 4-[3-(1-adamantyl)-4-hydroxyphenyl]-3-chlorocinnamic acid (3Cl-AHPC), that was found to bind to SHP and increase its interaction with LRH-1 [65]. Along with SHP and FGF15/19, HNF4α can suppress *CYP7A1* expression; chromatin immunoprecipitation (ChIP) assays suggest that bile acids can suppress transcription of the *CYP7A1* through blocking the association of HNF4α with the coactivators PGC-1α and CREB-binding protein (CBP) [66]. Similarly, *CYP8B1* is regulated by FXR via its target gene SHP. The *CYP8B1* promoter also contains a negative bile acid response element harboring overlapping binding sites for HNF4α [67] and LRH-1 [68]. Induction of *SHP* by FXR can in turn inhibit *CYP8B1* expression by

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negative interference of the positive transcriptional activity of HNF4α and LRH-1. However, another study indicated that there was no significant difference in repression of the *CYP8B1* gene by bile acids between the *Fxr*-null mice and wild-type mice, thus suggesting that feedback inhibition of CYP8B1 is FXR-independent [31]. In addition, CYP27A1, catalyzing the acidic pathway of bile acid biosynthesis, also can be suppressed by bile acids as revealed in a human cell line [69]. A negative bile acid response element in *CYP27A1* promoter can bind to HNF4α, suggesting a similar negative regulation of *CYP27A1* via the FXR-SHP pathway as found with the *CYP7A1* and *CYP8B1* genes [69,70]. CYP17A1, and enzyme involved in the 17α-hydroxylation of C21 steroids such as progesterone, is also suppressed by bile acids through a mechanism that likely involved FXR/SHP/LRH-1 pathway [71]. 17-Hydroxy steroid metabolites are involved in liver injury and thus suppression of CYP17A1 by high hepatic bile acids would be a protective mechanism.

Members of the CYP3A family of cytochromes P450 expressed in liver and intestine, are also involved in bile acid metabolism, by catalyzing hydroxylation of bile acids such as CDCA, LCA and DCA at different positions on the molecules [72,73]. Human hepatic CYP3A4. the dominant CYP3A in human liver, carries out the metabolism of xenobiotic compounds including many clinically-used drugs [74]. This enzyme is also highly expressed in the intestine where it plays an important role in first-pass metabolism of many orally dosed drugs [75]. FXR was shown to regulate the expression *CYP3A4*. For example, in HepG2 cells, mRNA encoding CYP3A4, was found to be elevated 24 hours after exposure to 100 μM CDCA and 1 μM GW4064 [76]. When wild-type, *Fxr*-null and *Pxr*-null mice were treated with GW4064, a significant induction of CYP3A11, the mouse equivalent of human CYP3A4, expression was observed in wild-type and *Pxr*-null mice, but not in *Fxr*-null mice [76]. In accordance with this result, secondary bile acids might activate transcription of the mouse *Cyp3a11,* the putative mouse homolog of *CYP3A4*, through FXR, although the presence of FXR regulatory elements have not been observed in the *Cyp3a11* gene. A recent study revealed that CDCA can induce *CYP3A4* expression in human liver, not human ileum [77]. These results provide evidence that FXR is involved in bile acid-induced CYP3A expression, although the participation of other receptors such as PXR, constitutive androstane receptor (CAR; NR1I3) and vitamin D receptor (VDR; NR1I1), activated by bile acids, that also regulate CYP3A expression, cannot be excluded. In addition to *CYP3A*, FXR was reported to up-regulate transcription of the human drug conjugating enzyme uridine 5’-diphosphate-glucuronosyltransferase 2B4 (UGT2B4) [78] that catalyzes production glucuronidated 6α-hydroxylated bile acids such as hyodeoxycholic acid [79], and sulfotransferase 2A1 (SULT2A1) [80] that catalyzes sulfate conjugation of many hydroxysteroid substrates, such as bile acids, pregnenolone, and estrogens [81]. Treatment of hepatocytes and HepG2 cells with the FXR agonists CDCA and GW4064 led to an increase in endogenous UGT2B4 expression and activity. Potential FXR induction of *SULT2A1* expression was assessed using a gene reporter system and endogenous SULT2A1 expression was decreased in HepG2 cells treated with CDCA or GW4064 [82]. FXR also regulates two enzymes involved in bile acid conjugation with taurine and glycine, bile acid-CoA synthetase (BACS, also called SLC27A5) and bile acid-CoA:aminoacid *N*-acetyltransferase (BAAT). The level of BACS and BAAT expression was activated in hepatocytes and Fisher rats treated by CDCA and GW 4064 [83]. Functional response elements were found in the proximal promoter of BACS and in the intronic region between exons 1 and 2 of the BAAT gene. Further mutational analysis confirmed that the inverted repeat-1 (IR-1) element of BACS and BAAT genes binds the FXR-RXR heterodimer.

FXR has a critical role in the elimination of hepatic bile acids through the regulation of the ATP-binding cassette (ABC) transporters. Bile salt exporting pump (BSEP, also termed ABCB11), is a major efflux transporter of bile acids from liver to gallbladder. BSEP

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deficiencies are associated with progressive familial intrahepatic cholestasis type 2 (PFIC2), benign recurrent intrahepatic cholestasis type 2 (BRIC2), and several acquired forms of cholestasis [84]. *Fxr*-null mice fed CA-supplement diet showed intrahepatic cholestasis, similar with that of the human BSEP deficiency. Reporter gene assays showed that the *BSEP* promoter was positively controlled by FXR and bile acids [85]. Mutation of the FXR regulatory element strongly represses the FXR-dependent induction of *BSEP* expression. Consistent with a role for FXR in *BSEP* regulation, an IR-1 element in the *BSEP* promoter specifically binds FXR/RXRα heterodimers [86]. In *Fxr*-null mice, BSEP expression was significantly reduced and the FXR agonist GW4064 cannot induce the expression *Bsep* gene thus confirming that FXR controls BSEP expression [87]. Expression of another ABC transporter ABCB4 (also called MDR3 in human and MDR2 in rodents), is significantly reduced in *Fxr*-null mice, where GW4064-induced *Abcb4* expression is not observed [87]. ABCB4 is a critical transporter of phospholipids, across canalicular membranes of hepatocytes [88]. After the phospholipids are transported to gallbladder from liver via ABCB4, the formation of mixed micelles containing cholesterol, bile acids, and phospholipids will increase their solubility and reduce their toxicity to the bile duct. Similar to BSEP, ABCB4 deficiency in humans can cause PFIC3 [89,90]. FXR regulates the *ABCB4* gene through binding a highly conserved IR-1 element at the distal promoter [91]. In primary human hepatocytes, ABCB4 mRNA is induced by CDCA and GW4064 in a time- and dose-dependent fashion. In rats, GW4064 treatment increases the expression of mouse *ABCB4* gene in the liver [13]. Thus, FXR can be a critical factor for bile acid homeostasis through regulating hepatic BSEP and MDR2/3 expression.

Role of FXR in the metabolism and transport of xenobiotics

In addition to bile acid homeostasis, FXR can contribute to the metabolism and elimination of the xenobiotics, through regulation of the phase I and II drug-metabolizing enzymes and drug transporters (Table 2). As noted above, FXR regulates several important enzymes involved in drug metabolism, such as CYPs, UGTs and SULTs, converting the hydrophobic compounds to more hydrophilic and less toxic conjugated derivatives that can more easily be eliminated from the body. A recent study reported that activation of FXR protects mice from acetaminophen (APAP)-induced heptotoxicity [92]. Under normal therapeutic dosing, APAP is metabolized in the liver mainly through direct conjugation by UGTs and SULTs. However, excessive APAP will saturate both glucuronidation and sulfation pathways, leading to accumulation of the toxic NAPQI metabolite produced by CYPs, notably CYP2E1 [93]. NAPQI is also subject to conjugation by glutathione S-transferase (GST) but under conditions of high doses of APAP, the amount of NAPQI exceeds the conjugation capacity and the cosubstrate for GST, glutathione, is depleted. The liver toxicity induced by high dose APAP could be attenuated by FXR up-regulation of several phase II enzymes. To identify which drug metabolizing enzymes might be regulated by FXR, three models were employed, a constitutively-active form of FXR (FXR-VP16), native FXR, and treating wild-type and *Fxr*-null mice with an FXR agonist. The expression levels of several GSTs (GSTα3, GSTα4, GSTμ1, GSTμ3), SULTS (SULT1A1 and SULT1A2), and UGTs (UGT1A1), were induced by FXR activation [92]. FXR response elements were found in some of these gene promoters by use of ChIP-Seq genome-wide binding site analysis. Others have also reported multiple binding sites for FXR in untreated [94], GW4064 ligand treated [95] and in high-fat diet-induced obese mice [96], thus revealing a surprisingly large number of genes with FXR binding sites, notably the characterized indirect repeat 1 (IR-1) element. While these genes were involved in multiple pathways, the functional relevance of the FXR binding for most of the genes has not been determined. Among the other notable findings by the *in vivo* binding analysis was the large degree of tissue-specific FXR binding; only 11% of total sites were shared between liver and intestine, the main sited of FXR activity [95]. The state of obesity also resulted in differential FXR chromatin binding thus

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suggesting a broader role of FXR in metabolism beyond the control of bile acid synthesis and transport [96].

Role of FXR in Cancer and Hepatotoxicity

While a critical role for FXR in bile acid homeostasis was established using mice with targeted disruption of FXR [31], FXR deficiency was also found to increase the development of liver and intestine cancer. A high incidence of hepatocellular adenoma, carcinoma, and hepatocholangiocellular carcinoma were detected in in 12-month-old male and female *Fxr*-null mice [97,98]. This was associated with an upregulation of genes involved in inflammation and cell cycle control in *Fxr*-null mice. Another study also indicated that FXR deficiency can promote cell proliferation, inflammation, and tumorigenesis in the intestine [99,100]. These results suggest that activation of FXR by its ligands may protect against liver and intestinal carcinogenesis. FXR expression was down-regulated in human hepatocellular carcinoma compared normal liver tissues and this was associated with increased expression of proinflammatory cytokines led to the hypothesis that decreased FXR expression through inhibition of FXR gene promoter promoted liver cancer [101]. However, others reported that FXR expression is downregulated by the microRNA miR-421 through inhibition of translation that could account for the decreased levels of FXR in liver tumors [102]. However, the precise nature of the FXR target genes involved in protection against liver cancer is not known. The possibility exist that the protection is the indirect result of alteration in bile acids and/or suppression of inflammation.

Previous studies indicated that wild-type mice exposed to 1% CA diet led to slightly decrease body weight as compared to control mice without CA, whereas there were no significant differences in alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels in the CA-treated group and control group [103]. Although the CA diet does not cause toxicity in wild-type mice, a challenge of CA diet to *Fxr*-null mice results in marked liver toxicity and cholestasis [104]. *Fxr*-null mice fed a CA-diet were further analyzed using metabolomics, revealing activation of adaptive metabolic pathways upon bile acid challenge (Figure 2). Urine of *Fxr-null* mice fed a 1% CA diet had increased p-cresol, corticosterone and several other metabolites, as compared to wild-type mice [103]. Among all metabolites, taurine-conjugated tetrahydroxy bile acids, likely generated through induction of CYP3A11, were highly increased in *Fxr*-null mice. In LCA-induced cholestasis, the excretion of the similar taurine-conjugated tetrahydroxy bile acid was also greatly increased in urine [103]. The excreted tetrahydroxy bile acid in LCA-treated *Fxr*-null mice that are resistant to LCA-induced intrahepatic cholestasis was greater than in LCA-treated wild-type mice, thus suggesting that hydroxylation of bile acids contributes to the detoxification of cholestatic bile acids in *Fxr*-null mice. These results also demonstrate that tetrahydroxyl bile acids are potential biomarkers for hepatotoxicity and cholestasis. In addition, the enhanced serum corticosterone in cholestatic animal models and humans also was observed in CA-treated *Fxr*-null mice. The urinary biomarker of abnormal corticosterone metabolism in CA-treated *Fxr*-null mice, characterized by the increased excretion of corticosterone metabolites HDOPA, DHOPA, and their hydroxylation metabolites. Future studies will be required to further determine the molecular mechanism linking liver injury (cholestasis, hepatitis) with hepatic and adrenal steroid metabolism.

The potency of FXR ligand as clinic therapeutic agents

Since FXR is a modulator of the metabolism and transport of bile acids and xenobiotics in liver and intestine, and FXR deficiency impairs bile acid and lipid homeostasis [31], therapeutic activation of FXR could be used to protect against intra- and extra-cholestasis [13]. Inflammatory bowel disease [15], and type 2 diabetes [16] have also been proposed as

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therapeutic targets for FXR ligand-based drugs. By use of animal models, GW4064, an aromatic ligand for FXR, shows effects on several types of diseases through activation of FXR. GW4064 protects against cholestatic liver damage in a rat model of intrahepatic and extrahepatic cholestasis. The naphthylisothiocyanate and bile duct-ligation (BDL) models are represent intrahepatic and extrahepatic cholestasis, respectively. Both cholestasis models treated with GW4064 result in significant reductions in serum ALT, aspartate aminotransferase, and lactate dehydrogenase, as well as other markers of liver damage [13]. GW4064-treated cholestatic rats also had decreased expression of the bile acid biosynthetic enzymes such as CYP7A1 and CYP8B1, and increased expression of genes encoding the bile acid transporters BSEP and MRP2. Thus, compounds with FXR agonist activity and favorable bioavailability such as GW4064 could have potential in the prevention of cholesterol gallstone disease that has a high prevalence in the United States. In the C57BL6/J mouse model susceptible to cholesterol-induced gallstones, GW4064 treatment can reduce cholesterol precipitation and gallstone formation through induction of *Abcb11* and *Abcb4* and the resultant increased biliary concentrations of bile salts and phospholipids [87]. In CYP7A1-overexpressing mice with high biliary and fecal cholesterol, GW4064 treatment induces hepatic ABCG5/G8 expression through FXR activation, thus suggesting that GW4064 could reduce gallstone formation by increasing the transport of cholesterol in the liver [105]. In addition, GW4064 may prevent epithelial deterioration and bacterial translocation in patients with impaired bile flow. In the BDL model in mice, populations of a number of aerobic and anaerobic bacteria in the ileum and cecum are increased. After administration of GW4064 to BDL mice, bacterial overgrowth in the ileum and cecum is completely blocked [106]. Finally, GW4064 holds promise for the treatment of type 2 diabetes mellitus. Treatment with GW4064 can significantly decrease the level of plasma glucose, triglycerides, and cholesterol in wild-type and genetically obese, diabetic, leptin receptor-deficient db/db mice, which is dependent on FXR activation [16].

6-ECDCA, also known as INT-747, is another efficacious FXR agonist under testing for various diseases associated with bile acid dysfunction, such as liver fibrosis [14] and inflammatory bowel disease [15]. The FDA and the EMEA have granted this agent orphan drug status for the treatment of PBC [107]. 6-ECDCA has been evaluated in phase I clinical trials in healthy volunteers, and phase II clinical trials in patients with type 2 diabetes mellitus, non-alcoholic fatty liver disease (NAFLD) and PBC. 6-ECDCA shows antifibrotic activity in three liver fibrosis models through activation FXR. In the porcine serum-induced rat liver fibrosis model, 12-week administration of 6-ECDCA can reduce expression of α1(I) collagen, transforming growth factor-β1 (TGF-β1), and α-smooth muscle actin in liver [14]. In the BDL rat model, 6-ECDCA can reduce liver fibrosis and α1(I) collagen, TGF-β1, α-SMA as well as tissue metalloproteinase inhibitor (TIMP)-1 and 2 mRNA by 70%–80% [14]. In the CCl_4 liver toxicity model, 6-ECDCA administration results in induction of SHP, prevents up-regulation of TIMP-1 mRNA, and accelerate collagen elimination. 6-ECDCA also shows anticholeretic activity on two cholestasis models [108]. In LCA-induced cholestasis, 6-ECDCA treatment can fully reverse the reduced bile flow and transient protect against the liver injury [10]. In estrone-induced cholestasis, administration of 6-ECDCA reduces serum ALP activity and improves the cholestatic changes caused by estrogen and partially abrogates the reduction of bile acid output through increased MCA and TCDCA secretion [109]. Additionally, 6-ECDCA treatment can decrease the level of glucose, free fatty acid and HDL in plasma, and the triglyceride, free fatty acid, cholesterol, and glycogen content in the liver via FXR activation [110], thus suggesting that 6-ECDCA is a potential therapy for non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD). In clinical trials, 6-ECDCA can protect against a broad range of chronic liver diseases [111]. 6-ECDCA treatment of patients with type 2 diabetes mellitus and NAFLD can increase glucose disposal rates and reduce body weight [112]. FXR ligands could provide therapeutic benefit for bile acid-related human diseases in

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the clinic via its regulation of FXR targets. By combination with increasing bile acid export from liver through elevation of BSEP expression and suppression of bile acid uptake from blood through down-regulated NTCP and OATPs, FXR agonists can decrease bile acid levels largely through activation of hepatic FXR-SHP and intestinal FXR-FGF15/19 pathways, potentially contributing to the alleviation of cholestasis-induced liver injury. Cholesterol-induced gallstones could also be inhibited by FXR agonists through increased biliary concentration of bile salts and phospholipids following the induction of BSEP and ABCB4 [87]. In addition, FXR agonists can inhibit inflammation and preserve the intestinal barrier function in inflammatory bowel disease through the suppression of key proinflammatory cytokines expression, such as tumor necrosis factor α (TNF-α) [15]. Similarly, the antagonists of FXR can lower serum low density lipoprotein cholesterol and triglyceride levels and increased high density lipoprotein levels through its regulation of a subset of FXR targets, including BSEP [113]. Lack of FXR results in reduces obstructive cholestasis in a cholestasis model via the regulation of the hepatic and intestinal bile acid transporters, such as BSEP and IBABP [114], thus suggesting that FXR antagonists might perform the function through their effects on bile acid transporters.

Conclusion

Intake endogenous chemicals, toxicants and xenobiotic compounds go though the small intestine and liver, and diffuse into whole body. In these two sites, exposed to high concentrations of bile acids, FXR plays an important role in endogenous chemical homeostasis and protection from potential toxicity (Figure 3). Recent discoveries suggest that alteration of hepatic and intestinal FXR signal transduction is involved in multiple diseases. Further understanding of FXR signaling in enterohepatic system can contribute to development of clinical agents for use in the therapy of metabolic diseases such as liver cholestasis, type 2 diabetes and atherosclerosis [17]. However, potent agonist for FXR would likely have unfavorable side effects such as lowering HDL levels and thus ligands will need to be carefully selected for the desired effects. One possibility is to develop selective FXR ligands or modulators similar to those under development for the estrogen receptor [115]. Another approach is to develope a gut-specific FXR activator that might have utility in the treatment of cholestasis by induction of FGF19 and reducing hepatic bile acid synthesis [116]. This would avoid any side effects from activation of the liver FXR. For example, a ligand for PXR, rifaximin, is gut specific and under trials for the treatment in intestinal inflammatory disorders [117,118]. However, it should be noted that FGF19-transgenic mice have increased hepatocytes proliferation and develop hepatocellular carcinoma [119], and thus chronic long-term treatment with and FXR agonist need to account for this possible side effect. In addition to gut-liver selective FXR modulators, perhaps, gene- and metabolic pathway-specific FXR modulators would be also important to consider as possible therapeutic strategies for treatment of cholestatic and metabolic diseases.

Abbreviations

ASBT	apical sodium dependent transporter
CA	cholic acid
CDCA	chenodeoxycholic acid
CREB	cAMP regulatory element-binding protein
DCA	deoxycholic acid
LCA	lithocholic acid

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I-BABP	intestinal bile acid binding protein
ERK1	extracellular signal-regulated protein
FGF	fibroblast growth factor
FXR	farnesoid X receptor
FGF1	fibroblast growth factor
GSK3	glycogen synthase kinase 3
IR-1	indirect repeat 1
IL-1β	interleukin-1β
NASH	non-alcoholic steatohepatitis
NAFLD	non-alcoholic steatohepatitis
NTCP	Na^+-taurocholate cotransporting polypeptide
OATP	organic anion-transporting peptides
PPAR	peroxisome proliferator-activated receptor
PGC-1α	peroxisome proliferators-activated receptor γ coactivator protein-1α
PXR	pregnane X receptor
PBC	primary biliary cirrhosis
RAR	retinoic acid receptor
RXR	retinoid X receptor
TNFα	tumor necrosis factor α
SHP	small heterodimer partner
TCDC	taurochenodeoxycholate
UDCA	ursodeoxycholic acid
VDR	vitamin D receptor

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HIGHLIGHTS

- Bile acids are critical for many hepatic and intestinal and metabolic functions
- Enterohepatic bile acid circulation is tightly regulated by farnesoid X receptor
- Farnesoid X receptor controls bile synthesis and transport in liver and intestine
- FGF15/19 hormone production in the intestine is regulated by farnesoid X receptor
- FGF15/19 from intestine circulates to liver and regulates hepatic bile acid synthesis

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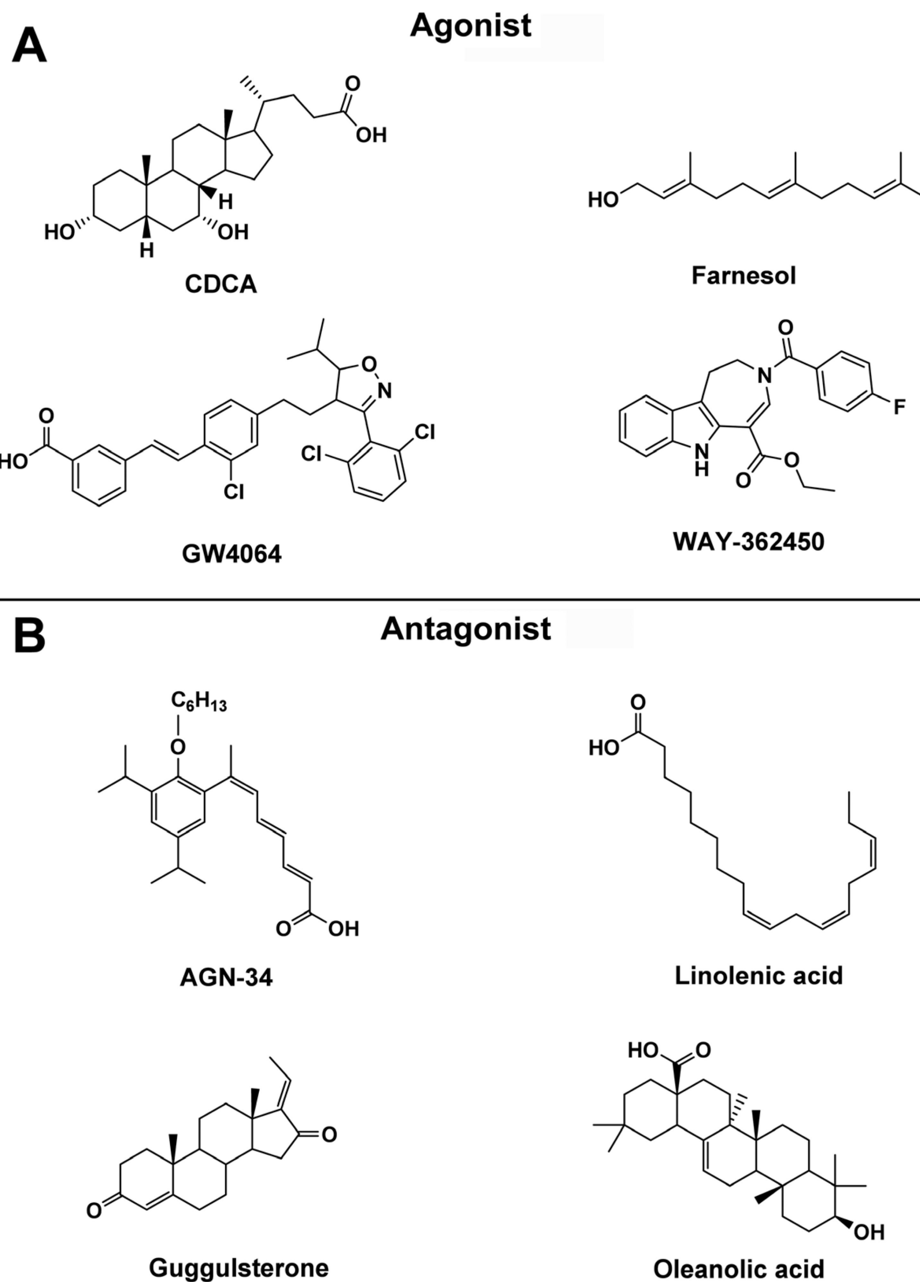

Figure 1. Reported FXR ligands

(A) FXR agonists, CDCA (steroid), farnesol (terpenoid), GW4064 (aromatics), and WAY-362450 (alkaloid). (B) FXR antagonists, AGN-34 (aromatics), linolenic acid (fatty acid), guggulsterone (steroid), oleanoic acid (terpenoid).

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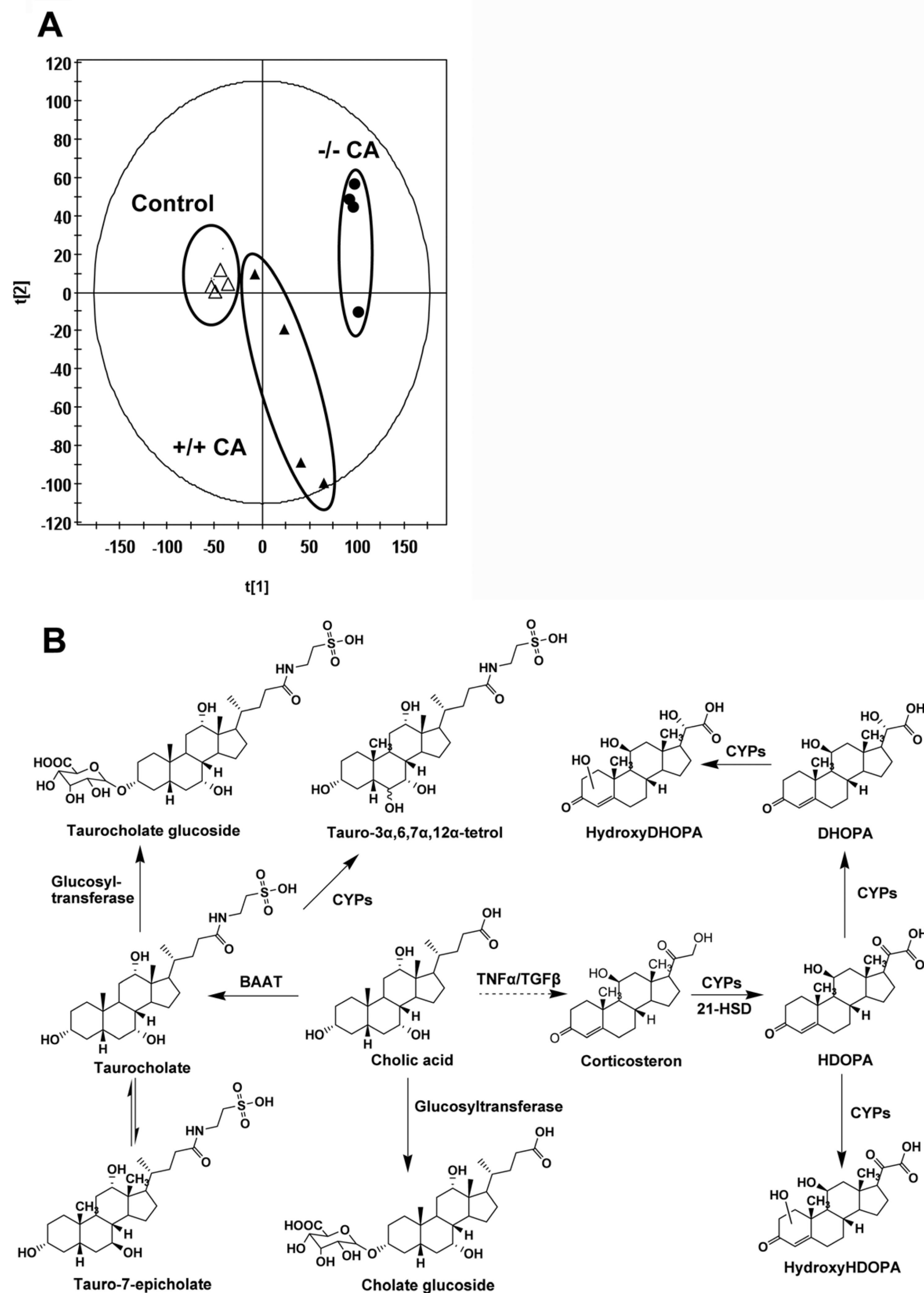

Figure 2. Metabolomic analysis of CA-induced and untreated wild-type (+/+) and *Fxr-null* (–/–) mice by ultraperformance™ liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry

(A) Scores scatter plot of principal components analysis (PCA) model of urine from the control and CA-treated groups in both wild-type (+/+) and *Fxr*null (–/–) mice. (B) The adaptive metabolic pathways of *Fxr*-null mice upon CA challenge. Under the effect of bile acid-CoA:amino acid *N*-acyltransferase (BAAT) and UDP-glucuronosyltransferase (UGT), cholic acid (CA) is converted to taurocholate and cholate glucoside. Taurocholate can be further metabolized into taurocholate glucoside and tauro-3α,6,7α,12α-tetrol under the effect of glucosyl-transferase and cytochromes P450, respectively. The level of

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inflammatory cytokines TNFα and TGFβ increase in *Fxr*-null mice upon CA challenge, which enhances the level of corticosterone *in vivo*. Under the cytochromes P450 catalysis and 21-hydroxysteroid dehydrogenase (HSD), corticosterone is transformed to HDOPA. HDOPA can further be converted to DHOPA and hydroxy-HDOPA under by cytochrome P450. Hydroxy-DHOPA is generated from DHOPA.

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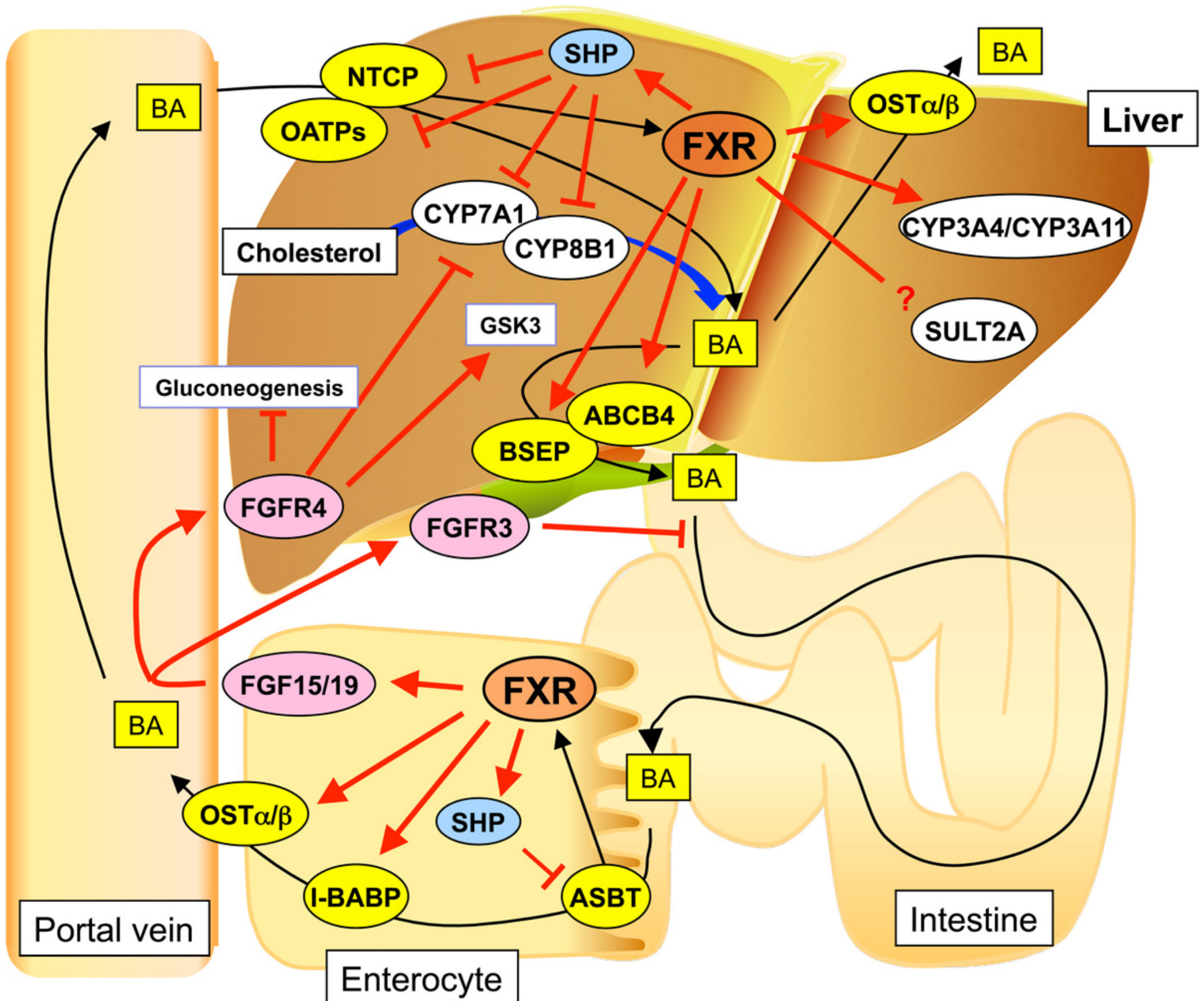

Figure 3.

Major roles of FXR signaling in the enterohepatic system. FXR accelerates bile acid export from liver through induction of BSEP and ABCB4 expression and decreasing bile acid uptake to liver by the suppression of NTCP and OATPs expression via hepatic FXR-SHP signaling, decreases bile acid absorption at the intestine through suppression of ASBT via FXR-SHP signaling, and attenuates cholesterol metabolism/bile acid synthesis by suppression of CYP7A1 and CYP8B1 expression via the hepatic FXR-SHP and intestinal FGF15/19 pathways. Thus, FXR action leads to decreased bile acid pool size. Intestinal FXR-FGF15/19 signaling decreases hepatic glucose metabolism through FGFR4 and induces gallbladder filling through FGFR3.

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Table 1

Reported FXR ligands

Year	Function	Compounds	Structure type	Source	Reference
1995	Agonist	Farnesol Farnesol metabolites	Terpenoid	Nature	[2]
1999	Agonist	CDCA DCA LCA TTNPB	Steroid Aromatics	Nature Synthesis	[4] [5]
1999	Agonist	CDCA CA DCA LCA	Steroid	Nature	[3]
2000	Agonist	Forskolin	Steroid	Nature	[7]
2000	Agonist	GW4064 GW9047	Aromatics	Synthesis	[120]
2001	Agonist	1,1-Bisphosphonate esters	Aromatics	Synthesis	[121]
2002	Agonist	6-ECDCA	Steroid	Semi-synthesis	[10]
2003	Agonist	fexaramine	Aromatics	Synthesis	[122]
2003	Antagonist	Guggulsterone	Steroid	Nature	[9]
2003	Agonist	Fexaramate Fexarene Fexaramine Fexarine fexarchloramide	Aromatics	Synthesis	[123]
2003	Agonist Antagonist	AGN29 AGN 31 AGN 34	Aromatics	Synthesis	[124]
2004	Agonist	UDCA	Steroid	Nature	[125]
2004	Agonist	CDCA derivatives	Steroid	Semi-synthesis	[126]
2004	Antagonist	Arachidonic acid Docosahexaenoic acid Linolenic acid	Fatty acid	Nature	[127]
2005	Agonist	Xanthohumol	Aromatics	Nature	[128]
2006	Agonist	22 (R)-hydroxycholesterol	Steroid	Nature	[129]
2006	Agonist Antagonist	Bile alcohols 5β-cyprinol Bile alcohols 5β-bufol Bile alcohols 5α-cyprinol Bile alcohols 5α-bufol	Steroid	Semi-synthesis	[130]
2006	Agonist	Androsterone	Steroid	Nature	[6]

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Year	Function	Compounds	Structure type	Source	Reference
2006	Agonist	Diphenylmethane skeleton	Steroid	Synthesis	[131]
2007	Antagonist	GW4064 derivatives	Aromatics	Semi-synthesis	[132]
2007	Antagonist	Stigmasterol	Steroid	Nature	[8]
2007	Agonist	Cafestol	Terpenoid	Nature	[133]
2008	Agonist	GSK8062	Aromatics	Semi-synthesis	[134]
2008	Agonist	Pyrazolidine-3,5-dione derivatives	Aromatics	Semi-synthesis	[135]
2008	Agonis	Coumestrol	Steroid	Nature	[136]
2008	Agonist	Methyl cholate, Methyl deoxycholate 5β-Cholanic acid, 5β-Cholanic acid-7α,12α-diol NIHS700 marchantinA marchantin E	Steroid Aromatics	Semi-synthesis Nature	[137]
2009	Agonist	Froglitazone Rosiglitazone Pioglitazone	Alkaloid	Synthesis	[138]
2009	Agonist	WAY-362450	Alkaloid	Synthesis	[139]
2009	Agonist	Pyrrole [2,3-*d*]azepines	Alkaloid	Semi-synthesis	[140]
2009	Agonist	N-oxide pyridine GW4064	Aromatics		[141]
2009	Agonist	Bile alcohols	Steroid	Semi-synthesis	[142]
2010	Antagonist	Oleanolic acid	Terpenoid	Nature	(20)
2011	Agonist	GSK2324	Aromatics	Semi-synthesis	[143]
2011	Antagonist	Sulfated sterols	Steroid	Semi-synthesis	[144]
2011	Agonist	6a-Ethyl-24-norcholanyl-23-amine derivate	Steroid	Semi-synthesis	[145]
2011	Antagonist	Tuberatolides	Terpenoid	Nature	[146]

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Table 2

FXR involved in the regulation of bile acid and xenobiotics metabolism

Metabolism	Gene	Substrate	Regulation	Manner	Reference
phase 0	NTCP	Bile acids	Suppression	Indirect effect	[51]
	OATP1B1	Bile acid, xenobiotics	Suprression	Indirect effect	[147]
	OATP1B3	Bile acid, xenobiotics	Activation	Direct effect	[148]
Phase I	CYP3A4	Bile acid, xenobiotics	Activation	Direct and indirect effect	[77,149]
	CYP7A1	Cholesterol	Suprression	Indirect effect	[59]
	CYP8B1	Cholesterol	Suprression	Indirect effect	[68]
	CYP27A1	Cholesterol	Suprression	Indirect effect	[69]
Phase II	UGT1A1	Bile acid, xenobiotics	Activation	Direct effect	[92]
	UGT2B4	Bile acid, xenobiotics	Activation	Direct effect	[78]
	UGT2B7	Bile acid, xenobiotics	Suppression	Direct effect	[150]
	SULT1a1	Bile acid, xenobiotics	Activation	Direct effect	[92]
	SULT1a2	Bile acid, xenobiotics	Activation	Direct effect	[92]
	SULT2A1	Bile acid, xenobiotics	Activation	Direct effect	[80]
	GSTα3	xenobiotics	Activation	Direct effect	[92]
	GSTα4	xenobiotics	Activation	Direct effect	[92]
	GSTμ1	xenobiotics	Activation	Direct effect	[92]
	GSTμ3	xenobiotics	Activation	Direct effect	[92]
	BACS	Bile acid	Activation	Direct effect	[151]
	BAT	Bile acid	Activation	Direct and indirect effect	[151,152]
Phase III	BSEP	Bile acid	Activation	Direct effect	[86]
	MRP2	Bile acid, xenobiotics	Activation	Direct and indirect effect	[153]
	MDR3	PC	Activation	Direct effect	[91]
	OSTα	Bile acid	Activation	Direct effect	[33,34]
	OSTβ	Bile acid	Activation	Direct effect	[33,34]
	FABP6	Bile acid	Activation	Direct effect	[30]
	ASTB	Bile acid	Suppression	Indirect effect	[24]