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Tissue-specific Function of Farnesoid X Receptor in Liver and Intestine

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Abstract

Nuclear receptors (NRs) are ligand-activated transcriptional factors that are involved in various physiological, developmental, and toxicological processes. Farnesoid X receptor (FXR) is a NR that belongs to the NR superfamily. The endogenous ligands of FXR are bile acids. FXR is essential in regulating a network of genes involved in maintaining bile acid and lipid homeostasis. It is clear that FXR is critical for liver and intestinal function. In mice FXR deficiency leads to the development of cholestasis, gallstone disease, nonalcoholic steatohepatitis, liver tumor, and colon tumor. Using mouse models where FXR is deleted either in the whole-body, or selectively in hepatocytes or enterocytes, we start to reveal the importance of tissue-specific FXR function in regulating bile acid and lipid homeostasis. However, a great challenge exists for developing tissue-specific FXR modulators to prevent and treat diseases associated with bile acid or lipid disorders. With further understanding of FXR function in both rodents and humans, this nuclear receptor may emerge as a novel target to prevent and treat liver, gastrointestinal and systemic diseases.

1. Introduction to nuclear receptor superfamily

Nuclear receptors (NRs) are a superfamily of ligand-activated transcription factors that regulate diverse biological functions. The NR superfamily is classified into three groups: the classical endocrine NRs, the orphan nuclear receptors (ONRs), and the adopted ONRs. Farnesoid X receptor (FXR) is an adopted ONR. The adopted ONR family, including the peroxisome proliferators-activated receptors (PPARs), liver X receptor, FXR, pregnane X receptor (PXR), and constitutive androstane receptor (CAR), is essential for the body to respond to endogenous or exogenous nutritional and environmental factors. Because the function of FXR in humans is not clear, the current review will mainly focus on research findings obtained from mice.

2. FXR cloning and structure

The FXR gene (gene symbol: *NR1H4/Nr1h4*) was first cloned from mouse and rat liver by using a degenerative probe corresponding to the highly conserved DNA binding domain of

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the NR superfamily [1,2]. The FXR gene is evolutionally conserved and similarity among different species is high. In drosophila the counterpart of FXR is the ecdysone receptor, EcR [3].

The human *NR1H4* and the murine *Nr1h4* gene are located on chromosome 12 and 10, respectively. Using alternative promoters and splicing, four FXR isoforms in humans and mice have been identified, FXRα1, FXRα2, FXRβ1, and FXRβ2 [4,5]. In C57BL/6J mice, FXRα is highly expressed in liver, kidney, intestine, and adrenal gland (www.nursa.org/10.1621/datasets.02001), and FXRβ is highly expressed in liver and testis (www.nursa.org/10.1621/datasets.02001). In humans, FXRα is highly expressed in adrenal and liver while FXRβ is highly expressed in colon, duodenum and kidney with a much lower expression level in liver [5].

FXR protein shares the common structure with other NR superfamily members (Fig. 1), including the DNA-binding domain in the N-terminal region and the ligand-binding domain in the C-terminal region. The ligand-independent activation function-1 (AF1) domain is located in the N-terminal region and the ligand-dependent activation function-2 domain (AF2) is located in the C-terminal region [6,7].

The most recognized DNA binding sequence by FXR is an inverted repeat separated by one nucleotide (IR1). This FXR response element (FXRE) has been detected in many FXR target genes [1,8–13]. In addition, FXR has been reported to bind to a few other response elements, such as an everted repeat separated by 8 nucleotides (ER8) in the Abcc2 gene [14], but the binding affinity may be lower compared to binding to IR1. Recently, our group has identified a novel FXR motif, ER2, which is present in many FXR binding sites, in addition to IR1 [15]. FXR normally binds to FXRE in gene regulatory regions as a heterodimer with 9-cis-retinoid acid receptor α (RXRα). It is commonly considered that without ligand binding, a co-repressor complex may be associated with the FXR/RXR dimer, which prevents the recruitment of the transcriptional activation machinery to get access to FXR target genes. Upon ligand binding, FXR undergoes conformational changes, which may result in the release of co-repressor(s) and subsequent recruitment of co-activator complex. The best characterized co-activators for the FXR/RXR dimer include steroid receptor coactivator-1, protein arginine(R)methyltransferase-1, PPARγ coactivator-1α and histone acetyl transferase [16,17]. How FXR interacts with co-activators (and co-repressors) and the mechanism by which FXR interacts with co-factors to regulate gene-specific transcription is largely unknown.

3. FXR modulators

3.1. Endogenous ligands of FXR

Bile acids are endogenous ligands of FXR [18–20], although FXR may be activated by high concentrations of farnesol and juvenile hormone III [1]. It is clear that several bile acids activate FXR with high potency, including chenodeoxycholic acid (CDCA) and its conjugates, lithocholic acid (LCA) and its conjugates, deoxycholic acid (DCA) and its conjugates and cholic acid (CA) and its conjugates. Synthesis and excretion of bile acids represent the most important mechanism to remove cholesterol from our body. The field of bile acid research has been greatly advanced by identifying bile acids as signaling molecules that activate a cohort of NRs, including FXR, PXR, CAR and vitamin D receptor [21–24]. Activating these receptors by bile acids collectively maintains bile acid homeostasis by regulating bile acid synthesis, transport and metabolism. In addition to activating NRs, bile acids activate a G-protein coupled membrane receptor, TGR5 [25]. Activation of TGR5 is critical in modulating thyroid hormone function and thermogenesis in the brown adipose

tissue as well as regulating glucagon-like peptide production in the L cells in small intestine to regulate glucose homeostasis [26,27].

3.2. Synthetic FXR ligands

Because of the innate detergent property, bile acids are toxic to cells at higher concentrations. In addition, bile acids activate multiple signaling pathways, thus acting as non-specific FXR ligands. A significant effort has been devoted to develop non-bile acid FXR modulators that may be used to treat bile acid and/or lipid related disorders. Increasing numbers of non-bile acid modulators that activate FXR with high potency have been developed, including GW4064 [28], 6α-ethyl-CDCA [29], fexaramine [30], methyl cholate, methyl deoxycholate, 5β-cholanic acid, 5β-cholanic acid-7α,12α-diol, NIHS700, marchantin A and marchantin E, and MFA-1 [31,32]. In animal studies some of these synthetic FXR modulators have been shown to reduce serum triglycerides [28], prevent cholestasis [33], and suppress liver fibrosis [34–36]. A comprehensive review of synthetic FXR ligands has been recently published for an update status of FXR modulator development [37]. However, without a comprehensive understanding of the biological functions of FXR, the clinical use of FXR full agonists needs to be cautioned because FXR full agonists may result in undesired toxicities. Based on prior experience and success in developing selective estrogen and androgen receptor modulators, it is reasonable to predict that selective FXR modulators will be more useful in the clinic. Indeed, effort has been spent in identifying gene-selective FXR modulators [38], however their pharmaceutical development and clinical use are not clear.

Research and development on FXR antagonists is limited. A main ingredient extracted from resins of the guggle tree, guggulsterone, has been identified as a potent FXR antagonist [39]. Further studies showed that the action of guggulsterone is not specific. In addition to antagonizing FXR, this guggle tree extract also acts on several steroid hormone receptors, including the mineralocorticoid receptor, the androgen receptor, as well as the glucocorticoid receptor [40].

4. Tissue-specific function of FXR in regulating bile acid, cholesterol, and triglyceride homeostasis

4.1. Synthesis and enterohepatic circulation of bile acids

The maintenance of cholesterol homeostasis is vital to survival and development. Disruption of cholesterol homeostasis results in serious cardiovascular and neurological disorders. The most important route in the body for cholesterol elimination is the conversion of cholesterol into bile acids in the liver [41]. Two pathways are responsible for bile acid synthesis in the liver: the neutral or classical pathway and the acidic or alternative pathway. Cholesterol-7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in the classic pathway and sterol 27-hydroxylase (CYP27A1) is the rate-limiting enzyme in the alternative pathway. The classical pathway generates both CA and CDCA, collectively called primary bile acids. The acidic pathway mainly synthesizes CDCA. Primary bile acids are conjugated to glycine or taurine before their excretion into the bile mediated by two canalicular transporters, bile salt efflux pump (Bsep) and multi-drug resistance-associated protein 2 (Mrp2) [42,43]. Bile is released into the small intestine upon food consumption. In the ileum, after facilitating lipid and lipid-soluble vitamin absorption, most of the primary bile acids are absorbed from intestinal lumen into enterocytes by an intestinal bile acid uptake transporter (Asbt) [44]. In ileal enterocytes bile acids are presumably bound to intestinal bile acid binding proteins (Ibabp) before being excreted into the portal vein from the basolateral side of enterocytes by the organic solute transporter (Ost) α and β heterodimer [45]. Through the portal circulation, bile acids reach the liver and are taken up mainly by the Na^+/taurocholate cotransporting

polypeptide (Ntcp) [46]. Most of the primary bile acids undergo continuous enterohepatic circulation and only 5% enter the colon where conversion from primary to secondary bile acids occurs by deamination and 7α-dehydroxylation by the intestinal bacteria with CA converted to DCA and CDCA to LCA. Under physiological conditions, DCA enters the enterohepatic circulation and LCA is mainly excreted from the feces.

4.2. Intestinal FXR is mainly responsible for FXR-mediated suppression of bile acid synthesis under physiological condition

Bile acids have detergent-like properties thus their synthesis is highly regulated. Feedback suppression of bile acid synthesis by bile acids is one of the major mechanisms in maintaining bile acid homeostasis. In ileum, where FXR is highly expressed, bile acids activate FXR to induce a growth factor-like peptide in the intestine, fibroblast growth factor 15 (Fgf15) in mice and FGF19 in humans [10,11]. Fgf15 is secreted from the enterocytes and travels to the liver, presumably via the portal circulation. In liver Fgf15 binds and activates its membrane receptor, FGFR4, which possesses endogenous tyrosin kinase activity. Activation of FGFR4 leads to activation of a cascade of signaling molecules, including cJun and ERK, which quickly turn off Cyp7a1 gene transcription [10,47,48]. The detailed signaling pathways in hepatocytes after FGFR4 activation still remain not clear. While FXR negatively regulates bile acid synthesis, this nuclear receptor induces bile acid efflux transporters, including Bsep and Ostα/β heterodimer, in both liver and intestine. This upregulation is believed to promote the enterohepatic bile acid circulation [49,50]. Under physiological condition, activation of intestinal FXR may be the major mechanism to suppress Cyp7a1 gene transcription by bile acids (Fig. 2).

4.3. FXR-mediated regulation of bile acid synthesis and transport under cholestatic condition

During cholestasis, bile flow is impaired and bile acids accumulate in the liver. In this situation, hepatic FXR plays a partial role in suppressing bile acid synthesis, likely via inducing an ONR in the liver, small heterodimer partner (SHP). SHP binds to liver receptor homologue 1 (LRH-1) to inhibit Cyp7a1 gene transcription [12,13]. In fact, suppression of bile acid synthesis during cholestasis may be mainly achieved by NR-independent signaling pathways, such as inflammatory cytokines, activated by higher concentrations of bile acids [41]. Although partially responsible for suppressing bile acid synthesis, activation of FXR during cholestasis may protect the liver by reducing hepatic bile acid levels via a direct induction of the gene expression of Bsep, the bile acid efflux transporter to promote biliary efflux, as well as via inhibition of the gene expression of Ntcp through the FXR-SHP-LRH-1 cascade to prevent bile acid uptake into the liver [51]. Ntcp is the major bile acid uptake transporter expressed in the hepatic sinusoids and is responsible for taking up the majority of conjugated bile acids into the liver [46]. Therefore the overall effect of FXR activation during cholestasis is to protect the liver from overt bile acid accumulation (Fig. 2). Toxic concentrations of bile acids in the liver also activate PXR and CAR to promote bile acid detoxification and sinusoidal efflux [21,24]. The use of non-bile acid FXR activators may be useful in the future to treat intrahepatic cholestasis to suppress bile acid synthesis and to promote bile acid efflux from hepatocytes. However, for extrahepatic cholestasis where the bile ducts are blocked, the use of FXR activators may be detrimental because increased bile acid release into a blocked bile duct will result in more damage. In agreement with this concept, FXR deficiency results in less severe hepatic injury following common bile-duct ligation [52]. Overall, activation of FXR serves two functions in regulating bile acids: to maintain bile acid homeostasis under physiological condition and to protect the liver from overt bile acid toxicity during cholestasis.

4.4. FXR in lipid metabolism

Deficiency of FXR in mice highly indicates that an important function of FXR is to maintain cholesterol and triglyceride homeostasis [53]. Although deficiency of FXR increases bile acid synthesis, which in turn should have removed more cholesterol from the body, FXR deficiency leads to increased serum levels of VLDL, LDL, and HDL, as well as triglycerides. The increased serum cholesterol levels in FXR knockout mice indicate FXR regulates cholesterol homeostasis in addition to regulating the conversion of cholesterol to bile acids [53,54]. It has been reported that FXR promotes reverse transport of cholesterol by increasing hepatic uptake of HDL-cholesterol via two independent mechanisms. The first is FXR-mediated suppression of hepatic lipase expression [55]. Hepatic lipase reduces HDL particle size by hydrolyzing its triglycerides and phospholipids in hepatic sinusoids, which facilitates hepatic uptake of HDL-cholesterol. The second mechanism is that FXR has been shown to induce the gene expression of scavenger receptor B1, the HDL uptake transporter in the liver [54]. Therefore one of the mechanisms by which FXR deficiency leads to increased serum cholesterol levels may be to decrease reverse cholesterol transport to the liver.

Bile acids are essential in facilitating intestinal absorption of lipid and lipid-soluble vitamins but the role of FXR in intestinal absorption of lipid remains unknown. However, it is known that activation of FXR reduces liver *de novo* fatty acid synthesis and VLDL transport. Studies have shown that FXR suppresses sterol regulatory element-binding protein-1c (SREBP-1c) and microsomal triglyceride transfer protein (MTP) via a SHP-mediated inhibition of co-activator recruitment to the SREBP1c and MTP promoters [56,57]. SREBP-1c is a central transcription factor in activating hepatic *de novo* fatty acid synthesis. MTP is a microsomal transport protein facilitating VLDL efflux from the liver. Furthermore, bile acids induce PPARα in humans via a FXR-dependent mechanism [58]. PPARα activation reduces lipid levels by increasing fatty acid oxidation. In addition, FXR activation induces the expression of apoCII [59], but inhibits the expression of apoCIII [60]. ApoCII is a co-activator for lipoprotein lipase and promotes lipoprotein lipase-mediated hydrolysis of triglycerides. On contrary, apoCIII is a co-inhibitor for lipoprotein lipase. Therefore FXR activation results in lowering circulating triglycerides by reducing hepatic fatty acid synthesis and transport as well as enhancing oxidation, meanwhile FXR activation may result in promoting VLDL and chylomicron triglyceride hydrolysis in the circulation.

5. Implication of FXR in hepatic, gastrointestinal, and systemic diseases

As mentioned above, FXR is clearly important in the liver and intestine to maintain bile acid, cholesterol and triglyceride homeostasis. Studies in experimental animals, especially using genetically modified mice, demonstrate that dysfunction of FXR is implicated in several hepatic, gastrointestinal and systemic abnormalities, including gallstone disease, nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), liver fibrosis, liver regeneration, liver tumors, intestinal tumors, and atherosclerosis.

5.1. Functional FXR prevents gallstone formation

By quantitative trait locus analysis the *Nr1h4* gene has been identified as one of the litho-related genes contributing to gallstone formation [61]. In mice, activation of FXR has been shown to prevent gallstone formation by increasing the bile acid to cholesterol ratio in bile [62]. However, in humans polymorphism of the *NR1H4* gene has not been linked to increased gallstone disease incidence [63]. One reason would be that humans have different ethnic background, which may lead to more complicated interaction with other risk factors for gallstone formation.

5.2. FXR is implicated in NAFLD and NASH development

NAFLD is a well recognized component of the metabolic syndrome, characterized by increased serum levels of lipids and glucose, increased incidence of type II diabetes, atherosclerosis, hypertension, and breast and colon cancer. Although many NAFLD cases have benign prognosis, some develop NASH, liver fibrosis, cirrhosis, and tumor. The disruption of the *Nr1h4* gene in mice showed that FXR deficiency results in fatty liver formation following feeding with a high-cholesterol diet [53]. In addition, FXR deficiency renders the mice more susceptible to NASH formation in a diet-induced obese mouse model [64]. The exact mechanism by which FXR deficiency enhances NAFLD to NASH transition is not clear, but likely involves a FXR-dependent disruption of lipid and bile acid homeostasis, which leads to lipid accumulation and bile acid-induced chronic injury in the liver. FXR deficiency also results in increased collagen expression [64], and increased collagen expression is an early event in liver fibrosis development. In agreement, activation of FXR has been shown to suppress liver fibrosis development [36]. Advanced liver fibrosis leads to cirrhosis, portal hypertension and liver failure. The treatment of choice is liver transplantation because no effective pharmaceutical agents are available to halt or reverse liver fibrosis. Liver fibrosis is associated with activation of hepatic stellate cells. FXR is expressed in stellate cells and activation of FXR interacts with the PPARγ to suppress stellate cell activation, which collectively reduces the expression of pro-fibrotic genes, including transforming growth factor β1 and αI collagen [34].

5.3. FXR has been implicated in hepatic and gastrointestinal cancer development

FXR deficiency in mice leads to spontaneous cancer development in the liver, including hepatocellular adenoma, carcinoma and hepatocholangiocellular carcinoma [65,66]. Moreover, in contrast to other liver tumor models where the male gender is mainly affected, 100% of both male and female FXR knockout mice develop liver tumors, indicating FXR deficiency disrupts estrogen-protected pathway(s) in the liver to promote tumor development. Studies have shown that a gender specific expression of IL-6 in the liver determines female-specific resistance to hepatocarcinoma development [67]. Both FXR deficient male and female mice express similar amount of IL-6 mRNA in the liver (unpublished data), whereas male wild-type mice have more IL-6 than female wild-type mice. These data indicate that FXR may regulate IL-6 expression and thus subsequently affecting gender-dependent development of liver tumor.

FXR deficiency also enhances intestinal tumor development in mice. Using both adenomatosis polyposis coli multiple intestinal neoplasia mice (APC^{min} mice), and mice treated with azoxymethane, a well-known colon carcinogen, FXR deficiency increased intestinal tumor incidence and tumor size [68,69]. The mechanism by which FXR reduces tumorigenesis has been investigated and it is likely that activated FXR mediates both a pro-apoptotic as well as anti-inflammatory effects to suppress tumor development [69,70]. In humans, FXR expression is associated with colon cancer development. The expression of intestinal FXR and its target genes are decreased with the progression of colon cancer as well as with increased malignancy of colon cancer cell lines [71]. This association indicates FXR may play a role in colon cancer development or at least the loss of FXR expression may be used as a marker for colon cancer formation and the degree of malignancy.

5.4. FXR in atherosclerosis

FXR regulates lipid homeostasis and deficiency of FXR in mice increases systemic and liver lipid levels. However, FXR deficiency has been shown to increase atherosclerotic plaque formation in male ApoE knockout mice but protect female ApoE mice from atherosclerotic plaque formation [72–74]. The reduction of atherosclerotic plaque in the aorta area of female mice may be due to a decreased CD36 expression and foam cell formation. CD36 is

a long-chain fatty acid transporter and is mainly responsible for taking up oxidized LDL into macrophages. Lipid-laden macrophages become foam cells, the hall mark for atherosclerosis plaque development. This gender difference in the role of FXR in atherosclerosis development indicates again that FXR may interact with estrogen-related pathway(s) to modulate biological responses.

6. Novel genome-wide research tools for cutting-edge FXR research

FXR is a transcription factor that binds to DNA, likely[75], in the entire genome. In the past, we have made substantial advances in indentifying FXR target genes and regulated pathways. However the complicated mechanisms by which FXR and its target gene products both regulate transcription make it difficult to identify direct target genes, especially at the genomic level. The emerging cutting-edge technology, chromatin immunoprecipitation coupled with deep sequencing (ChIP-seq), is a non-biased method to identify genomic regions bound by transcription factors [76]. We and others have recently published FXR ChIP-seq analysis [15,77]. These ChIP-seq studies show that FXR binds to broader genomic regions, promoters, introns, exons and intergenic regions. In addition, there is only 11% FXR binding sites overlap between liver and intestine, indicating epigenetic mechanism that may be responsible for tissue-specific FXR binding. The novel ChIP-seq data allow us to discover that binding of FXR to both upstream and downstream gene regulatory regions forms head-to-tail chromatin looping to increase the efficiency in gene transcriptional activation [78]. Motif analysis of FXR binding site indicates pioneer transcription factors may facilitate FXR-mediated gene transcriptional activation. Binding of a pioneer transcription factor to DNA helps to remodel chromatin structures and convert heterochromatin to euchromatin, to which other transcription factors will bind. The important roles of pioneer factors in facilitating estrogen receptor binding to DNA have been recently documented [79,80]. In agreement, Chong et al. showed that LRH-1 binds adjacently to FXRE and enhances FXR-mediated transcriptional activation [77]. Using the cutting edge technology we have provided an unbiased and comprehensive analysis of potential FXR target genes, which may be used as the basis to discover novel biology pathways regulated by FXR.

7. Future directions for FXR research

The last decade has just turned the page for an in-depth research on nutrition-related NRs, including FXR. The intensive research on FXR regulated biological pathways made breakthroughs in understanding bile acid, liver, and intestinal biology. It is likely that FXR is involved in regulating diverse biological functions to affect hepatic, gastrointestinal and systemic homeostasis. It is clear that there are multiple layers of regulation for FXR function. Besides regulating FXR ligand availability and concentration, the regulation of FXR expression and function also likely leads to more insight into bile acid homeostasis and pathophysiology of diseases associated with bile acid and lipid abnormalities [81]. In addition, the interaction of FXR with other components of the transcriptional machinery at the chromatin level will likely add profound understanding of FXR target gene regulation. With an in-depth understanding of FXR function and regulation at the cell-, gene- and tissue-specific levels, we will have more tools and be more confident in designing FXR modulators in the future to prevent and/or treat bile acid and lipid related abnormalities in humans.

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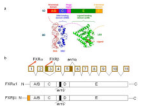

Fig 1. Gene and protein structure of FXR

(a) General structure of nuclear receptors (NRs). A typical NR contains several domains. The N-terminal region (A/B) contains the ligand-independent AF1 transactivation domain. The C region contains the conserved DNA binding domain. A hinge region (D) connects the DNA- and ligand-binding domain. The E region contains the ligand-binding domain. (b) Schematic diagram of the murine *Nr1h4* genomic structure. The mouse *Nr1h4* gene is composed of 11 exons and 10 introns. FXRα and FXRβ are transcribed from exon 1 and 3, respectively. A 12 base-pair (bp) insert is located at the 3′ end of exon 5. Alternative splicing from exons 5 and 6 produces two FXR isoforms that either contain the 12-bp insert or not. FXRα and FXRβ have the same amino acid sequence, except for an additional 37 amino acid at the N-terminal of FXRβ. FXRα1 and FXRβ1 have a 4 amino acid insert (MYTG) that is located at the hinge region.

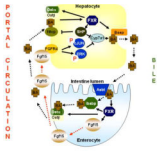

Fig 2. Regulation of bile acid homeostasis by FXR

Under physiological conditions, bile acids in the intestine activate FXR that induces Fgf15, which reaches hepatocytes and activates FGFR4. Activation of FGFR4 leads to activation of signaling cascades, including cJUN and ERK, which collectively turn off the transcription of the Cyp7a1 gene. FXR not only suppresses bile acid-mediated feedback regulation of Cyp7a1, but also induces bile acid transport in both the liver and intestine. In the liver, FXR induces the expression of Bsep and in the intestine FXR induces the expression of Ibabp and Ostα/β dimer, to promote enterohepatic bile acid circulation. Under cholestatic conditions, increased bile acids in the liver activate FXR as well as other signaling pathways. Activated FXR induces SHP, which inhibits the transcription of Cyp7a1 and Ntcp. Meanwhile, FXR induces Bsep and Ostα/β dimer in the liver to promote hepatic efflux of bile acids into the bile and blood, respectively.