

## Review

# FXR, a target for different diseases

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**Summary.** Great progress has been made in the understanding of the physiological roles of the nuclear receptor farnesoid X receptor (FXR) during the last several years. Roles for FXR were initially identified in the regulation of bile acid, cholesterol, triglyceride, and glucose metabolism. More recently, our group has identified additional functional roles of FXR. Specifically, we have shown that FXR regulates normal liver regeneration and plays a protective role in liver carcinogenesis. These exciting findings suggest that FXR has a broader role than previously thought, and also highlight potential new opportunities for using FXR as a drug target for different diseases. Here we summarize the latest results from studies on FXR response elements, target genes and functions in different diseases.

**Key words:** FXR, Nuclear receptor, Bile acid, Metabolism

### Introduction

The farnesoid X receptor (FXR, NR1H4) was first isolated from a rat liver cDNA library in 1995 (Forman et al., 1995). FXR was originally considered an “orphan” nuclear receptor because its natural ligands were unknown (Forman et al., 1995). Subsequently, FXR was “adopted” following the discovery that metabolites of bile acid bind to and activate this receptor at physiological concentrations (Makishima et al., 1999; Parks et al., 1999). FXR is highly expressed in the liver, intestine, kidney, and adrenals, and was subsequently identified as an important regulator for diverse metabolic pathways, including the bile acid, lipid and glucose metabolisms. Recent studies indicate that FXR, and possibly other nuclear receptors, regulate the size and/or regeneration of the liver by sensing the levels of liver

metabolites such as bile acids (Huang et al., 2006). In addition, it was observed that FXR null mice spontaneously develop liver tumors as they age, which suggests that FXR plays a key role in the suppression of liver tumors (Yang et al., 2007). Therefore, FXR has become a promising target not only for the treatments of cholesterol gallstone disease, type 2 diabetes, hypertriglyceridemia, steato-hepatitis, and metabolic syndrome, but also possibly for liver carcinoma.

Nuclear hormone receptors are transcription factors that are involved in numerous processes, including reproduction, development and general metabolism (Chawla et al., 2001). Most of these receptors are comprised of a ligand-independent transcriptional activation domain (AF-1) at the amino terminus, a DNA binding domain, a hinge region, a ligand binding domain, a dimerization interface, and a ligand-dependent activation domain (AF-2) at the carboxyl terminus (Glass, 1994; Mangelsdorf et al., 1995). FXR, as a transcription factor, binds to DNA as heterodimers with its partner, retinoid X receptor (RXR, NR2B1) to regulate the levels of various gene transcripts involved in bile acid, lipid and glucose metabolisms (Zhang et al., 2003). There are four known FXR isoforms (FXR $\alpha$ 1, FXR $\alpha$ 2, FXR $\beta$ 1 and FXR $\beta$ 2) in humans and mice (Huber et al., 2002; Zhang et al., 2003). Some FXR target genes, including intestinal bile acid binding protein (IBABP) and the three fibrinogen subunits (FBG, -alpha, -beta and -gamma), are regulated in an isoform-dependent manner (Anisfeld et al., 2005); but most FXR target genes, including bile salt export pump (BSEP, ABCB11) and small heterodimer partner (SHP), are regulated in an isoform-independent manner. The four isoforms are expressed in a tissue-dependent manner (Zhang et al., 2003). FXR $\alpha$  and FXR $\beta$  were most abundantly expressed in the liver. The liver was the only organ that expressed similar levels of both isoforms. FXR $\alpha$  was moderately expressed in ileum and adrenal gland. FXR $\beta$  was abundantly expressed in ileum, moderately in kidney, and at low levels in stomach, duodenum, and jejunum (Zhang et al., 2003).

Over the past decade, a number of studies on FXR have established FXR as a key regulator of metabolism.

This review will focus on the recent advances in our understanding of the roles of FXR in homeostasis and physiology. We will also discuss the links between FXR and different diseases, which suggest intriguing possibilities for using FXR as a drug target.

### Diverse FXR response elements in different target genes

FXR regulates the expression of a wide variety of target genes by binding either as a monomer or as a heterodimer with RXR to FXR response elements (FXREs) that consist of an inverted repeat (IR) of the canonical AGGTCA hexanucleotide core motif spaced by 0 bp (IR-0) (Song et al., 2001) or 1 bp (IR-1) (Ananthanarayanan et al., 2001; Li et al., 2005). The highest level of transactivation by FXR/RXR was mediated by an IR-1 where one half-site had the sequence GGGTCA. In 1999, Grober et al. showed that bile acids (BAs) induce expression of the human IBABP gene through the binding of the FXR/RXR heterodimer to an IR-1 element in the proximal IBABP promoter (Table 1). The physiological relevance of these data is supported by the observation that BA-mediated regulation of IBABP expression is also found in vivo in the mouse (Grober et al., 1999). Goodwin et al. (2000) reported that FXR directly binds and activates the SHP-1 promoter by binding to an IR-1 element

(GAGTTAaTGACCT) (Table 1). SHP-1, in turn, interacts with liver receptor homolog 1 (LRH-1) and represses the expression of cholesterol 7 alpha-hydroxylase (CYP7A1) and cytochrome P450 sterol 12-hydroxylase (CYP8B1). Ananthanarayanan et al. (2001) showed that FXR directly activates the transcription of BSEP by binding to an IR-1 element in the BSEP promoter (Table 1). FXR also induces bile acid-CoA:amino acid N-acetyltransferase (BAT) and phospholipid transfer protein (PLTP) via IR-1 elements in the promoters of these genes (Urizar et al., 2000; Pircher et al., 2003) (Table 1). In addition, FXR activates other genes that are critical for bile acid enterohepatic circulation, such as human organic anion transporting polypeptide 8 (OATP8) and organic solute transporters  $\alpha$  and  $\beta$  (OST $\alpha/\beta$ ), via IR-1 elements (Jung et al., 2002; Lee et al., 2006). To summarize, IR-1 is the primary binding sequence for FXR. Identification of more FXR target genes by IR-1 element has rapidly expanded our understanding of FXR function.

Additionally, FXR has also been shown to bind to an IR-0 in the dehydroepiandrosterone sulfotransferase (STD) gene, which encodes an enzyme with bile acid sulfo-conjugating activity (Song et al., 2001) (Table 1). Moreover, in addition to IR-1 and IR-0 elements, FXR/RXR heterodimers can also recognize other DNA motifs with varying affinity, such as direct repeats (DRs) of the hexanucleotide core sequence with different

**Table 1.** Genes regulated by FXR and the related FXREs.

Metabolism/Gene	Regulation	FXREs	Refs
<b>Cholesterol and Bile acid</b>			
SHP	Induced	IR-1, GAGTTAaTGACCT(human)	Goodwin et al., 2000
CYP7A1	Repressed	-	Goodwin et al., 2000
CYP8B1	Repressed	-	Goodwin et al., 2000
IBABP	Induced	IR-1, GGGTGAaTAACCT	Grober et al., 1999
BSEP	Induced	IR-1, GGGACAiTGATCCT	Ananthanarayanan et al., 2001
rMRP2	Induced	ER-8, TGAACtctaaccaAGTTCA	Kast et al., 2002
BAT	Induced	IR-1, AGGTCAaGTGCCT	Pircher et al., 2003
STD	Induced	IR-0, GGGTCA TGAACCT	Song et al., 2001
ASBT	Repressed	-	Li et al., 2005
Hepatic insig-2	Induced	IR-1, AGGTCAaCGACCT, AGGACAiTGCCCC	Hubbert et al., 2007
OATP8	induced	IR-1, AGGACAaTGACCT	Jung et al., 2002
hOST $\alpha$ /hOST $\beta$	induced	OST $\alpha$ , IR-1, GGGTGAaTGACCT,AGGCCAgTGACCC, GGGTCAg GGCCCT, GGGTAAiTAAACC	Lee et al., 2006
BACS	Induced	OST $\beta$ , IR-1, AGGTCAgTCACCC,AGGTGAiTACACCT	Pircher et al., 2003
<b>Lipid</b>			
MDR3	Induced	IR-1, ATGTCAaTAACCT	
Huang et al., 2003			
Human Complement C3	Induced	IR-1, AGGTTAaTCACCC	Li et al., 2005
SDC1		DR-1, AGAGCAhAGGGGA	Anisfeld et al., 2003
VLDLR	Induced	-	Sirvent et al., 2004a
PLTP	Induced	IR-1, GGGTCAgTGACCC	Urizar et al., 2000
Apo-I	Repressed	monomeric form, GATCCT TGAACCTCT	
Claudel et al., 2002			
hepatic lipase	repressed	-	Sirvent et al., 2004b
<b>Glucose</b>			
PEPCK	Induced or repressed	-	Stayrook et al., 2005; Ma et al., 2006; Zhang et al., 2006; Cariou et al., 2005

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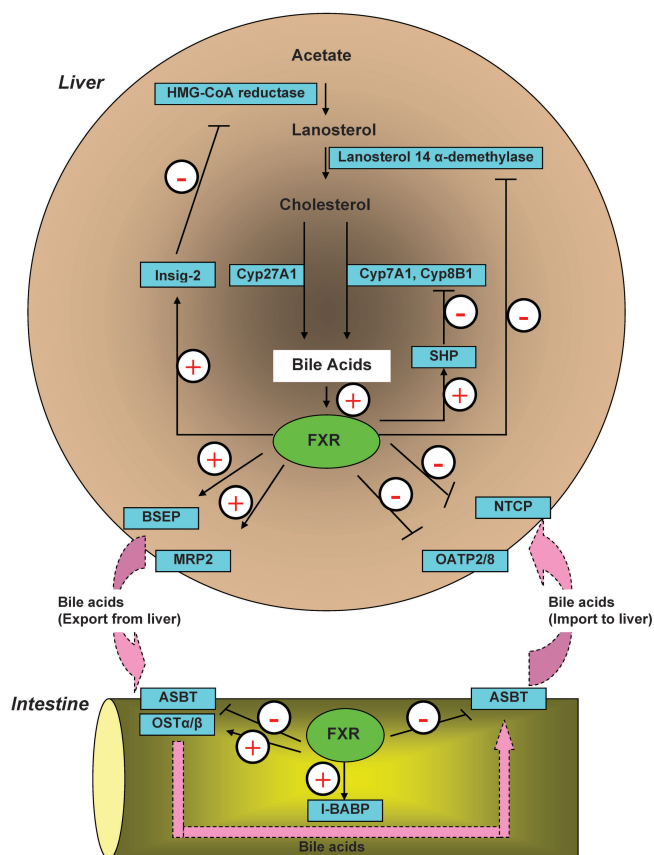
spacing (Laffitte et al., 2000). For example, FXR uses a DR-1 element to bind to the promoter of the syndecan-1 (SDC-1) gene, a transmembrane heparan sulfate proteoglycan that participates in the binding and internalization of extracellular ligands (Anisfeld et al., 2003) (Table 1). Furthermore, an everted repeat (ER) of the core motif separated by eight nucleotides (ER-8) was shown to mediate the induction of the multidrug resistant-associated protein 2 (MRP2) by BAs (Kast et al., 2002). Finally, Claudel et al. (2002) showed that apolipoprotein A-I (apoA-I) is regulated by FXR via the DNA binding of a monomeric form. In summary, FXR can bind to a variety of FXREs with differing affinities. This allows FXR to regulate the expression of different genes involved in lipid, lipoprotein and glucose metabolisms, in addition to regulating genes involved in bile acid metabolism (Table 1).

### FXR regulates diverse metabolic pathways

As described above, the diverse FXR response elements provide clues about the functions of FXR not only in bile acid homeostasis, but also in lipoprotein metabolism and glucose homeostasis.

Bile acids are amphipathic molecules with detergent-like properties that are essential for their physiological functions. Cells, especially those of organs that participate in enterohepatic circulation, must be able to sense intracellular bile acid levels and to elicit an adequate response in case of depletion or over-load. Physiological concentrations of both primary and secondary bile acids efficiently activate FXR. For example, the primary bile acid, chenodeoxycholic acid (CDCA), strongly activates FXR. FXR can also be activated by the secondary bile acids, lithocholic acid (LCA) and deoxycholic acid (DCA) (Parks et al., 1999). Elevated bile acid levels are toxic, and therefore their synthesis and enterohepatic circulation is tightly controlled. It is now well established that FXR functions as a primary bile acid sensor. In accordance with this role, many FXR-target genes have been identified that are involved in bile salt and cholesterol metabolism (Table 1) (Song et al., 2001; Lee et al., 2006; Hubbert et al., 2007). FXR not only downregulates the expression of CYP7A1, the rate-limiting enzyme in the classic pathway of bile acid synthesis from cholesterol, but also represses the expression of another key enzyme in bile acid synthesis, CYP8B1 (Moschetta et al., 2004) (Fig. 1). Recently, Hubbert et al. (2007) reported that FXR $\alpha$  induces the expression of hepatic Insig-2, which represses lanosterol 14 $\alpha$ -demethylase, and reduces HMG-CoA reductase protein levels. This FXR $\alpha$ -mediated regulation results in the repression of cholesterol synthesis (Fig. 1). These findings indicate that FXR not only directly represses the synthesis of bile acids, but also inhibits the synthesis of cholesterol, the precursor for bile acids. In addition, FXR controls enterohepatic circulation of bile acids by regulating genes involved in bile acid secretion, such as BSEP, the

major hepatic bile salt exporter in liver, and MRP2, which mediates the efflux of several conjugated compounds across the apical membrane of the hepatocyte into the bile canaliculi (Ananthanarayanan et al., 2001; Kast et al., 2002), as well as proteins involved in bile acid transport, such as IBABP, an intestinal protein that binds bile salts with high affinity in the cytosol of enterocytes; the apical sodium-dependent bile acid cotransporter (ASBT/SLC10A2), which is the primary bile salt uptake protein in the intestine; and the sodium-dependent taurocholate cotransporting protein (NTCP), the major hepatic bile salt importer. Moreover,



**Fig. 1.** FXR as a master regulator of bile acid homeostasis in liver and intestine. In the liver, FXR negatively regulates bile acid production by repressing CYP7A1, the rate-limiting enzyme of the synthetic pathway of bile acids, and inhibiting HMG-CoA reductase and lanosterol 14 $\alpha$ -demethylase, which play key roles in the synthesis of cholesterol. FXR induces the expression of BSEP and MRP2, which are involved in bile acid export, and simultaneously represses bile acid import by downregulating NTCP and OATP2/8. In the intestine, FXR induces the expression of IBABP, which provides the enterocytes with a shuttling device for the delivery of bile acids from the apical to the basolateral membrane. FXR does not directly regulate the ASBT gene, but it may influence the network of transcription factors that are involved in a response to bile acids. FXR activates the expression of OST $\alpha/\beta$ , which serves to transport bile acids from the gut to the enterohepatic circulation where they are transported back to the liver.

FXR also regulates genes involved in bile acid detoxification, such as BAT, dehydroepiandrosterone-sulfotransferase (SULT2A1) and bile acid CoA synthetase (BACS) (Russell, 2003). In conclusion, FXR is a master regulator of the homeostasis of bile acids.

Subsequent studies demonstrated that FXR also regulates a set of genes that participate in lipid and glucose metabolism. Sinal et al. originally proposed that FXR controls plasma lipid levels (Sinal et al., 2000). Furthermore, the studies of Edwards et al. (2002) showed that FXR alters the transcription of several genes involved in fatty acid and triglyceride synthesis, as well as lipoprotein metabolism. These genes include the phospholipid transfer protein (PLTP), the syndecan-1 (SDC-1) and the very low density lipoprotein receptor (VLDLR) (Edwards et al., 2002; Anisfeld et al., 2003; Sirvent et al., 2004a). These results, together with the finding that activation of FXR leads to repression of SREBP-1c, a transcription factor that controls genes involved in fatty acid and triglyceride synthesis, provide a mechanism to account for the triglyceride-lowering effects of bile acids and some synthetic FXR agonist ligands (Watanabe et al., 2004; Zhang et al., 2004).

Early studies showed that expression of FXR is reduced in animal models of diabetes and the expression of FXR is regulated by glucose, likely via the pentose phosphate pathway (Duran-Sandoval et al., 2004). Although recent observations of glucose levels in FXR<sup>-/-</sup> mice have produced conflicting data, suggesting that glucose levels are either unchanged (Zhang et al., 2006), increased (Ma et al., 2006) or repressed (Cariou et al., 2006) compared to wild-type littermates indicate that other crucial factors have yet to be identified, these reports have provided direct evidence that activation of FXR in wild-type or diabetic [db/db or KKA-(y)] mice promotes hypoglycemia and increases insulin sensitivity. Ma et al. (2006) showed that loss of FXR function in the liver results in increased hepatic lipid accumulation and elevation of non-esterified fatty acids (FFAs) in the serum. The development of insulin resistance in the liver, which fails to suppress gluconeogenesis, and in the skeletal muscle, which reduces glucose uptake, contributes to the dysregulation of glucose homeostasis in FXR<sup>-/-</sup> mice. Therefore, FXR is essential for normal glucose homeostasis. Zhang et al. (2006) demonstrated that activation of FXR lowers plasma glucose levels by sensitizing insulin action, which provides further evidence that FXR may be involved in the regulation of glucose homeostasis.

## FXR and different diseases

### FXR and cholestasis

Cholestatic injury is associated with the accumulation of bile acids and activation of pro-inflammatory cytokines in liver. Cholestasis causes systemic and intrahepatic retention of potentially toxic bile acids that results in liver injury, and ultimately leads

to biliary fibrosis and cirrhosis (Trauner et al., 1998). Miyata and colleagues (2005) explored the FXR-dependent protective mechanism for cholic acid-induced toxicity. FXR-null mice and wild-type mice were fed a diet that contained cholic acid. The investigators found that BSEP mRNA and protein levels increased in the wild-type mice, but decreased in the FXR-null mice. As the concentration of cholic acid in the diet increased, the wild-type mice had a compensatory increase in bile acid output rate. In FXR-deficient mice, the bile acid output significantly decreased in the face of rising cholic acid concentrations. This study demonstrates that FXR-mediated adaptive enhancement of canalicular bile acid excretion is a critical protective mechanism to prevent cholic acid-induced toxicity in cholestasis. Similarly, Liu et al. (2003) found that a ligand for FXR, GW4064, protected the liver from cholestatic injury in the bile duct-ligation and alpha-naphthylisothiocyanate models of cholestasis.

Estrogens can cause intrahepatic cholestasis in susceptible women during pregnancy and when prescribed for oral contraception or postmenopausal hormone replacement therapy. Fiorucci and colleagues (2005) found that in a rat model of estrogen-induced cholestasis, administration of 6-ethyl chenodeoxycholic acid, a semi-synthetic bile acid and potent FXR ligand, protected against cholestasis by dramatically increasing the expression of basolateral and canalicular bile acid transporters (BSEP, MRP2 and MDR2) and repressing bile acid biosynthesis. The investigators concluded that the development of FXR ligands might provide a new approach for treatment of cholestatic disorders. Also, together with the xenobiotic receptors, pregnane X receptor (NR1H2) and constitutive androstane receptor (NR1H3), FXR prevents and ameliorates cholestasis through the activation of hepatic CYP450s, phase II enzymes that are able to decrease the cholestatic xenobiotic noxae and to detoxify the bile acid pool (Barbier et al., 2003; Jung et al., 2006).

### FXR and diabetes

Several key processes regulated by FXR, such as bile acid and triglyceride metabolism, are impaired in diabetic individuals (Boland et al., 2002). This observation has led many investigators to assess the potential role of FXR in animal models of type 1 and 2 diabetes. Duran-Sandoval et al. observed that the expression of FXR was decreased in the livers of streptozotocin-induced diabetic rats as well as in diabetic Zucker rats, which suggested a link between FXR and diabetes (Duran-Sandoval et al., 2004). Furthermore, FXR expression was shown to be regulated by glucose via the pentose phosphate pathway, which provided an unexpected link between bile acid and carbohydrate metabolism. Stayrook et al. (2005) reported that phosphoenolpyruvate carboxykinase (PEPCK) expression and glucose production are regulated by FXR, which provides an evidence for an additional link



between carbohydrate metabolism and the well characterized lipid metabolism pathways regulated by FXR. The findings that FXR may regulate gluconeogenesis, coupled with the discovery of Duran-Sandoval et al. (2004) that FXR expression is regulated by glucose levels, suggest that a feedback loop may be operating. In addition, Stayrook et al. (2005) found that treatment of C57BL6 mice with GW4064 significantly increased hepatic PEPCK expression. Therefore, they suggested that activation of FXR may actually be unfavorable in diabetics. However, despite this observation, most researchers suggest that activation of FXR may be useful for inhibiting hepatic gluconeogenesis in diabetics (De Fabiani et al., 2003). Zhang et al. (2006) reported that FXR null mice exhibited glucose intolerance and insulin insensitivity. They proposed that the development of FXR agonists might prove useful for the treatment of diabetes. A recent study by Ma et al. (2006) showed that, in contrast to the results in FXR<sup>-/-</sup> mice, bile acid-induced activation of FXR in wild-type mice repressed expression of gluconeogenic genes and decreased serum glucose. They demonstrated that FXR is required for the maintenance of glucose homeostasis *in vivo*. Based on these findings, FXR selective agonists are potential pharmaceutical candidates for the management of type 2 diabetes and hypertriglyceridemia, which are two major symptoms of metabolic syndrome.

#### *FXR in liver regeneration and carcinogenesis*

Liver regeneration after the loss of hepatic tissue is a fundamental parameter of liver response to injury. It is now defined as an orchestrated response induced by specific external stimuli and involving sequential changes in gene expression, growth factor production, and morphologic structure. Normal liver regeneration is important for restoring the liver mass following liver injury. However, irregular regeneration of hepatocytes, which develops as a result of repeated cycles of necrosis and regeneration of hepatocytes in chronic hepatitis, has been reported as an important factor in the hepatocarcinogenesis (Ueno et al., 2001).

In addition to controlling the levels of BAs, FXR also helps accelerate normal liver regeneration in response to increased BA stress after 70% hepatectomy, which suggests that FXR has a parallel role in promoting liver growth after injury (Huang et al., 2006). Numerous secreted factors, including growth factors and cytokines, have been implicated in regulating hepatocyte proliferation. Huang et al. (2006) reported that bile acids are stimulatory signals for liver regeneration in mice. An increase in bile acids stimulates liver regeneration, which requires the normal function of the bile acid receptor FXR. The authors proposed a homeostatic mechanism for determination of liver size, in which FXR and possibly other nuclear receptors sense the levels of endogenous metabolites to determine the liver's functional capacity. When liver function is decreased as

a result of injury, the resulting accumulation of bile acids activates FXR, which stimulates signaling pathways to protect the liver from bile acid toxicity and also promote liver growth to handle the overload. However, the mechanism and signaling pathways involved in FXR function in liver regeneration remain to be identified.

The hepatoprotective role of FXR is essential for the maintenance of normal liver physiology and prevention of the deleterious effects of bile acids. Indeed, FXR null mice spontaneously develop liver tumors due to chronic liver injury as they age (Yang et al., 2007). In the absence of FXR, Yang et al. detected significant hepatocellular apoptosis, chronic liver injury and irregular liver regeneration, which resulted in spontaneous liver tumor formation (Yang et al., 2007). The findings indicate that FXR is an important factor in an intriguing link between metabolic regulation and hepatocarcinogenesis. However, the mechanisms by which FXR suppresses liver cancer remain to be investigated.

#### **Concluding remarks**

FXR affects numerous signaling pathways via the genes it regulates directly and by its interference with other nuclear receptor signaling pathways. This creates a unique integrative mechanism to regulate the metabolism of bile acids, cholesterol, triglycerides, and glucose. Because FXR functions in diverse metabolic pathways, it is a promising therapeutic target for treating or preventing type 2 diabetes, hypertriglyceridemia, cholesterol gallstone disease, steato-hepatitis, and metabolic syndrome. The recent discovery that FXR is also involved in liver regeneration and liver carcinogenesis suggests that FXR is a potential target for the treatment of liver cancer.

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