The Role of FXR in Disorders of Bile Acid Homeostasis

As ligands for the nuclear receptor FXR, bile acids regulate their own synthesis, transport, and conjugation, thus protecting against bile acid toxicity. Recently, the role of genetic variants in FXR itself, FXR target genes, and regulators of FXR in the pathophysiology of the liver and intestine has become increasingly evident.

Members of the nuclear receptor superfamily of transcription factors are the chief regulators of a wide variety of important metabolic pathways (28). Their ability to sense and respond to changes in intracellular metabolic environment is largely due to the fact that the transcriptional potential of most nuclear receptors is crucially dependent on small lipophilic ligands, such as bile acids, fatty acids, lipophilic vitamins, and steroidal hormones. Binding of agonistic or antagonistic ligands leads to allosteric changes in the ligand-binding domains of nuclear receptors, thus resulting in alterations in the interactions of nuclear receptors with their coactivators and corepressors, and consequently affecting transcriptional rates of target genes (89). Most nuclear receptors bind to their DNA response elements as either hetero- or homodimers, and their consensus DNA-binding motifs typically contain two hexameric half sites. These hexameric motifs, the consensus sequence for which is AGGTCA, can be arranged as direct (DR), inverted (IR), or everted (ER) repeats, separated by a variable and receptor-specific number of base pairs.

The suggested role of altered nuclear receptor activity in several metabolic diseases, together with the potential of modulating their activity with specific ligands, has made them attractive targets for pharmacological intervention in these diseases (29). Given that the majority of nuclear receptors are involved in regulating a variety of metabolic processes, and accordingly have logical intervention in these diseases (29). Given that the majority of nuclear receptors can interact with their DNA-binding sequences to modulate transcriptional activity, they are ideal targets for pharmacological manipulation.

The Nuclear Receptor for Bile Acids, FXR

The farnesoid X receptor (FXR), also known as the bile acid receptor or BAR, gene symbol NR1H4 is a member of the nuclear receptor family of transcription factors. FXR functions as the chief sensor of intracellular levels of bile acids (the end products of cholesterol catabolism) and the main executor of bile acid-induced transcriptional programmes. Bile acids directly interact with the ligand-binding domain of FXR and enhance or antagonize the transactivation function of FXR. In accordance with its function as the bile acid receptor, FXR is most abundantly expressed in the tissues commonly exposed to bile acids in normal physiology: liver, intestine, and kidneys (25). Along the intestinal tract, higher FXR levels can be found in the ileal epithelium, the main site of intestinal bile acid absorption, than in the epithelium of proximal small intestine or the colon (39). The preferred DNA-binding sequence for FXR within its target promoters is typically a variant of the so-called "inverted repeat-1" motif (IR-1; inverted hexameric AGGTCA-like repeat separated by one base pair) (57), to which FXR binds as a heterodimer with the nuclear retinoid X receptor (RXR). Although in lower mammals there are two FXR genes, Nr1h4 (encoding FxrH9252) and Nr1h5 (encoding Pxr), the latter gene product employing lanosterols as its ligands, in humans and other primates the homolog of Pxr is a pseudogene (76). Thus, in this review, focusing on the properties and function of this nuclear receptor in humans, we use the phrase FXR/Fxr to refer to FXR/Nr1h4.

Four isoforms of FXR, termed FXR1–4, can be translated from the single NR1H4 gene in humans, generated by both alternative promoter usage and by alternative splicing (37, 109). FXR1 and FXR2, the most abundant FXR isoforms in the human liver, differ from FXR3 and FXR4 at the NH2 terminus, since the mRNAs for these two isoform pairs are transcribed by separate promoters. In addition, the isoforms FXR3 and FXR4 contain an additional stretch of four amino acids, MYTC, due to a differential splicing event at the end of exon 5. All four FXR isoforms harbor identical ligand-binding domains but may exhibit different coactivator recruitment, DNA-binding, or RXR heterodimerization properties. In the context of at least a subset of target genes, the FXR isoforms appear to exhibit differential DNA-binding and transactivation properties (4, 109), although in the context of most FXR target genes this remains to be comprehensively studied.

FXR functions typically as an agonist-dependent transcriptional activator of its direct target genes. Numerous transcriptional coactivators recruited to agonist-bound FXR, mediating its transcriptional function, have been proposed (reviewed in Ref. 20). FXR can also negatively regulate transcription of specific target genes in an agonist-dependent manner. Although there is evidence from the studies on the human apolipoprotein B promoter that binding by FXR can act in concert with the LXR promoter to inhibit transcription (86). The SHP mutant of FXR inactivates this effect by altering the typical nuclear receptor activator function of the receptor family belonging to the nuclear receptor superfamily (20). SHP may also modulate transcriptional activity of other nuclear receptors acting as transcriptional coactivators or corepressors (50). The consequences of the FXR SHP mutation in the context of FXR dependent transcriptional activity are currently unknown.

The first FXR ligand identified, human primary bile acids, was recently shown to be such FXR ligands that act as potent FXR agonists (62, 66, 79). Identification of human primary bile acids directly leads to enhanced FXR transcriptional activity, leading to enhanced bile acid excretion (66). The first FXR antagonist identified, chenodeoxycholic acid (CDCA), is a potent FXR antagonist (62, 66, 79). FXR antagonists have been used therapeutically to enhance bile acid excretion and expression of ileal bile acid transporters (31).

Since the identification of several other FXR ligands (94), other compounds have been shown to act as FXR activators or agonists, such as atypical nuclear receptors or other nuclear receptors in the nuclear receptor superfamily. The identification of FXR agonists and antagonists has driven the development of FXR-targeted therapeutics for the treatment of diseases associated with bile acid homeostasis, including chronic liver disease, obesity, diabetes, and cancer.

Jyrki J. Eloranta and Gerd A. Kulak-Ulblick
Division of Clinical Pharmacology and Toxicology, University Hospital Zurich, Zurich, Switzerland
jyrki.eloranta@usz.ch
human apolipoprotein A-I and apolipoprotein C-III promoters that may be achieved through direct binding by FXR to negative bile acid response elements (11, 13), more often FXR downregulates target genes via an indirect mechanism involving another nuclear receptor, small heterodimer partner (SHP) (88). The SHP (NR2BE2) gene is directly transactivated by FXR in response to FXR ligands and encodes an atypical nuclear receptor, lacking the DNA-binding domain. However, SHP does contain the dimerization domain and a putative ligand-binding domain. SHP can interact with, and negatively affect, the transcriptional activity of several other members of the nuclear receptor family, as well as transcription factors belonging to other protein families (reviewed in Ref. 20). SHP may achieve this by either blocking access of a transcriptional coactivator to the DNA-binding transactivator (42, 60, 61), or actively recruiting transcriptional corepressor complexes to the target promoters (50, 61), or both. No endogenous or exogenous ligands for SHP have been confirmed to exist. However, a synthetic retinoid termed CD437/ADPHN was recently shown to both directly interact with SHP and enhance the recruitment of a corepressor complex (21), although it is not yet clear whether the interaction with this compound is in fact mediated by the putative ligand-binding pocket of SHP.

FXR Ligands: Bile Acids and Beyond

Nuclear receptors, for which no ligands have yet been identified, have been termed "orphan nuclear receptors." The first attempt to rescue FXR from the orphanage (i.e., to find its ligand) was made in 1995, when farnesol metabolites were suggested as candidates to be such FXR ligands; however, these compounds only activate FXR at concentrations clearly exceeding physiological conditions and do not appear to act as bona fide direct ligands for FXR (25, 107). FXR was finally identified, have been termed "orphan nuclear receptors." The preferred FXR isoforms, FXRα and FXRβ, are encoded by two closely related genes belonging to two protein families (reviewed in Ref. 57), to which FXR fits. FXRα is expressed in the liver and intestine, the renal cortex, and the brain, while FXRβ is expressed in the liver and intestine, the heart, and the brain. Both isoforms lack a canonical N-terminal stretch of potential splice variants encoding isoforms harboring long introns may exhibit greater differences in expression and function in the context of endogenous bile acids than those occurring in the two species.

For FXR, the ligands of choice are bile acids (9, 14), it has been reported to be more sensitive to activation by FXR than the mouse Fxr (101). It was suggested that androsterone induces an overlapping but distinct subset of FXR target genes from CDCA, indicating ligand-dependent target gene selectivity as previously shown in a study employing synthetic FXR ligands (18).

In addition to endogenous molecules, recent reports have suggested that compounds present in our dietary intake may act as FXR ligands. For example, a soy lipid-derivatized phytosterol, stigmastanol, has been reported to function as an antagonist of FXR activity, possibly contributing to the cholestatic phenotype associated with neonatal parenteral nutrition employing soy-derivatized lipid emulsions (89). Additionally, a diterpene compound found in coffee beans and present in unfiltered coffee brews, called cafestol, was found to function as an agonistic FXR ligand (84). Previously, cafestol had been shown to be responsible for hypercholesterolemia and increased risk of coronary heart disease associated with high intake of unfiltered coffee (92, 93, 102), and it is possible that this association is dependent on the ability of cafestol to activate FXR. For example, application of cafestol decreased the expression of the rate-limiting enzyme of cholesterol to bile acids and thus impaired elimination of cholesterol from the body.

Besides naturally existing FXR ligands, an intense interest in designing pharmacologically effective synthetic FXR agonists and antagonists has developed over the last few years. In rodent models, some of these have already shown promising hepatoprotective qualities in rat models, the synthetic agonist GW4064 (67) provides hepatoprotection against intra- and extrahepatic cholestasis (64) and a semi-synthetic bile acid derivative 6-ethyl chenodeoxycholic acid (6-ECDC) protects against liver fibrosis (24). When evaluating the therapeutic efficiency of FXR agonists in rodent models, one should bear in mind that there appears to be a certain degree of species dependence in the ligand-specificity of FXR/Fxr: for example, although the mouse Fxr is less responsive to CDCA than the human FXR ortholog (14), it has been reported to be more sensitive to activation by androsterone than the human variant (101). Also, concerning experimental rat models, it is worth noting that, unlike humans, rats do not have a gall bladder, and certain regulatory aspects of bile acid homeostasis may thus be fundamentally different in the two species.
Therapeutic usage of cholesterolic FXR ligands that leads to increased bile flow via the regulatory mechanisms described below may not be advantageous in cholestatic diseases that frequently have an obstructive component: in a mouse study, increased bile flow and biliary pressure in fact aggravated bile inapproaches in mice suffering from obstructive cholestasis upon bile duct ligation (23).

**FXR Controls and Fine-Tunes Bile Acid Homeostasis**

Bile acids are the end products of hepatic cholesterol catabolism, thus providing a major pathway of excess cholesterol elimination from the body. Approximately 500 mg of bile acids are synthesized every day in the adult human liver. Enterohepatic cycling of bile acids, mediated by plasma membrane transporters expressed in hepatocytes and enterocytes in a polarized manner, is highly efficient in healthy individuals, with approximately only 5% escaping ileal reabsorption and being lost into feces. Functioning as physiological deterrents, bile acids promote absorption of lipophilic nutrients and vitamins in the intestine. Furthermore, bile acids contribute to the solubilization of cholesterol in bile, thus protecting against precipitation of cholesterol crystals and preventing the formation of cholesterol gallstones. Despite these crucially important roles in normal physiology, elevated levels of bile acids, such as observed in cholestatic disease, can be cytotoxic due to their deterrent properties. To avoid cellular damage, bile acids function as homeostatic regulators and signaling molecules to adjust their own intracellular levels. Bile acids exert their regulatory effects chiefly by acting as FXR ligands, although they do also elicit other signaling pathways, such as those involving a G-protein-coupled receptor TRGS (48) and the c-Jun NH2-terminal kinase (JNK) (30), which are likely to contribute to the protection against bile acid-induced damage. It is interesting to note that different bile acids have differing affinities to FXR and also been suggested to be bile acid-responsive genes are induced by transactivation of FXR. BSEP, the major biliary canalicular bile salt export pump of the ATP-binding cassette (ABC) transporter family (90), is a target for direct transactivation by FXR (28, 81, 87) [FIGURE 1; Table 1]. Thus, in conditions of increased bile acid load in hepatocytes, bile acids enhance their own efflux into bile by activating FXR and consequently increasing BSEP expression. Although BSEP is responsible for the efflux of innocuous bile acids from hepatocytes into bile, the multidrug resistance-associated protein 2 (MRP2, ABCG2) contributes to the overall canalicular bile acid efflux by exporting divalent and sulphated or glucuronidated bile acids into bile (reviewed in Ref. 53). Both the human and rodent ABCG2/Abcc2 promoters can be activated by FXR in the presence of bile acids (47). Yet another ABC transporter gene that is transactivated by FXR is ABCB4, encoding MDR3 (35), which is thought to be a flavoprotein oxidase within the canalicular membrane of hepatocytes. Via induction of ABCB4 expression, FXR enhances the function of MDR3 in counteracting the toxicity of biliary bile acids by promoting formation of mixed micelles that contain cholesterol, bile acids, and phospholipids in bile canaliculi. The SLC10A3 gene encoding a member of the organic anion transporting polypeptide (OATP) family, OATP1B3, is directly transactivated by FXR in a ligand-dependent manner through an IR-1 element (43). OATP1B3 is an uptake system at the sinusoidal membrane of hepatocytes for numerous drugs and peptides, such as digoxin and cholecystokinin (40, 55). OATP1B3 may also transport bile acids in a sodium-independent manner (31), although the extent of its contribution to overall bile acid transport into hepatocytes has not been established. As discussed below, the activity of another liver-specific basolateral uptake system with an overlapping substrate specificity, OATCP, is suppressed by FXR-dependent pathways in cholestatic, bile acid-enriched environment (44). Induction of OATP1B3 expression by FXR may serve to maintain sufficient hepatic extraction of organic solutes and xenobiotics in conditions that lead to decreased expression of other basolateral drug uptake transporters, such as OATP1B1 (56).

Table 1. Hepatic genes discussed in the review, the FXR-dependence of their expression, and their functions

<table>
<thead>
<tr>
<th>Gene</th>
<th>FXR Target</th>
<th>Known or Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP</td>
<td>+</td>
<td>Major canalicular bile acid efflux system</td>
</tr>
<tr>
<td>NTCP</td>
<td>-</td>
<td>Major sinusoidal bile acid uptake system</td>
</tr>
<tr>
<td>MDR3</td>
<td>+</td>
<td>Canicular phospholipid efflux system</td>
</tr>
<tr>
<td>MRP2</td>
<td>+</td>
<td>Canicular drug and bile acid efflux system</td>
</tr>
<tr>
<td>OATP1B1</td>
<td>-</td>
<td>Sinusoidal drug and bile acid uptake system</td>
</tr>
<tr>
<td>OATP1B3</td>
<td>+</td>
<td>Sinusoidal drug and peptide uptake system</td>
</tr>
<tr>
<td>OSTA/OSTj</td>
<td>-</td>
<td>Sinusoidal alternative bile acid efflux system</td>
</tr>
<tr>
<td>CYP7A1</td>
<td>+</td>
<td>Bile acid synthesis via cholesterol catabolism</td>
</tr>
<tr>
<td>CYP8B1</td>
<td>-</td>
<td>Bile acid synthesis via cholesterol catabolism</td>
</tr>
<tr>
<td>UGT2B4</td>
<td>+</td>
<td>Glucuronidation of bile acids</td>
</tr>
</tbody>
</table>

For details and references to literature, please see the main text. +, induced by FXR; -, suppressed by FXR.
the divergent
pathways.
A recent review has detailed the potential for bile acid receptors to affect the expression of bile acid transporters. These have been shown to be involved in the expression of both bile salt export pump (BSEP) and sodium taurocholate cotransporting polypeptide (NTCP), which are both involved in the uptake of bile acids. BSEP is responsible for the efflux of bile acids from the liver, while NTCP is the major transporter of bile acids into the liver.

FXR negatively regulates bile acid uptake systems and bile acid synthesis
The Na+-taurocholate cotransporting polypeptide (NTCP) is the predominant transporter responsible for bile acid uptake from portal blood across the basolateral membrane of hepatocytes. In rodent models of cholestasis, expression of the Ntcp mRNA and protein is notably decreased (22, 27, 112). Thus, in addition to enhancing bile acid efflux by treatment of cultured cells with bile acids (75), two different pathways have been proposed for this phenomenon: it has been suggested that SHP interferes with the retinoic acid receptor (RAR)-RXR-dependent transactivation (75) of the NTCP promoter (19). Given that the FXR-SHP pathway also negatively targets GR on the human NTCP promoter, the latter

The organic anion transporter polypeptide OATP1B1, which is a Na+-independent bile salt uptake system at the basolateral hepatocyte membrane in humans, can function as a sodium-dependent bile acid transporter (ASBT) belongs to the same family of transporter proteins as NTCP, and it is also expressed in the ileal epithelium, the major site of intestinal bile acid absorption. The ASBT gene is also suppressed by treatment of cultured cells with bile acids (75). Two molecular mechanisms, potentially operational in parallel, have been proposed for this phenomenon: it has been suggested that SHP interferes with the RAR-RXR-dependent transactivation (75) or GR-dependent transactivation (19) of the human ASBT promoter. Given that the FXR-SHP pathway also negatively targets GR on the human NTCP promoter, the latter

The authors of the review also noted that bile acids, through their interaction with FXR, can also affect the expression of other genes involved in bile acid metabolism, including those encoding enzymes involved in the synthesis of bile acids and those involved in the conjugation of bile acids. FXR is known to transactivate genes encoding enzymes that can metabolize and thus detoxify bile acids. One such FXR target is the gene encoding the human uridine 5'-diphosphate-glucuronosyltransferase 2B4 (UGT2B4), which converts hydrophobic bile acids to more hydrophilic glucuronide derivatives via an increase in the negative charge of a bile acid molecule (4). Expression of the human UGT2B4 gene is suppressed by FXR-induced SHP, strongly suppressing enzyme activities in ileal epithelium, the major site of intestinal bile acid absorption. The ASBT gene is also suppressed by treatment of cultured cells with bile acids (75).

The authors then went on to discuss the potential for bile acid receptors to affect the expression of bile acid transporters. These have been shown to be involved in the expression of both BSEP and NTCP, which are both involved in the uptake of bile acids. BSEP is responsible for the efflux of bile acids from the liver, while NTCP is the major transporter of bile acids into the liver. The authors noted that there is evidence to suggest that bile acid receptors can affect the expression of these transporters. For example, the authors noted that bile acids, through their interaction with FXR, can also affect the expression of other genes involved in bile acid metabolism, including those encoding enzymes involved in the synthesis of bile acids and those involved in the conjugation of bile acids. FXR is known to transactivate genes encoding enzymes that can metabolize and thus detoxify bile acids. One such FXR target is the gene encoding the human uridine 5'-diphosphate-glucuronosyltransferase 2B4 (UGT2B4), which converts hydrophobic bile acids to more hydrophilic glucuronide derivatives via an increase in the negative charge of a bile acid molecule (4). Expression of the human UGT2B4 gene is suppressed by FXR-induced SHP, strongly suppressing enzyme activities in ileal epithelium, the major site of intestinal bile acid absorption. The ASBT gene is also suppressed by treatment of cultured cells with bile acids (75). Two molecular mechanisms, potentially operational in parallel, have been proposed for this phenomenon: it has been suggested that SHP interferes with the RAR-RXR-dependent transactivation (75) or GR-dependent transactivation (19) of the human ASBT promoter. Given that the FXR-SHP pathway also negatively targets GR on the human NTCP promoter, the latter

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FGF19/Fgf15 pathway of repression of bile acid synthesis, since the effect seems clearly attenuated in Shp-deficient mice (38). The FGF19/Fgf15-mediated endocrine-type loop may explain the previous observations that intestinal administration of bile acids leads to decreased hepatic CYP7A1 expression in rats, whereas intravenous or portal administration does not (73, 78).

Genetic Variation in the FXR Gene in Liver and Biliary Diseases

Genetic mutations and polymorphisms in several members of the nuclear receptor family, such as peroxisome proliferator-activated receptors-α and γ (PPARα, PPARγ, Refs. 76, 91), vitamin D receptor (VDR, Ref. 95), and hepatocyte nuclear factor-4α (HNF-4α, Ref. 105) have been associated with specific metabolic disorders. There are currently approximately 400 genetic single nucleotide polymorphisms (SNPs) or mutations within the NR1H4 gene encoding FGF19/Fgf15 pathway of repression of bile acid synthesis.

In the study by van Mil et al. (97), both the coding regions and exon/intron boundaries of the NR1H4 gene were studied in 92 British women of varied ethnic origins, who were suffering from intrahepatic cholestasis of pregnancy. Four heterozygous SNPs, 1G>T, 1A>G (M1V), 238T>C (W80R), and 518T>C (M173T), were shown to lead to reduced FXR protein expression and decreased level of translactation of a FXR-dependent promoter construct in transfected human embryonic kidney HEK293T cells compared with the wild-type FXR. The 518T>C (M173T) variant, harboring an amino acid substitution within the zinc finger DNA-binding domain of FXR, also led to a reduction in the transactivation ability of FXR, but not in the transactivation ability of PXR, suggesting to be associated with intrahepatic cholestasis of pregnancy (ICP).

Consistent with these findings, the ICP phenotype is rare disorder that occurs primarily in the first and second trimester of pregnancy, and in such genetic cases, further investigation into the etiology nature of the disease may be needed to be conducted.

Intrahepatic cholestasis of pregnancy

In the study by van Mil et al. (97), both the coding regions and exon/intron boundaries of the NR1H4 gene were studied in 92 British women of varied ethnic origins, who were suffering from intrahepatic cholestasis of pregnancy. Four heterozygous SNPs, 1G>T, 1A>G (M1V), 238T>C (W80R), and 518T>C (M173T), were shown to lead to reduced FXR protein expression and decreased level of translactation of a FXR-dependent promoter construct in transfected human embryonic kidney HEK293T cells compared with the wild-type FXR. The 518T>C (M173T) variant, harboring an amino acid substitution within the zinc finger DNA-binding domain of FXR, also led to a reduction in the transactivation ability of FXR, but not in the transactivation ability of PXR, suggesting to be associated with intrahepatic cholestasis of pregnancy (ICP).

Cholesteryl ester transfer protein (CETP) and apolipoprotein E (apoE) have been associated with gallstone formation, but the exact genetic role of these factors remains unclear. A recent study by van Mil et al. (97) identified a novel genetic variant, 518T>C (M173T), in the NR1H4 gene encoding FGF19/Fgf15, which may contribute to the development of gallstone formation.

The authors propose that this genetic variation may provide insight into the complex genetic basis of gallstone formation and the development of gallbladder disease. This finding highlights the importance of genetic variation in the development of gallstone disease and suggests potential targets for future research. Further studies are needed to confirm these findings and to investigate the role of this genetic variant in the development of gallbladder disease.
Intrahepatic cholestasis

The FIC1–FXR Connection and Intrahepatic Cholestasis

This study investigated the functional activity of the -1G>T variant in mice (103), Kovace et al. (54) recently proceeded to genotype the human NR1H4 gene in 481 gallstone carriers and 523 control individuals from three different ethnic populations. While no polymorphisms leading to an amino acid change were identified in this study, the haplotype termed NR1H4_L, containing the more frequent alleles in three base positions (-1G>T, T110G, and -1G>T in the 5′ region of the gene, and IVS7–31A>T within intron 7), was associated with gallstone prevalence in the Mexican male group but not in the German or Chilean populations. It will be interesting to establish whether these haplotypes lead to altered levels of expression of FXR in the liver and in the intestine, thus affecting the amount of cholesterol available for biliary excretion. No significant difference in FXR mRNA levels was reported in liver biopsies obtained from 11 untreated cholesterol cholestasis patients compared with 9 cholesterol gallstone-free subjects (6). However, in this latter study, the allelic status of the NR1H4 gene was not determined in the patients. Although the expression of FXR itself was not altered in this group of gallstone patients, the transcriptional coactivator PGC-1α was significantly less expressed in the livers of patients with cholesterol cholestasis than in controls with nonsulfate-gallstone patients. PGC-1α can function as a coactivator of FXR, mediating the activation of FXR target genes (46, 108), and may also enhance the expression of the NR1H4 gene itself via coactivator of the nuclear receptors PPARγ and HNF4α (108). Reduced expression of PGC-1α in gallstone patients could thus lead to decreased expression of both FXR and its target genes BSEP and MDRI, the actions of which help to maintain cholesterol in its soluble form in the bile.

In further support of the role for FXR in cholelithiasis, FXR-null mice exhibit the typical characteristics of cholesterol gallstone disease, such as supersaturation of cholesterol in bile, precipitation of cholesterol crystals in the gallbladder, and increased hydrophobicity of bile salts (32, 71). Furthermore, in a gallstone-susceptible FXR-null type mouse strain, application of the specific FXR ligand GW4064 reduced cholesterol precipitation by increasing biliary bile salt and phospholipid concentrations and restoring cholesterol solubility. It is clear that other regulatory factors are also involved in the development of the undoubtedly multifactorial gallstone disease. In a recent report of a mouse study (7), the winged helix/forkhead transcription factor FoxO1 was shown to be a positive regulator of cholesterol efflux system ABCG5/ABCG8 at the canalicular membrane and at the apical membrane of enterocytes, in addition to many other tissues (94). Another group has suggested that FoxD3, a PGC-1α target, would be one of the factors that control cholesterol metabolism in hepatocytes. Disinhibition of FoxD3 action may, in a concerted manner with compromised FXR function, promote cholesterol gallstone formation.

The FIC1–FXR Connection and Intrahepatic Cholestasis

The familial intrahepatic cholestasis-1 (FIC1) protein, encoded by the FIC1 gene, is expressed at the liver canalicular membrane and at the apical membrane of enterocytes, in addition to many other tissues (94). PIC1 acts as an amidophospholipid Bissap, translocating phosphatidylserine from the outer leaflet of the lipid bilayer of the plasma membranes, thus contributing to the lipid asymmetry of the membrane. The exact significance of PIC1 in normal physiology is not known in detail, but it is believed to contribute to the detergent-resistant properties of the liver canalicular
plasma membrane. Genetic mutations and polymorphisms in the ATP8B1 gene have been associated with familial intrahepatic cholestasis, characterized by low γ-glutamyltransferase plasma levels (reviewed in Refs. 41, 96). A disease entity termed progressive familial intrahepatic cholestasis type 1, or PFIC1 (also known as “Byler’s disease”) is the severe form of FXR-related liver disease, characterized by fat malabsorption, intense pruritus, and frequently leads to the cirrhosis of the liver. The milder form of PFIC-related disease is known benign recurrent intrahepatic cholestasis type 1, or BRIC1. In BRIC1, discrete cholestatic episodes are separated by asymptomatic periods, and BRIC1 does not typically lead to progressive liver injury. PFIC1 and BRIC1 are associated with distinct sets of genetic variants in the ATP8B1 gene, and it could be hypothesized that the severity of the disease may correlate with the degree of corresponding effect in FIC1 function.

In 2004, two groups reported that, in PFIC1 patients, there is a tendency for decreased hepatic and intestinal mRNA levels of FXR and of genes transactivated by FXR (1, 10), implying that FXR may influence the expression and/or function of FXR, possibly thus contributing to the pathogenesis of the liver disease. Intriguingly, in a more recent report, it was shown that, whereas the wild-type FIC1 was capable of potent activation of the BSEP promoter, the PFIC1-associated FIC1 variants were inactive, and the BRIC1-associated FIC1 variants activated the BSEP promoter to a moderate degree (26). The authors further hypothesized that the wild-type FIC1 protein induces nuclear localization of FXR through stimulation of a phosphorylation cascade targeting FXR and that FIC1-related disease may be caused by the compromised ability of the associated FIC1 variants to influence FXR localization and function.

FXR-Mediated Indirect Mechanisms Affecting Intestinal Bacterial Growth

It was previously known that bile acids can protect against bacterial overgrowth in the small intestine via their anti-microbial activity, thus helping to maintain the integrity of the intestinal epithelium and to prevent systemic infections caused by bacterial translocation across the mucosal barrier (5, 65). In mice, bile acids exert this protection against intestinal mucosal injury via FXR (39). In wild-type mice, bile duct ligation resulted in a significant increase in the number of intestinal bacteria and led to bacterial invasion of the mucosa. Administration of the synthetic and potent FXR ligand GW4064 alleviated these effects of bile duct ligation in wild-type but not FXR-deficient mice. In gene profiling studies in mice, several candidate GW4064-induced genes were identified that could potentially be involved in the intestinal mucosal defence. Perhaps one of the most notable of these is the gene encoding the inducible nitric oxide synthase (iNOS), giving the anti-mucosal properties of nitric oxide, as well as its role in epithelial barrier function (74, 98). Another gene identified as GW4064-inducible was the gene encoding angiogenin, which also exerts anti-bacterial effects (34). It may be that agonistic ligands for FXR could be therapeutically useful in patients with reduced bile flow and consequently elevated bacterial growth and invasion across the intestinal mucosa.

Outlook

At present, the physiological significance of FXR function and expression is largely supported by observations in FXR-deficient mice. As crucial as such rodent whole-organism studies are, their results should be interpreted with a certain degree of caution: even slight differences in the complex promoter regions of FXR target genes between species may result in differential transcriptional consequences of FXR activation, and thus differential physiological downstream effects. Furthermore, since FXR-null mice have been devoid of any FXR function, the possibility of modulating FXR activity in pathogenic settings should assist us in our understanding of the pathobiology of these diseases and point to new therapeutic targets.

In addition to the bile acid homeostasis in the liver and intestine discussed here, FXR has been identified as a key player in several other metabolic processes, such as glucose and lipid metabolism (12), as well as carcinogenesis (52, 106) and liver cell proliferation (36). Although this has raised much excitement about the potential of modulating FXR activity in pathogenic processes beyond those involving disturbed bile acid homeostasis, this has also highlighted the potential difficulties in achieving tissue, process, and even gene specificity in therapeutic targeting of FXR. Since the range of FXR ligands, endogenous and synthetic, is rapidly increasing, the likelihood of discovering FXR agonists and antagonists that may function in a more specific manner may be increased, and modeling of novel synthetic FXR ligands displaying such specificity may be facilitated.

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