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 reverted (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 most nuclear receptors are involved in regulating a variety of metabolic processes, and accordingly have multiple target genes, the major challenge will be to achieve functional selectivity by therapeutic ligands leading to enhanced transcriptional activity with specific target genes in specific tissue sites.

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 mammmals there are two FXR genes, Nr1h4 (encoding Fxr1) and Nr1h5 (encoding Fxr2), the latter gene product employing lanosterols as its ligands, in humans and other primates the homolog of Fxr1 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 isoforms.

Four isoforms of FXR, termed FXR1a–4, can be translated from the single NR1H4 gene in humans, generated by both alternative promoter usage and by alternative splicing (37, 109). FXR1a and FXR1b, the most abundant FXR isoforms in the human liver, differ from FXR3a and FXR3b at the NH2 terminus, whereas the mRNAs for these two isoform pairs are transcribed from separate promoters. In addition, the isoforms FXR3a and FXR3b contain an additional stretch of four amino acids, MTTY, due to a differential splicing event at the end of exon 5. All four FXR isoforms harbour identical ligand-binding domains but may exhibit different conformational 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 transactivation function, have been proposed (reviewed in Ref. 28). 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 apo-
human apolipoprotein A-I and apolipoprotein C-III promoters that this 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 (NR0B2) 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 therefore function as a transcriptional corepressor, or actively recruiting transcriptional activity of several other members of the nuclear receptor family, as well as of 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/AHPN 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 as a target for the diterpenic compound found in coffee beans and present in unfiltered coffee brews, called cafestol, was found to function as an agonistic ligand for FXR (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 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-ECDCA) 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 choleretic 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 inlets 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 enteroocytes 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 detergent 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 also do elicit other signaling pathways, such as those involving a G-protein-coupled receptor TRIG (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 TRIG (85), providing one mechanism for the divergent downstream signaling events that they elicit.

In the liver and intestine, the transcriptional events exerted by bile acid-activated FXR lead to increased cellular bile acid efflux and detoxification and decreased bile acid uptake and synthesis, as described below.

**FXR induces bile acid efflux and detoxification machinery**

The ABCB1 gene encoding BSEP, the chief liver canalicular bile salt export pump of the ATP-binding cassette (ABC) transporter family (90), is a target for direct transactivation by FXR (2, 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 monovalent bile acids from hepatocytes into bile, the multidrug resistance-associated protein 2 (MRP2, ABCC2) 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 ABCC2/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 flippase for phospholipids within the canalicular membrane of hepatocytes. Via induction of ABCB4 expression, FXR enhances the function of MDR3 in countering the toxicity of biliary bile acids by promoting formation of mixed micelles that contain cholesterol, bile acids, and phospholipids in bile canaliculi.

The SLC22A10/ABCC2 gene encoding a member of the organic anion transporting polypeptide (OATP) family, OSTa/OSTb, is directly transactivated by FXR in a ligand-dependent manner through an IR-1 element (45). OSTb is a major sinusoidal bile acid uptake system at the sinusoidal membrane of hepatocytes for numerous drugs and peptides, such as digitoxin and cholecystokinin (40, 55). OSTa/OSTb 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, OATP1B1, is suppressed by FXR-dependent pathways in cholestatic, bile acid-enriched environment (44). Induction of OATP1B1 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).

In an analogous manner to the ABCB1 gene, FXR induces the expression of the two genes encoding the essential heterodimeric bile acid efflux system at the basolateral membrane of ileocytes, OSTa/OSTb (Ref. 58, 59, 82; Table 2). The OSTa/OSTb heterodimer has also been suggested to mediate biliary excretion of bile acids in biliary cirrhosis (34) and to contribute to the detoxification of FXR-RXR heterodimer-activated promoters (55). The bile acid transporters MRP2 and MDR3 are increased in cholestasis, primarily from primary bile salt-retaining compounds (4). The OATP1B1 promoter contains a bile acid-responsive element, which is more important in the bile acid receptor (4).

**FXR negatively regulates bile acid uptake**

The Na+-taurine cotransporter (NTCP) is the major bile acid uptake transporter and is necessary for the maintenance of bile acid homeostasis in both the liver and intestine. NTCP is notably decreased in patients with cholestatic diseases with a concomitant rise in plasma bile acid concentrations and reduced enterohepatic circulation (43). NTCP is located at the basolateral membrane of hepatocytes and is notably decimated in conditions of bile acid excess and cholestasis, such as those observed in cholestatic, bile acid-enriched environment (44). NTCP is directly transactivated by FXR under conditions of FXR-RXR heterodimer activation, as indicated by 10.220.32.246 on April 19, 2017 http://physiologyonline.physiology.org/ Downloaded from Reversing the bile acid homeostasis of the liver in both vivo and ex vivo systems is possible in bile duct-ligated rats through induction of NTCP expression by the bile acid receptor (B). This likely occurs in the duodenum through activation of the FXR-RXR heterodimer, which can also associate with the bile acid signal transducer SHP (82). In bile duct-ligated rats, the bile acid receptor (B) downregulates NTCP expression in the liver when present, whereas in the case of FXR knockouts, NTCP is greatly increased. SHP decreases the total amount of NTCP protein in the liver in a FXR-dependent manner, possibly by inducing NTCP degradation (45).
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 from hepatocytes, FXR also suppresses the expression of the major bile acid uptake system in the liver or intestine, the expression of which is regulated by FXR.

The location of the membrane proteins at the correct membrane domain is shown in orange. Membrane transporters and other proteins expressed in the liver or intestine, the expression of which is regulated by FXR, are shown in green, whereas those whose expression FXR suppresses are shown in red.

The organic anion transporter polypeptide OATP1B1 can function as a Na+-independent bile salt uptake system at the basolateral hepatocyte membrane in humans. FXR-mediated repression of the OATP1B1 promoter takes place through a multistep cascade (44). FXR-induced SHP interferes with the nuclear receptor hepatocyte nuclear factor-4α, which is a transactivator of the gene encoding the homeodomain transcription factor hepatocyte nuclear factor-1α (HNF-1α). HNF-1α, in turn, is a strong direct DNA-binding transactivator of the OATP1B1 promoter (43).

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proposed pathway emphasizes the coordinated regu-
lization of the biliary uptake systems in both human liver and inte-
testine.

In addition to downregulating the expression of bile acid uptake transporters in the liver and the intestine, FXR also represses transcription of three genes coding for bile acid synthesizing enzymes, namely choles-
terol-7α-hydroxylase (CYP7A1), sterol-12α-hydroxy-
ase (CYP8B1), and sterol-27-hydroxylase (CYP27A1), in a ligand-dependent manner (reviewed in Ref. 20).

Thus elevated levels of bile acids can suppress their own de novo production through a negative feedback loop. All three CYP promoters contain a negative bile acid response element, which is targeted by the FXR-
induced repressor SHP. In the CYP7A1 and CYP8B1
promoters, the targeted DNA-binding transactivators are the monomeric nuclear receptor liver receptor homolog-1 (LXR-1) and homodimeric HNF-4α, which have overlapping DNA-binding motifs on both pro-
moters. In the case of the CYP27A1 gene, the negative bile acid response element contains a DNA-binding site only for HNF-4α but not for LXR-1.

Although SHP-deficient mice exhibit impaired nega-
tive feedback regulation of bile acid synthesis, this is not completely abolished, implying that additional or parallel SHP-independent repression pathways may lead to reduced bile acid synthesis (51, 100). One such alternative pathway involving signaling between the liver and the intestine has been proposed. In response to bile acids, FXR directly activates the gene encoding fibroblast growth factor-19 (FGF19; mouse ortholog Fgfl5) in the intestine (33, 38). From the intestine, FGF19/Fgf15 is released to portal blood, and upon reaching the liver it elicits a signaling cascade by bind-
ing to the fibroblast growth factor receptor-4 (FGFR4) tyrosine kinase on the surface of hepatocytes. Activated FGFR4 stimulates the intracellular JNK kinase pathway-
way, which eventually suppresses the CYP7A1 promot-
er in the nucleus. A complex mechanism has been proposed for this downstream effect, according to which JNK signaling induces expression of the tran-
scriptional coactivator PGC-1α, which may block the recruitment of the tran-
scriptional repressor SHP somehow contributes to the FXR-
FGF19/Fgf15 pathway of repression of bile acid synthe-
sis, since the effect seems clearly attenuated in SHP-
deficient mice (30). The FGF19/Fgf15-mediated endocrine-type loop may explain the previous observa-
tions 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 per-
oxisome proliferator-activated receptors-α and -γ (PPARα, PPARγ; Refs. 70, 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 approximate-
ly 400 genetic single nucleotide polymorphisms (SNPs) or mutations within the NR1H4 gene encoding FGF19 submitted to the NCBI (http://www.ncbi.nlm.nih.gov/snp) and HapMap (http://www.hapmap.org) SNP databases, most of them located in the intronic regions or in regions flanking the FGF19 coding sequence. Only recently have reports been published on associations between genetic variants in the NR1H4 gene and human pathobiology, and these are summarized below.

**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 eth-
ic origins, who were suffering from intrahepatic cholestasis of pregnancy (ICP). ICP is a reversible form of a cholestatic disease, which is frequently associated with adverse pregnancy outcomes, such as premature birth, fetal distress, and intra-uterine death. Four heter-
zygous NR1H4 variants were identified: –1G>T, 1A>G (M1V), 238T>C (W80R), and 518T>C (M173T). The first two FXR variants, –1G>T and 1A>G (M1V), were shown to lead to reduced FXR protein expression and decreased level of transcriptional activation 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 in cell-based assays, even if the degree of protein expression, DNA-binding, and heterodimerization with RXR remained apparent-
antly unaffected by the change of residues. Another group published simultaneously an independent study on FXR polymorphisms in populations of European, African, Chinese, and Hispanic descent (68). One of the four FXR variants identified in this latter study also contained the −1G>T substitution in the base position adjacent to the translational initiation site. Although in

**Table 2. Intestinal 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>OSty/OSTj</td>
<td>+</td>
<td>Heterodimeric basolateral bile acid efflux system</td>
</tr>
<tr>
<td>ASBT</td>
<td>−</td>
<td>Apical bile acid uptake system</td>
</tr>
<tr>
<td>FGF19</td>
<td>+</td>
<td>Bile acid-inducible growth factor</td>
</tr>
<tr>
<td>iNOS</td>
<td>+</td>
<td>Nitric oxide synthase, anti-microbial</td>
</tr>
<tr>
<td>angiogenin</td>
<td>+</td>
<td>Anti-microbial</td>
</tr>
</tbody>
</table>

For details and references to literature, please see the main text. +, induced by FXR; –, suppressed by FXR.
In this study the functional activity of the -1G>T variant also appeared to be compromised, the level of transcription and translation efficiency of the variant was evaluated comparable to the wild-type in a cell-free assay and in transfected HEa cells. The difference between the two studies remains unclear but may simply be due to the different experimental setups. Interestingly, the mRNA expression levels of the FXR target genes SHP and OATP1B3 are significantly reduced in the livers of the heterozygote subject carrying the -1G>T allele, whereas the FXR mRNA expression is similar to that in controls, further indicating that this polymorphism may lead to compromised function but not expression level of FXR (68).

Also in the above-mentioned report by van Mill et al. (97), a case-control study of a British cohort (293 ICP cases, 290 controls) was performed to determine the allelic frequencies of the NR1H4 variants previously identified. The variant 238T>C (W80R) was not present in subjects of Caucasian origin, and only one British ICP patient carried the 1A>G (M171) variant. Out of the two variants present in both the ICP group and the control group, -1G>T and 51HFT-C (M173T), only the latter exhibited significant associations with the ICP phenotype in the Swedish group. It is clear that in such genetic association studies larger cohorts will be needed to confirm the results and to discover further rare disease-associated variants. Furthermore, as cholesterol gallstone diseases may be complex, possibly requiring particular allelic variants in multiple susceptibility genes, these findings need to be confirmed in larger studies to fully determine the functional activity of the NR1H4 variants in gallstone carriers and 523 control individuals from the Swedish cohort. 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 the genes encoding cholesterol transporters OATP1B3 and MDR3, 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 wild-type mouse strain, application of the specific FXR ligand GW4064 reduced gallstone prevalence by increasing biliary bile salt and phospholipid concentrations and restoring cholesterol solubility.

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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). *FIC1* acts as an aminophospholipid translocase, 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 *FIC1* in normal physiology is not known in detail, but it is believed to contribute to the detergent-resistant properties of the liver canalicular membrane.
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 familiar intrahepatic cholestasis type 1, or PFIC1 (also known as “Byler’s disease”) is the severe form of PFIC-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 PFIC1 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 RXRβ promoter, the PFIC1-associated FIC1 variants were inactive, and the BRIC1-associated FIC1 variants activated the RXRβ 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 (35). 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), given the antimicrobial 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 these 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 throughout their embryonic development and adult life, compensatory mechanisms may have developed. These models may not accurately reflect the situation in human pathologies, where genetic variants of FXR may have altered, but by no means are absent in, function. Increased knowledge of the consequences of genetic variation in the human FXR gene itself, FXR target genes, or potential upstream regulators of FXR such as FIC1, and of association between these genetic events and human pathogenesis 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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The function of FXR is critical in the healthy liver to maintain bile acid homeostasis. FXR is activated by bile acids and plays a central role in the regulation of bile acid synthesis, secretion, and transport. The FXR activation leads to the suppression of genes involved in cholesterol and bile acid synthesis and the induction of genes involved in bile acid excretion and transport. This results in the maintenance of bile acid homeostasis and the prevention of liver diseases.

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