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REVIEW

FXR: a metabolic regulator and cell protector

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Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors. As a metabolic regulator, FXR plays key roles in bile acid, cholesterol, lipid, and glucose metabolism. Therefore, FXR is a potential drug target for a number of metabolic disorders, especially those related to the metabolic syndrome. More recently, our group and others have extended the functions of FXR to more than metabolic regulation, which include anti-bacterial growth in intestine, liver regeneration, and hepatocarcinogenesis. These new findings suggest that FXR has much broader roles than previously thought, and also highlight FXR as a drug target for multiple diseases. This review summarizes the basic information of FXR but focuses on its new functions.

***Keywords*:** FXR, bile acid, metabolism, liver regeneration, hepatocarcinogenesis

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Introduction

Nuclear receptors (NRs) are ligand-activated transcription factors that have central roles in nearly every aspect of development and adult physiology [1]. This family contains 48 members in humans. Because of the importance of NR functions in different metabolic pathways, they have become attractive targets for drug discovery.

Initially cloned and named as the farnesoid X receptor (FXR, NR1H4) in 1995 [2], FXR belongs to a subcluster of metabolic receptors that also include vitamin D receptor (VDR, NR1I1), constitutive androstane receptor (CAR, NR1I3), pregnane X receptor (PXR, NR1I2), and liver X receptor alpha and beta (LXRα, NR1H3; LXRβ, NR1H2). As a transcription factor, it binds to DNA either as a monomer or as a heterodimer with a common partner for NRs, retinoid X receptor (RXR, NR2B1), to regulate the expression of various genes involved in bile acid (BA), lipid, and glucose metabolisms [3]. FXR is highly expressed in the liver, intestine, kidney, and adrenals, but with lower expression in fat and heart [2, 4, 5]. FXR protein has the typical NR structure composed of modular domains, the N-terminal ligand-independent transcriptional activation AF1 domain (AB), the DNA-binding C-domain, a D domain and hinge region, and the C-terminal ligand-binding E domain containing the ligand-dependent AF2 activation domain [6-8]. There are two FXR genes (FXRα (NR1H4) and FXRβ (NR1H5)) in mammals [9]. FXRβ is a functional receptor in mice, rats, rabbits, and dogs, but constitutes a pseudogene in humans and primates [10]. The functional role of FXRβ is not clear yet. A single FXRα gene encodes FXRα1 or α2 and FXRα3 or α4 isoforms resulting from the differential use of two promoters and an alternative splicing by using two different sites in exon 5 [5, 11, 12]. The four isoforms are expressed in a tissue-dependent manner [5]. FXRα is most abundantly expressed in the liver. FXRα1 and FXRα2 are moderately expressed in ileum and adrenal gland. FXRα3 and FXRα4 are abundantly expressed in ileum, moderately in kidney, and at low levels in

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Abbreviations: BACS (bile acid CoA synthetase); BAT (bile acid-CoA:amino acid N-acetyltransferase); BSEP (bile salt export pump); IBABP (intestinal bile acid binding protein); MRP2 (multidrug resistant-associated protein 2); OATP8 (organic anion transporting polypeptide 8); hOSTα/hOSTβ (human organic solute transporters α/β); SHP (small heterodimer partner); STD (dehydroepiandrosterone sulfotransferase); ASBT (apical sodium-dependent bile acid cotransporter); CYP7A1 (cholesterol 7α-hydroxylase); CYP8B1 (cytochrome P450 sterol 12α-hydroxylase); MDR3 (multidrug resistance protein 3); PLTP (phospholipid transfer protein); SDC1 (Syndecan-1); VLDLR (the very low density lipoprotein receptor); ApoA-I (apolipoprotein A-I); Apo CIII (apolipoprotein CIII); PEPCK (Phosphoenolpyruvate carboxykinase); G6Pase (glucose-6-phosphatas)

stomach, duodenum, and jejunum [5]. By regulating the expression of genes involved in diverse metabolic pathways, FXR is becoming an attractive drug target for different metabolic diseases. More recently, we showed that FXR regulates liver regeneration, thereby linking BA signaling to liver re-growth [13]. In addition, FXR null mice spontaneously developed liver tumors as they aged [14, 15]. These new findings suggest that FXR has much

Table 1 Summary of related FXR information

Gene	NR1H4 12q23.3
Expression	Liver Small intestine Kidney Adrenals Vascular smooth muscle Adipose tissue Breast cancer
Natural agonists	**Primary bile acid:** CA, CDCA **Secondary bile acid:** LCA, DCA **Polyunsaturated fatty acids:** arachidonic acid; docosahexaenoic acid, and linolenic acid (endogenous and selective bile acid receptor modulators that specifically regulate expression of certain FXR targets) [21] **Bile acid metabolites:** 26- or 25-hydroxylated bile alcohols [22] **Oxysterols:** oxysterol 22(R)-hydroxysholesterol [19] **Androsterone** (very weak activity) [20] **The order of potency of these ligands:** 26- or 25-hydroxylated bile alcohols=CDCA>LCA=DCA>CA
Synthetic agonists	GW4064 (high-affinity agonist), 6ECDCA (semisynthetic bile acid), AGN29 [23], AGN31 [23] **The potency of these ligands:** GW4064 and 6ECDCA are more potent than the bile acids AGN29 and AGN31 are FXR-selective ligands and 25-fold more potent than naturally occurring ligands
Antagonists	Guggulsterone, lithocholate, AGN34 [23]
Response elements	**IR-1:** GAGTTAaTGACCT GGGTGAaTAACCT GGGACAtTGATCCT AGGTCAaGTGCCT GGGTCAgTGACCC **DR-1:** AGAGCAnAGGGGA **ER-8:** TGAACTcttaaccaAGTTCA **Monomer binding site:** GATCCTTGAACTCT TGAACT
Relevant diseases	Cholestasis Diabetes Atherosclerosis Cholesterol gallstone disease Liver regeneration Liver inflammation Hepatocarcinogenesis Breast cancer Colon cancer

broader roles than previously thought.

In this review, we summarize the basic properties of FXR including its ligands and target genes, but focus on its new functions. Specifically, we will discuss the impact of these new findings on the studies of liver regeneration and hepatocarcinogenesis.

The ligands of FXR

Ligand identification for NRs always greatly facilitates their research. FXR was originally proposed to be a receptor for an intermediary metabolite, farnesol [2]. However, the supraphysiological concentrations required to activate FXR impede the use of farnesoid as a ligand. The major breakthrough in FXR biology was the discovery that BAs are endogenous ligands for this NR [16-18]. In fact, both conjugated and unconjugated bile salts are able to activate FXR at physiological concentrations. The hydrophobic BA chenodeoxycholic acid (CDCA) is the most effective activator of FXR. Deoxycholic acid (DCA) and lithocholic acid (LCA) can both activate FXR, but to a much lesser extent than CDCA, whereas hydrophilic ursodeoxycholic (UDCA) and muricholic acids cannot activate FXR [17]. Recently, Deng *et al*. [19] and Wang *et al*. [20] reported that oxysterol 22(R)-hydroxycholesterol and androsterone are natural FXR ligands, respectively (Table 1). However, whether they represent *bona fide* endogenous ligands of FXR and the physiological consequences of FXR activation by them remain to be established (Table 1). Some polyunsaturated fatty acids such as arachidonic acid and decosahexaenoic acid [21] and BA metabolites such as 26- or 25-hydroxylated bile alcohols [22] were also identified as weak FXR ligands. In addition, several synthetic FXR ligands have been generated. They include GW4064, 6ECDCA, AGN29 [23], and AGN31 [23]. The most widely used FXR ligand is the non-steroidal isoxazole analog GW4064 [24]. But the potential cell-toxic effect and uncertain bioavailability restrict its further use. Instead, 6α-ethyl-chenodeoxycholic acid (6-ECDCA), a novel compound derived from the natural FXR ligand CDCA, has become an alternative agonist ligand for FXR [25-27].

FXR and its target genes

The function of FXR has been shown to be related to different diseases including cholestasis, diabetes, atherosclerosis, and cholesterol gallstone disease. A number of excellent reviews on roles of FXR in these diseases have been published recently [12, 28-31]. FXR fulfills its regulatory role by controlling the expression of a variety of genes in cognate metabolic pathways. Here we briefly summarize the identified FXR-binding elements and its 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). Typical FXREs consist of an inverted repeat (IR) of the

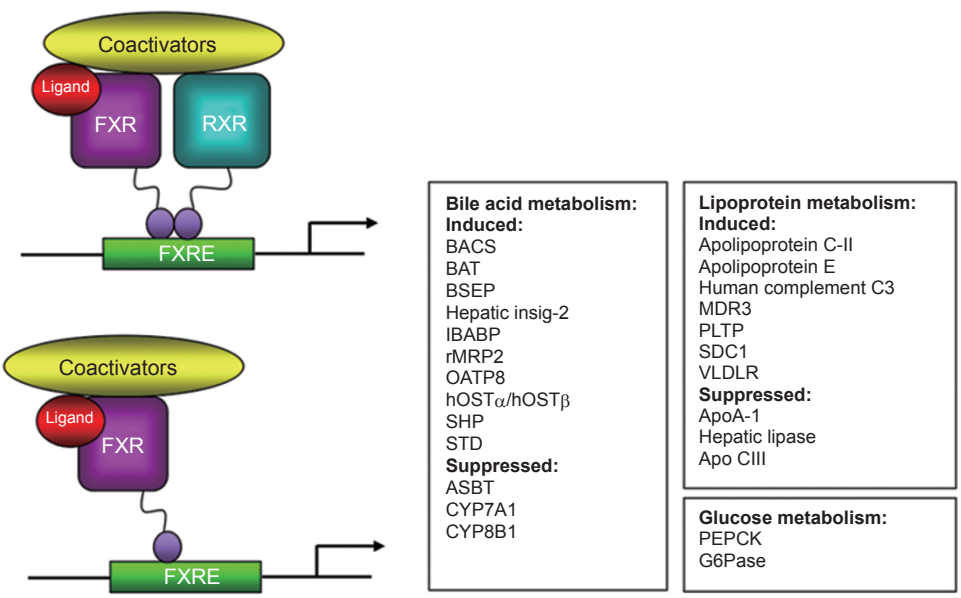

Figure 1 FXR regulates a large number of target genes involved in bile acid, lipoprotein and glucose metabolisms. FXR binds to DNA either as a heterodimer with RXR or as a monomer to regulate the expression of various genes.

canonical AGGTCA hexanucleotide core motif spaced by 0 bp (IR-0) [32] or 1 bp (IR-1) [33, 34]. IR-1 is the primary binding sequence for FXR. FXR regulates human intestinal bile acid binding protein (IBABP), small heterodimer partner (SHP), bile salt export pump (BSEP), BA-CoA:amino acid *N*-acetyltransferase (BAT) [35] and phospholipid transfer protein (PLTP) via IR-1 elements in the promoters of these genes [36-39]. Besides IR-1, other FXREs include IR-0, direct repeat (DR), everted repeat [13] of the core motif separated by eight nucleotides (ER-8) and monomeric binding sites [32, 40-43] (Table 1). In summary, FXR can bind to a variety of FXREs with varied affinities.

By binding to FXREs, FXR regulates many genes belonging to different metabolic pathways (Figure 1). Activation of FXR alters the expression of different groups of genes involved in BA homoeostasis, lipid metabolism, and glucose balance. FXR is the primary sensor of BAs. FXR activates the expression of short heterodimer partner (SHP), which in turn binds to and inactivates liver receptor homolog 1 (LRH-1, NR5A2), thus potently inhibiting the expression of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in BA biosynthesis [37, 44]. In addition, it is clear that BA activation of FXR in the intestine leads to the induction of mouse Fgf15 [45] or its human ortholog FGF19 [46], thereby suppressing CYP7A1 expression through a JNK-dependent signaling cascade. BAs are end products of cholesterol catabolism. As such, FXR also activates the expression of hepatic Insig-2, which represses cholesterol synthesis [47]. These findings indicate that FXR not only directly suppresses the synthesis of BAs, but also inhibits the synthesis of cholesterol, the precursor for BAs. A constant pool of BAs was maintained by enterohepatic circulation. In addition to regulating BA synthesis, FXR controls the recycling of BAs by regulating genes involved in BA secretion, transportation, absorption, conjugation, and detoxification [34, 42]. The remarkable ability of FXR to regulate BA metabolism is confirmed by loss-of-function studies on FXR in animal model [15, 48, 49]. Therefore, FXR is also called the BA receptor due to its master effect on BA homeostasis.

Analyses of FXR knockout animals also reveal an unexpected role of FXR in lipid metabolism. It was shown that FXR also regulates a set of genes that participate in lipoprotein metabolism. These include genes for PLTP, SDC-1, the very low density lipoprotein receptor (VLDLR), apolipoprotein C-II, and apolipoprotein E [50-52]. All these genes are involved in the metabolism of plasma lipoproteins. In addition, activation of FXR leads to repression of SREBP-1c, a transcription factor that controls genes involved in fatty acid and triglyceride synthesis [53, 54]. Therefore, FXR plays an important role in regulating lipid metabolism.

Because of the intrinsic interaction between lipid and glucose metabolism, it was not surprising to find that FXR was also involved in the regulation of glucose levels. FXR regulates gene expression of phosphoenolpyruvate carboxykinase (PEPCK) [55], which is a key enzyme of the hepatic gluconeogenesis pathway, by catalyzing a critical step in gluconeogenesis. However, recent observations of glucose levels in $FXR^{-/-}$ mice have produced conflicting results. Glucose levels are shown to be unchanged [56], increased [57] or repressed [58] in FXR null mice compared to the wild-type littermates. This may be due to the different genetic backgrounds of experimental animals used or different experimental approaches. In contrast, two reports have provided consistent results that activation of FXR in wild-type or diabetic [db/db or KKA-(y)] mice promotes hypoglycemia and increases insulin sensitivity [56, 57].

New functions of FXR

Recently, several new functions of FXR beyond its roles in metabolism were discovered. For example, activation of FXR induces the expression of several genes involved in enteroprotection, which may explain the previous observation that BAs can inhibit bacterial growth in the intestine [59, 60]. Because higher levels of BAs have been linked to human colon cancer, it will be interesting to know if FXR plays a role in colon carcinogenesis. In addition, our group and others have identified novel functions of FXR in liver regeneration and hepatocarcinogenesis.

FXR and liver regeneration

Liver regeneration after the loss of hepatic tissue is an important function of liver to repair injury. It is an adaptive response induced by specific external stimuli, and executed through subsequent sequential changes in gene expression, growth factor production, and morphologic reconstruction. Liver regeneration consists of several well-orchestrated phases, with rapid induction of proliferative factors that activate the quiescent hepatocytes and prime their subsequent progression through the cell cycle, followed by re-establishment of normal liver size and renewed hepatocyte quiescence [6, 61]. Although many genes and signaling pathways are involved in liver regeneration, the essential circuitry required for this process is defined mainly by three major networks: cytokines, growth factors, and metabolic signaling [62]. Secretion of several growth factors and cytokines such as HGF, TGF-α, TNF-α, and IL-6 is an important early

response in liver regeneration. Activation of these potent signaling pathways increases expression of many downstream target genes via activation or induction of several transcription factors, including Stat3, NF-κB, AP-1, and c-Myc [61]. Compared to the cytokine and growth factor networks, little is known about the roles of metabolic signals in liver regeneration. It has been suggested that the increased metabolic demands on the residual hepatocytes after partial hepatectomy (PH) may be critical signals to activate the machinery specific for hepatocyte replication. Also, metabolic signals may function as a sensor that calibrates the regenerative response according to the body demands [62]. Therefore, identification of the key metabolic signaling pathways in liver regeneration will help us better understand the mechanism of this process and provide novel approaches to manipulate liver regeneration.

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 in chronic hepatitis, has been reported as an important factor in hepatocarcinogenesis [63]. We will further discuss this issue later.

Previous reports indicate that 70% hepatectomy increases BA flux and changes expression of several NRs and enzymes involved in BA metabolism [64-66]. We recently showed that normal liver regeneration is dependent on and regulated by FXR [13]. Liver regeneration was accelerated in mice in which BA pools were increased by feeding with a 0.2% cholic acid (CA) diet. In contrast, decreasing BA pool by feeding with a diet supplemented with the BA sequestering resin, cholestyramine, strongly decreased the rate of liver regeneration. The effects of both CA and cholestyramine feeding on liver regeneration were absent in $FXR^{-/-}$ mice, suggesting that FXR is the mediator of the effect of BA signaling on liver regeneration. Furthermore, the rate of liver growth was much slower in the early stages of liver regeneration in $FXR^{-/-}$ mice. Activation of FXR by BAs increased the expression of a Forkhead Box transcription factor, FoxM1b, which was shown to regulate cell cycle progression during liver regeneration [67]. The results thus suggest that FXR is required for liver regeneration after damage possibly by regulating FoxM1b expression. However, whether FoxM1b is a direct target of FXR or indirectly regulated by FXR is still unknown. It is well known that FXR can activate FGF signaling in intestine, which consequently suppresses BA synthesis in liver [45, 46]. However, the potential role of this FGF signaling axis in liver regeneration is still unclear. In addition, the relationship between the FXR-dependent BA signaling and cytokine or growth factor signaling pathways is also unclear. Nonetheless, the identification of a metabolic signaling pathway for liver regeneration suggests that releasing metabolic burden after liver injury could be a critical and integrated part of liver regeneration. It will be interesting in the future to further understand the impact of metabolic signals in liver regeneration. In addition, given the rapidly increasing demand of liver transplantation, targeting FXR pharmacologically may provide a novel approach to accelerate liver regeneration after liver transplantation or surgery.

We now have a more complete picture of FXR function in hepatoprotection. On the one hand, FXR controls the balance of liver metabolism, thereby preventing the deleterious effect of accumulation of toxic metabolic products in liver. On the other hand, once the liver has been subjected to injury, FXR will further participate in liver repair by promoting regeneration and helping restore organ homeostasis. This extraordinary power of hepatoprotection by FXR is essential for the maintenance of normal liver physiology and prevention of liver pathogenesis. Indeed, in the absence of FXR, $FXR^{-/-}$ mice spontaneously developed liver tumors when they aged [14].

FXR and hepatocarcinogenesis

Liver cancer is one of the most common forms of cancer worldwide. In the United States, the incidence of liver cancer has doubled during the last two decades and liver cancer has become the most rapidly increasing form of cancer. Hepatocellular carcinoma (HCC) is the primary liver tumor that accounts for more than 80% of all liver tumors. Among the major etiological causes of HCC, hepatitis B and hepatitis C infection, chemicals, alcoholic and nonalcoholic fatty liver diseases, and some metabolic genetic diseases including hemochromatosis and alpha-1-antitrypsin deficiency, have been implicated in liver cancer development, but the exact mechanism of hepatocarcinogenesis remains unknown [68, 69]. HCC is characteristically associated with the pathologically chronic liver diseases of hepatitis and cirrhosis, in which, triggered by chronic liver injury, hepatocytes proliferate continuously and at higher rates than normal liver cells. This uncontrolled proliferation or irregular liver regeneration is in contrast to normal liver regeneration, in which the liver resumes its normal size and stops cell proliferation within a short period of time. Therefore, irregular liver regeneration may constitute a common mechanism of hepatocarcinogenesis regardless of cancer etiology [35, 63].

The development of cancer is a multi-step process. Recently, NF-κB and the inflammatory pathways have

been shown to be involved in liver tumorigenesis [70]. In a chemical-induced hepatocarcinogenesis model, the role of NF-κB in hepatocyte protection is shown to be critical to prevent liver injury and the consequent irregular liver regeneration [71-73]. In the absence of NF-κB, the induced hepatocyte proliferation during irregular liver regeneration provides an HCC-promoting environment that favors the selection of transformed cells and promotes tumor formation. Similarly, several metabolic diseases have also been shown to result in chronic liver injury and irregular liver regeneration, thereby promoting hepatocarcinogenesis [68, 69, 74, 75]. These common pathological changes were also observed during hepatocarcinogenesis in $FXR^{-/-}$ mice.

$FXR^{-/-}$ mice spontaneously developed liver tumors including hepatocellular adenoma and carcinoma between 13-15 months of age [14]. Both serum and liver BA levels were significantly higher in $FXR^{-/-}$ mice compared to the wild-type controls. BAs have been implicated in the induction of liver apoptosis and injury [76]. BAs can promote liver tumors in a HBV transgenic mouse model and are thought to induce inflammation and liver tumorigenesis in mdr-2 knockout mice [77-79]. This is now further confirmed in our study as feeding of a cholic-acid-diet promoted chemical-induced hepatocarcinogenesis [14].

In parallel, Kim *et al*. [15] reported very similar findings that, at 12 months of age, both male and female $FXR^{-/-}$ mice had a high incidence of degenerative hepatic lesions, altered cell foci, and liver tumors including hepatocellular adenoma, carcinoma, and hepatocholangiocellular carcinoma. The major findings between these two studies are very similar, especially the pathological changes of liver in $FXR^{-/-}$ mice including liver injury, irregular regeneration, and strong inflammation. These results suggest that FXR may provide an intriguing link between metabolic regulation and hepatocarcinogenesis. However, the mechanism by which FXR suppresses liver cancer remains to be investigated. The fact that FXR is required for both liver regeneration and protection against hepatocarcinogenesis suggests an intrinsic link between liver regeneration and hepatocarcinogenesis. We hypothesize that FXR has dual roles in helping the liver maintain normal homeostasis: one is by controlling the BA level in liver, the other is by promoting liver repair through regeneration (Figure 2). However, the FXR-dependent liver regeneration is to prevent further liver injury and proliferation. Therefore, FXR is rather working as a tumor suppressor. In the absence of FXR, the cycle of injury and compensatory liver regeneration (irregular liver regeneration) provides a tumor-prone environment. This is consistent with a recent report regarding the role of NF-κB in liver injury and hepatocarcinogenesis [70].

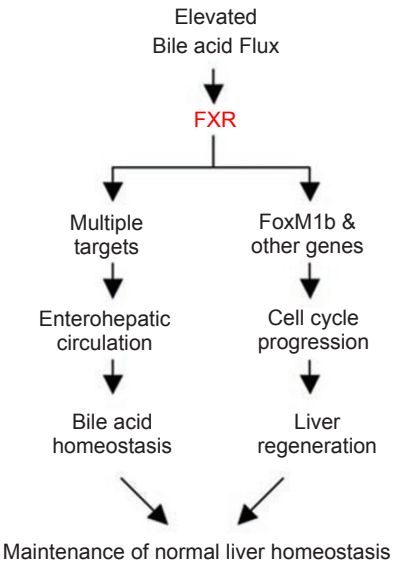

Figure 2 A model of dual effects of FXR function. In response to the increased bile acid flux, FXR regulates genes involved in both bile acid homeostasis and liver regeneration, which helps maintain normal liver homeostasis.

The precise roles of FXR in hepatocarcinogenesis need to be further defined. However, a potential contribution of FXR in tumor suppression may be attributed to its anti-fibrosis function in liver [80, 81]. Chronic liver fibrosis has been linked to hepatocarcinogenesis when it finally develops into cirrhosis. Fiorucci *et al*. [80, 81] used a novel FXR ligand, 6-ECDCA, to demonstrate that activation of FXR in stellate cells inhibited pro-fibrosis gene expression in cooperation with two other NRs, Shp and PPARα.

The roles of FXR in carcinogenesis are not necessarily restricted to liver. FXR is expressed in non-enterohepatic tissues, including at high levels in the kidneys and adrenal gland, which are "non-classic" BA targets [4], and low levels in the heart, vascular tissue, thymus, ovary, spleen, testes, and adipose tissue [5, 82]. The functions of FXR in these non-enterohepatic tissues are poorly understood, particularly in humans. It has been reported that FXR is expressed in human breast cancer tissues and cell lines [61,83]. Breast cancer is epidemiologically linked to high-fat diets and high levels of BAs in the body [84]. BAs are present at high concentrations in the plasma of postmenopausal women with breast cancer and in breast cysts. Swales *et al*. [83] showed that FXR activation by CDCA and GW4064 induced breast cancer cell apoptosis. In contrast, Journe *et al*. [61] showed that the FXR activator, farnesol, induced breast cancer cell growth.

We also found that FXR ligands CDCA and GW4064 induced proliferation of MCF-7 breast cancer cells (unpublished data). Further studies are needed to understand FXR function in breast cancer cell growth and to decipher the roles of FXR in breast cancer development.

Prospects

The function of FXR is expanded rapidly from initial roles in controlling metabolism to regulating cell growth and malignancy. The novel roles of FXR in promoting liver regeneration and protecting against hepatocarcinogenesis, however, are consistent with its previously defined functions in regulating BA metabolism and defending against BA toxicity. Therefore, we conclude that FXR is an important cell protector. We expect that further investigation of FXR function in these new areas will provide novel insights into the complex mechanism of liver regeneration and hepatocarcinogenesis.

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