FXR Friend-ChIPs in the Enterohepatic System

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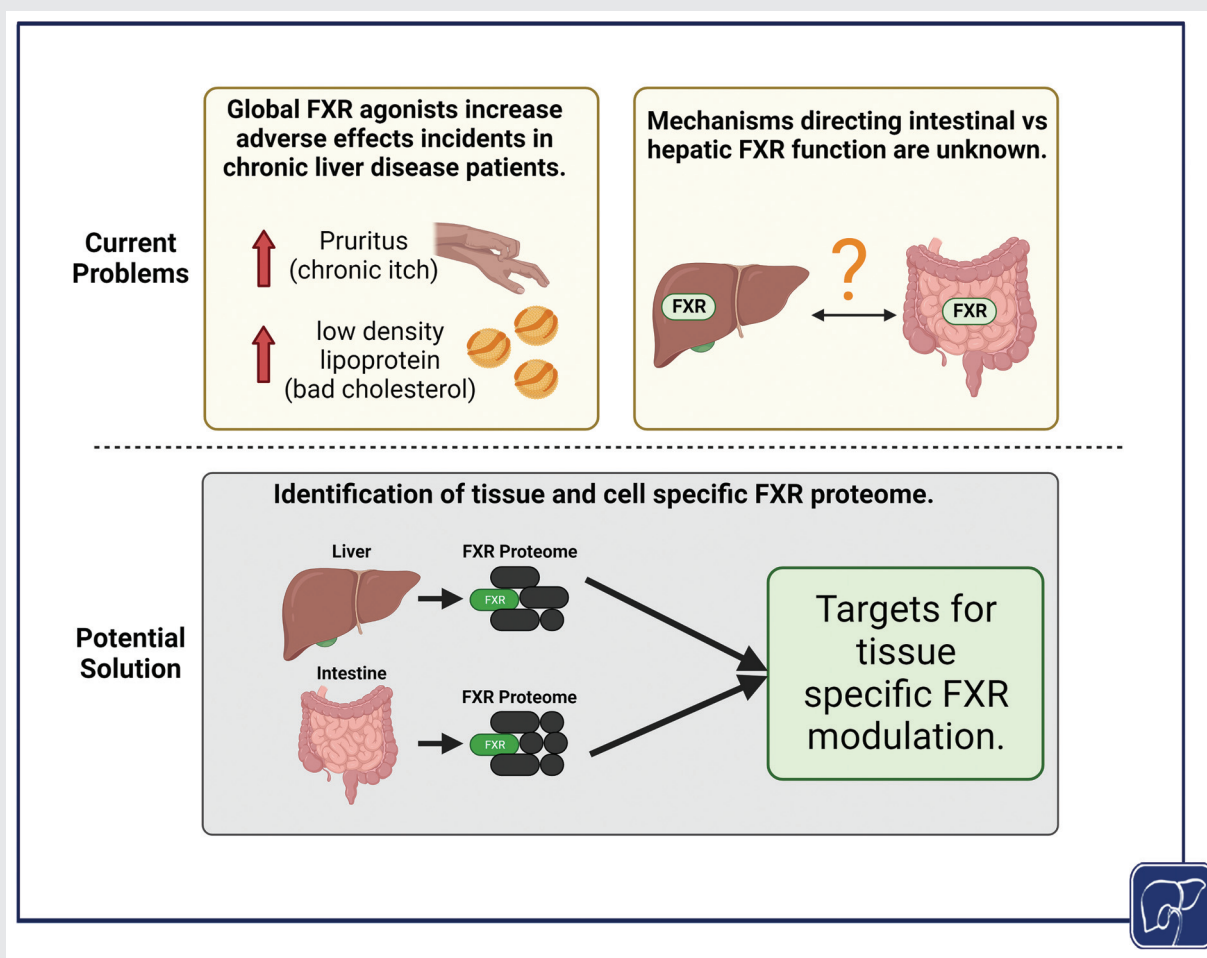
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Graphical Abstract

Abstract

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Chronic liver diseases encompass a wide spectrum of hepatic maladies that often result in cholestasis or altered bile acid secretion and regulation. Incidence and cost of care for many chronic liver diseases are rising in the United States with few Food and Drug Administration-approved drugs available for patient treatment. Farnesoid X receptor (FXR) is the master regulator of bile acid homeostasis with an important role in lipid and glucose metabolism and inflammation. FXR has served as an attractive target for management of cholestasis and fibrosis; however, global FXR agonism results in adverse effects in liver disease patients, severely affecting quality of life. In this review, we highlight seminal studies and recent updates on the FXR proteome and identify gaps in knowledge that are essential for tissue-specific FXR modulation. In conclusion, one of the greatest unmet needs in the field is understanding the underlying mechanism of intestinal versus hepatic FXR function.

Lay Summary

Occurrence and treatment cost of chronic liver disease are increasing in the United States with few Food and Drug Administration-approved drugs available for patients. A common symptom of liver disease is reduced or blocked bile flow from the liver, which is regulated by farnesoid X receptor (FXR), a nuclear receptor protein that is important for regulating liver function. FXR must bind other proteins to control bile acid synthesis and bile flow and has unique organ-dependent roles. Understanding how FXR activity is controlled in different organs is an urgent unmet need in liver and intestinal disease research. In this review, we summarize the first findings of FXR-associated proteins and highlight recent studies addressing the knowledge gap for organ-specific FXR research.

Chronic liver disease encompasses a spectrum of liver diseases with cost burden of $81.1 billion for its related care and hospitalizations in the United States.[1] Patients often experience long asymptomatic lapses and are diagnosed at a late stage leading to poor prognosis and high mortality. While great effort has been made in identifying biomarkers and tests to aid in earlier diagnosis,[2] the heterogeneity of chronic liver diseases and subsequent comorbidity complicate this endeavor.

One of the major symptoms of chronic liver disease is cholestasis or impaired bile flow and secretion. Bile is an aqueous heterogenous mixture that contains bile salts, bilirubin phospholipid, cholesterol, amino acids, bicarbonate, vitamins, exogenous drugs, and xenobiotics.[3] Bile salts possess strong detergent properties allowing for fatty acid micelle formation and intestinal absorption.[4,5] Bile allows for the removal of harmful toxicants and serves as the major route of cholesterol elimination through bile acid formation and secretion.[3] Bile acids are amphipathic sterols and serve as the end-product of cholesterol catabolism, mainly synthesized by hepatocytes, to aid in fat and fat-soluble vitamin absorption. Bile is readily altered by cholangiocytes, bile duct epithelial cells, through secretion of water, bicarbonate, secretin, and other signaling hormones.[6] Cholangiocytes can circumvent normal bile acid circulation through the cholehepatic shunt prior to secretion to the gall bladder for storage or small intestine postprandially.[6] In this process, cholangiocytes transport bile acids from the bile duct lumen to hepatocytes for further modification. However, up to 95% of bile acids are recirculated through enterocyte absorption and secretion into portal circulation, a process called enterohepatic circulation. This circulation and synthesis of bile acids are tightly regulated by a ligand-activated nuclear receptor, FXR, and disruption leads to severe consequences. Much like other nuclear receptors, FXR function relies on interactions with various cofactors and transcriptional regulators. Cofactors are considered promising targets for liver disease therapeutics; however, ubiquitous expression, transient complex formation, and poor antibody performance all pose significant challenges that impede progress in this field of research.

FXR

FXR is a member of the nuclear receptor superfamily and is widely recognized as the master regulator of bile acid synthesis and transport.[7–11] First discovered as a binding partner for retinoid X receptor (RXR),[12] FXR is highly expressed in the liver and intestine, where it carries out a major role in suppressing bile acid synthesis via downstream effectors, fibroblast growth factor (FGF) 15 (murine ortholog of human FGF19), and to a less extent small heterodimer partner (SHP).[13,14] Four isoforms of FXR (FXRα1–4) arise in humans and mice with alternative splicing of a 4-amino acid extension of the DNA binding domain, which separates FXRα1 and FXRα3 isoforms from FXRα2 and FXRα4.[15–17] Human liver preferentially expresses FXRα1 and FXRα2, while mouse liver tissue preferentially expresses FXRα2 or FXRα4, and human and mouse intestines preferentially express FXRα3 and FXRα4.[15,17] In both human and mouse livers, hepatic FXRα2 is the dominant driver of FXR agonism functions.[15,18] It is still unclear if FXR is a type I (cytoplasmic)

or type II (nuclear) nuclear receptor, but its transcriptional activation has been extensively studied in the liver and intestine.

Canonical Function

In the gastrointestinal tract, FXR is highly expressed in the distal ileum and is critical in regulating enterohepatic bile acid homeostasis, including suppressing bile acid synthesis, and promoting bile acid transport. In the intestine, FXR is activated by bile acids to initiate the expression and secretion of FGF15/19 into portal circulation. Intestinal FXR activation regulates enterohepatic bile acid circulation through complex regulation of intestinal bile acid transporters, specifically promoting efflux and inhibiting influx of bile acids. In the ileum, apical sodium bile acid transporter expression is decreased while the expression of fatty acid binding protein 6 and organic solute transporter α and β expression are increased.[19–21] Further, FXR promotes epithelial layer integrity following activation through increased intestinal tight junction protein expression[22] and mucus production.[23] Moreover, FXR may modulate the ceramide production in the ileum to regulate metabolic diseases.[24,25] It is important to note that bile acids are metabolized and modified by the intestinal microbiome and there is a mutual relationship between bile acids and microbiome composition.[26] Bacteria create secondary bile acids via deconjugation, dihydroxylation at carbon 7, oxidation, and epimerization of primary bile acids to dampen antimicrobial function, alter intestinal immune microenvironment, and improve bacterial fitness.[26,27]

In the liver, circulating FGF15/FGF19 binds to hepatic β-klotho and FGF receptor 4 dimer to inhibit gene expression of cytochrome P450 7a1 (*Cyp7a1*/CYP7A1) and 8b1 (*Cyp8b1*/CYP8B1), ultimately suppressing bile acid synthesis.[13,14,28,29] Circulating bile acids activate hepatic FXR leading to induction of SHP that mainly functions to inhibit *Cyp8b1* expression.[14,29–32] Hepatocyte canalicular bile acid efflux transporter, bile salt export pump (BSEP), and sinusoidal uptake transporter, sodium taurocholate co-transporting polypeptide, are both regulated by hepatic FXR activation, serving as the main mechanism for hepatic bile acids to be transported from portal circulation into the bile canaliculi.[14,33] Hepatic FXR activation also results in reduced fatty acid synthesis[30,34,35] and hepatic inflammation.[36–38] Since CYP7A1 mediates the rate-limiting step of bile acid synthesis, and CYP8B1 determines bile acid hydrophobicity, it is generally considered that intestinal FXR is critical for regulating the bile acid pool and hydrophobicity, while hepatic FXR is critical in determining hydrophobicity of bile acids.[14,39,40] There are major differences between the murine and human bile acid speciation, which lends complexity to current studies of bile acid effects in disease states.[41] Overall, humans display a hydrophobic bile acid pool and mice exhibit hydrophilic bile acid pool with unique bile acid species, muricholic acids.[42] CYP2C70 has been identified as the enzyme responsible for α- and β-muricholic acid formation from chenodeoxycholic acid.[41] Murine models of CYP2C70 deficiency demonstrate a humanized bile acid pool with increased hepatic damage that is ameliorated following FXR activation.[41,43]

Noncanonical Function

In recent years, our understanding of the impact of FXR activation has expanded from the enterohepatic system. FXR activation has been found to reduce lung macrophage activation following nitrogen mustard exposure[44] and increase β-oxidative gene expression in cardiomyocytes.[45] In the brain, FXR expression is correlated with Alzheimer's disease and loss of FXR reduces β-amyloid-induced brain injury.[46] FXR increases water reabsorption and promotes renal medullary collecting duct cell survival, ultimately affecting urine concentration during dehydration.[47] Besides, adipose-specific overexpression of FXR promotes brown adipose tissue whitening and fibrosis.[48] There is little information on FXR function in important sensory cells like cholangiocytes, tuft cells in the intestine, or chromaffin cells of the adrenals (PMID: 24068255, PMID: 35245089, PMID: 17963822). The ubiquitous expression of FXR in various organs, while less than in hepatocytes and ileal enterocytes, makes it crucial to understand FXR activation in a whole-body setting (PMID: 36988391).[44–47,49]

FXR Function in Disease

The role of FXR in intestinal inflammation and fibrosis has been of increasing interest. Whole-body activation of FXR with obeticholic acid (OCA) in mice reduces dextran sodium sulfate (DSS) and trinitrobenzenesulfonic acid-induced colitis including immune cell infiltration and inflammatory cytokine expression.[22] Further, OCA, also known as INT-747, reduces proinflammatory cytokine secretion in activated mononuclear cells and monocytes derived from inflammatory bowel disease patients.[22] Notably, murine models of whole-body FXR loss demonstrate an enhanced inflammatory phenotype following DSS treatment with increased innate lymphoid cell presence within the damaged intestine and increased inflammatory cytokine expression.[50] Similarly, inhibition of ileal FXR by *Parabacteroides distasonis* improves hepatic fibrosis in mice fed methionine and choline-deficient diet.[51] Prophylactic FXR activation in the intestine, with tissue-specific FXR agonist fexaramine, prevents DSS-induced intestinal villus damage, serum interleukin 17 (IL-17) secretion, and immune cell infiltration of the intestine.[50] Function of fexaramine, and other fex-derivatives, is thought to be gastrointestinal-specific with heterogeneity of FXR activation depending on route of administration.[52,53] Oral administration of fexaramine is able to activate ileal FXR, with little to no activation in other colon, liver, and kidney, which is likely due to its increased interactions with helix 3 of the FXR protein and deeper penetration and filling of the ligand binding pocket due to fexaramine's hydrophobic rings and larger volume.[52,53] Fexaramine's intestine-specific activation is likely due to poor absorption into circulation.[53]

While ileal FXR activation is widely regarded to contribute to hepatic function, liver FXR activation may also influence gut permeability. Hepatic FXR loss results in increased colonic mucus secretion and enhanced bacterial response gene expression profile.[23] Further, loss of hepatic FXR shifts the microbiome toward mucosal protection by reducing abundance of mucin-degrading genera (*Turicibacter*) and increasing abundance of mucus barrier-enforcing bacteria (*Roseburia, Bifidobacterium,* and *Clostridium sensu stricto 1*).[23] FXR activation antagonizes nuclear factor kappa B (NF-κB) signaling which results in reduced hepatic inflammation.[37] Mice lacking FXR display increased hepatic inflammation following treatment with lipopolysaccharide, a bacterial cell wall component, which is ameliorated following transfection with FXRα2-adenovirus.[37] FXR activation prevents NF-κB activity through interference of NF-κB and DNA binding.[37]

The effect of FXR activation on hepatic fibrosis is considered disease-dependent.[54–57] Loss of FXR has been shown to have no effect on hepatic fibrosis in mice following carbon tetrachloride treatment, a classical model of liver injury; however, in common bile duct ligated and 3,5-diethoxycarbonyl-1,4-dihydrocollidine-fed mice, loss of FXR directs protection against portal fibrosis in the liver.[54] FXR expression, as shown by immunohistochemistry, is found mainly in hepatocytes and cholangiocytes and minimally in murine myofibroblasts.[54] Conversely, it has been shown that FXR activation by OCA attenuates collagen deposition, α smooth muscle actin positive staining, and hepatic hydroxyproline content in mice treated with carbon tetrachloride and rats treated with thioacetamide.[55–57] The protective effects of FXR activation are thought to result from SMAD3 and FXR interaction following FXR activation.[55] Together these data suggest that FXR activation may have indirect effects on fibrosis, and loss of FXR improves portal fibrosis while global FXR activation improves noncholestatic hepatic fibrosis.

Known Mechanisms and Interactions of the FXR Proteome

Due to the synergistic roles of bile acids in lipid and glucose homeostasis, FXR regulation of bile acid synthesis and transport, and FXR antagonism effects on inflammation, FXR has been extensively researched as a therapeutic target for chronic liver diseases. This pursuit of global FXR agonists can be controversial in the context of disease treatment, largely due to our knowledge gaps in understanding mechanisms underlying tissue-specific FXR functions.[58–60]

Originally speculated to be an independent bile acid sensor, the complex role of FXR cofactors in directing tissue-specific FXR response has been of growing interest.[61–63] FXR can inhibit gene expression of apolipoprotein A-I (ApoA-I) as a monomer or homodimer[64]; however, FXR transcriptional activation is regarded to be a direct result of heterodimerization with other transcriptional regulators like RXR α (RXRα).[65,66] Interestingly, several factors are now shown to interact with and regulate FXR function. Hereon, we summarize a few of the suspected, and confirmed, members of the FXR proteome in the liver and intestine.

FXR Binding Partners

RXRα

RXRα is a nuclear receptor and promiscuous binding partner discovered to be the "missing factor" in various nuclear receptor transcriptional activity.[67] RXR isoforms, α, β, and γ,[68] are activated by 9-*cis*-retinoic acid and can act as a homodimer to activate the transcription of target genes. Heterodimerization of RXRs with other nuclear receptors can result in nonpermissive or silent partner function, which cannot be activated by RXR agonists, or in a permissive function, responding to ligand activation of either RXR or its partner nuclear receptor.[67,69] The FXR/RXR complex activated by RXR's endogenous ligands (e.g., 9-*cis*-retinoic acid) increased FXR-mediated transcriptional activation following activation by synthetic agonists (e.g., WAY-362450), suggesting that RXR activation promotes transcriptional activity of their permissive partners.[65] Like other RXR/nuclear receptor complexes, FXR/RXR heterodimer facilitates transactivation by binding to target sequences with RXR binding to 5′ half-site and its partner binding to the 3′ half-site of target sequences/response elements.[67] Interestingly, it has been found that FXR binding of the SHP promoter requires FXR interaction with the liver receptor homologue 1 (LRH-1) response element without LRH-1 binding; however, 9-*cis*-retinoic acid-dependent SHP expression requires RXRα occupation of the inverted repeat separated by 1 nucleotide (IR-1) site for subsequent SHP expression.[70] It was originally found that the FXR/RXR heterodimer can bind to the IR-1 sequence with high affinity; however, changes to the half-site sequences, spacing nucleotide, and flanking nucleotides are also bound by this heterodimer, shown pictorially in ►**Fig. 1**.[71] While the FXR/RXR complex upholds many known behaviors of nuclear receptor interactions, their differential expressions may rely on unique site binding and cofactor recruitment at the time of ligand activation.[72]

Hepatocyte Nuclear Factor 4 α

Hepatocyte nuclear factor 4 α (HNF4α) is an orphan nuclear receptor that is highly expressed in epithelial tissues of digestive organs such as the liver and intestine. HNF4α plays essential roles in enterohepatic development, hepatic metabolism, and regulation of hepatocyte cell fate of hepatic progenitor cells.[73–75] HNF4α is known to interact with other transcription factors to induce transcriptional regulation.[76] HNF4α and FXR share many target genes related to bile acid synthesis, albeit their actions are in an opposing manner, as HNF4α normally promotes, whereas FXR suppresses, the expression of genes in bile acid synthesis.[77] HNF4 regulates bile acid conjugation through expression of bile acid-CoA: amino acid *N*-acyltransferase and bile acid-CoA ligase.[78] The existence of the FXR/HNF4α complex has been established in mouse[77] and human hepatocytes.[79] Despite these findings, the mechanism or biological significance of the interaction between FXR and HNF4α remains unclear.

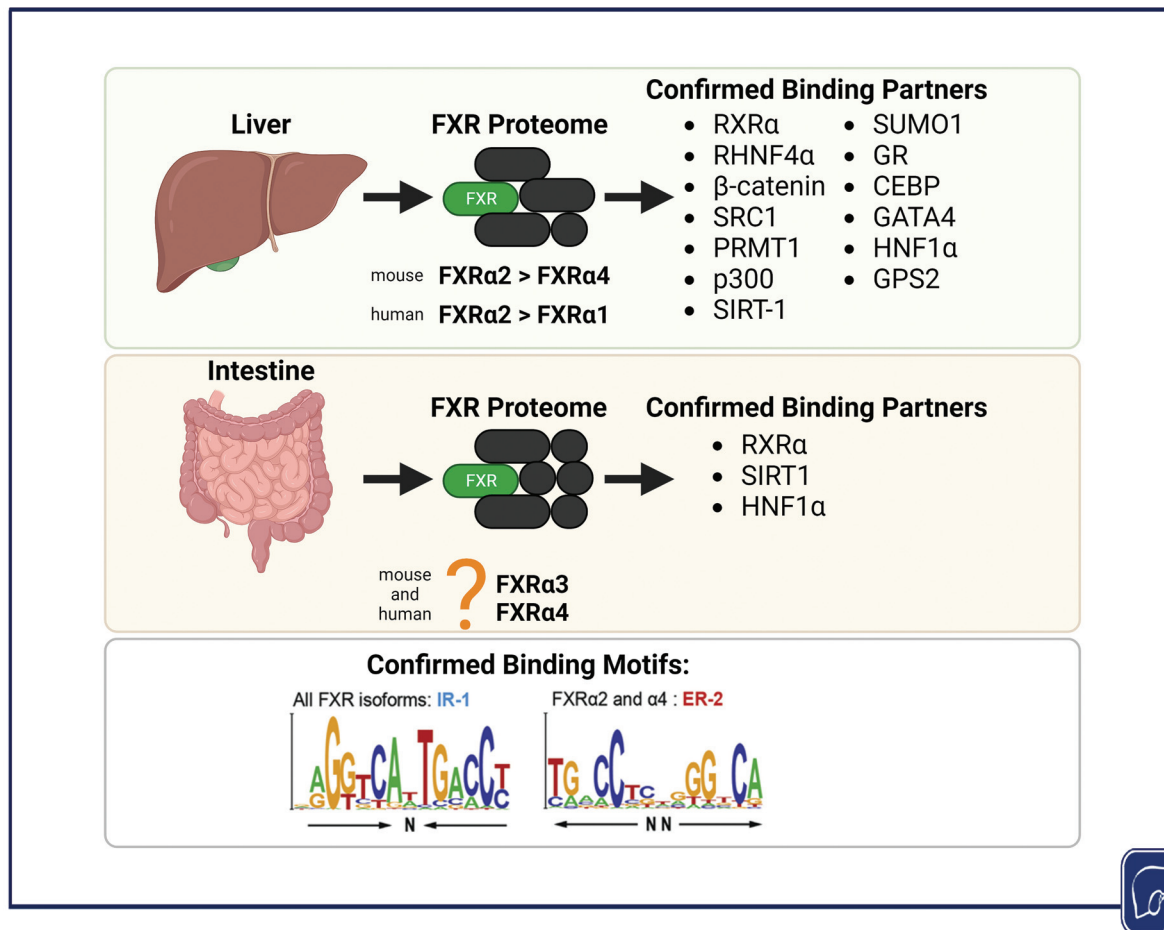HNF4α not only interacts with FXR but also induces FXR gene expression. In the fasting state, peroxisome

Fig. 1 Current understanding of the FXR proteome. The liver in human and mice preferentially expresses FXRα2 to perform ligand-activated transcriptional activity. It is unknown which FXR isoform, FXRα3 or FXRα4, is preferentially expressed in the intestine. All FXR isoforms bind to the IR-1 motif while only FXR2 and FXR4 have been shown to bind the ER-2 DNA binding motifs. Identification of confirmed binding partners of the hepatic and intestinal FXR proteome, and studies on confirmed DNA binding motifs the FXR isoforms, may provide ideal targets for tissue-specific FXR therapeutics. Figure created with biorender.com and confirmed DNA binding motif sequences are repurposed with permission.[15]

proliferator-activated receptor-gamma coactivator-1 α (PGC-1α) coactivates HNF4α to induce FXR transcription, favoring isoforms FXRα3 and FXRα4.[80] FXR competitively binds PGC-1α to inhibit transcriptional activation of sulfotransferase family 1E member 1 (*Sult1e1*) gene by HNF4α.[81] HNF4α and LRH-1 interaction keeps *Cyp7a1* gene in a transcriptionally active state, which can be reversed by SHP-FGF15/19-mediated suppression. Specifically, SHP inhibits LRH1 activity to prevent FGF15/19 activation of ERK and JNK pathways that activate *Cyp7a1* gene transcription.[14,82–84] SHP has been shown to directly interact with HNF4α at the cysteine sulfinic acid decarboxylase promoter to inhibit its transcription and reduce downstream taurine production.[85] Liver zonation transcriptomics data found that *Cyp7a1* expression is limited to pericentral hepatocytes, specifically referred to as layer 1, while HNF4α and FXR are equally expressed through all hepatocyte zones, layers 1 to 9.[86] Ubiquitous expression of HNF4α and FXR throughout the hepatocyte zones indicates that their downstream effects may rely on cofactor or ligand binding.

FXR Cofactors

It has been long suspected that FXR cofactors, unable to bind DNA but able to bind nuclear receptors, influence tissue-specific FXR activation. These regulatory cofactors often function in histone modification or chromatin remodeling capacities, inherently affecting the transcription of FXR target genes through their coactivator or corepressor function. Below we detail key studies that identify and investigate FXR/cofactor complexes in vitro and in vivo.

Cofactors via Posttranslational Modifications of FXR (PMRT, p300, SIRT1, SUMO1, SRC1, O-GlcNac Transferase)

Posttranslational modifications of proteins are important for cell homeostasis, proliferation, and stress response. Post-translational modifications of FXR direct its function by altering DNA binding, ligand binding, heterodimer formation, and subcellular localization.[87] Protein arginine methyl-transferase type I (PMRT1), p300, and sirtuin 1 (SIRT1) can regulate transcription through methylation, acetylation, and

deacetylation, respectively, of histone and nonhistone proteins.[88–90] Further, SIRT1 can interact with p300 to repress its transcriptional regulatory activity.[91] Small ubiquitin-like modifier (SUMO) proteins direct protein–protein interaction and cellular localization of nuclear receptors.[87] The steroid receptor coactivator 1 (SRC1) initiates p160 SRC family protein recruitment to regulate nuclear receptor function.[92] FXR is subject to methylation, phosphorylation, acetylation, SUMOylation, and O-GlcNAcylation at various sites including lysine 67, 122, and 127 and glutamate 277 and AF1 domain.[88,93]

Methylation of FXR and FXR target gene histones by PRMT1 is essential for FXR activation.[88] Following treatment with a synthetic bile acid, OCA, FXR recruits PRMT1, which methylates histone H4 protein near promoter regions of *BSEP* and *SHP*.[88] This FXR activation is ablated in the presence of methylation inhibitors, indicating that methylation is important in regulating FXR transcriptional activity. Reduced methylation of FXR target promoter regions results in decreased FXR transcriptional activity and subsequently the level of conjugated bile acids in the liver.[94] Conversely, FXR transcriptional regulation of bile acid homeostasis requires phosphorylation by nonreceptor tyrosine kinase, Src, at tyrosine 67.[95] Phospho-defective FXR, or Src downregulation, disrupts the expression of FXR target genes and impairs bile acid homeostasis following cholic acid feeding in wild-type (WT) mice.[95]

Acetylation of FXR and histones at the *Shp*/SHP promoter initiates SHP gene expression following FXR activation in mouse livers and HepG2 cells.[89] The recruitment of p300 is FXR-dependent as shown in FXR null mice who lack p300 recruitment and its subsequent acetylation at the *Shp* promoter. Interestingly, acetylation of FXR at lysine 157 and lysine 217 by p300 prevents the FXR/RXRα dimer formation.[66] Mutations at these acetylation sites result in retained RXR binding and ablated p300 acetylation. Further, it has been found that inhibition of p300, in vitro, resulted in increased ApoA-I and reduced G-6-Pase and phosphoenolpyruvate carboxykinase expression, which was unaffected by FXR activation with GW4064.[89] SIRT1 deacetylation of FXR promotes FXR/RXRα dimer formation with increased FXR transactivation.[66] Deletion of intestinal SIRT1 decreases FXR/HNF1α complex formation resulting in reduced bile acid transport and increased hepatic bile acid synthesis.[96]

Hepatic fibrosis resolution remains a key goal in liver disease research. OCA has been shown to be an effective prophylactic treatment against fibrosis.[97] Activated hepatic stellate cells (HSCs) display increased FXR SUMOylation, which renders FXR unable to bind OCA. Prevention of FXR SUMOylation, in combination with OCA treatment, effectively reduces HSC activation and hepatic fibrosis formation in mice.[97] In addition, FXR/SUMO1 complex formation decreases FXR binding and recruitment to the *BSEP* and *SHP* promoters in HepG2 cells.[98]

SRC1, along with other co-activators such as PGC-1α, is responsible for hepatocyte differentiation, metabolism, and homeostasis via HNF4α regulation.[99] Due to HNF4α-directed expression of *Cyp7a1*, it is unsurprising that SRC1 impacts FXR activity. SRC1 interacts with the FXR ligand binding domain following the formation of the FXR/RXR complex.[100]

FXR transcriptional activity is regulated by glucose and O-linked-N-acetylglucosaminylation (O-GlcNAc) of the N-terminus of the AF1 domain.[93] O-GlcNAc transferase regulates FXR activity during fasting and feeding through O-GlcNAcylation at serine 72 in murine FXRα1 and human FXRα3 and serine 62 in human FXRα2.[93] Further, O-GlcNAc transferase can also modify carbohydrate-responsive element-binding protein (ChREBP) to interact with O-GlcNAc–FXR under high glucose concentrations to express glycolysis and lipogenesis genes.[101] However, in the presence of bile acids, regardless of high glucose levels, ChREBP-target gene expression is inhibited.[101] In human hepatocytes, ligand activation of FXR inhibits glucose transcription of ChREBP genes.[79]

Taken together, studies demonstrate that FXR transactivation is not only cofactor-dependent but driven by post-translational modifications of FXR and target gene environments, including epigenetic modifications. Due to the complex nature of FXR regulation, targeting of individual cofactors will likely need to be disease- or cell-dependent. Understanding of FXR posttranslational modifications, as well as cofactors that induce them, will provide key insights into the regulation of FXR transcriptional activity in a tissue-specific manner.

Beta Catenin

Beta catenin (β-catenin) is a well-known and evolutionary conserved protein shown to be important in tight-junction formation, cell proliferation, and is integral to the Wnt signaling cascade.[102] In the liver, β-catenin regulates liver homeostasis, injury repair, and tumorigenesis, and protein expression is mainly found in pericentral hepatocytes.[103–106] While a relationship has been identified, the molecular mechanism of β-catenin and FXR interactions is undefined. In mouse models of hepatocellular carcinoma (HCC), FXR and β-catenin expression patterns display an inverse relationship.[107,108] HCC patients display decreased FXR expression,[108] while β-catenin expression increases in HCC patients and human-derived HCC cell lines[109,110] compared with controls.

In mouse hepatocytes, it is thought that β-catenin sequesters FXR resulting in reduced FXR availability to promote bile acid efflux via regulating bile acid transporter expression and coactivating pregnane X receptor to regulate *Cyp3a11* gene expression.[111] GW4064 treatment in β-catenin knock-out (KO) mice, subjected to bile duct ligation, demonstrates increased RXRα and FXR binding in hepatocytes.[111] Similarly, GW4064 treatment, in an α-naphthyl isothiocyanate model of biliary injury, shows increased FXR binding to RXRα and reduced β-catenin binding to FXR.[112] Bile duct ligation of transgenic mice overexpressing hepatocyte S45D-β-catenin and low-density lipoprotein receptor 5/6 double KO mice with deficient hepatocyte Wnt signaling demonstrates similar FXR/β-catenin complex levels to WT mice following immunoprecipitation pulldown.[113] Contradictory to these findings, patients with primary sclerosing cholangitis display reduced β-catenin protein expression and mRNA expression of SHP, FXR target gene, and *Cyp7a1*, SHP target gene.[104] However, there could be other mechanisms to

downregulate the expression of these genes, such as inflammation. Taken together, these studies indicate that the FXR/β-catenin complex inhibits hepatocyte FXR function, and due to peri-central protein expression pattern of β-catenin, exploration of interzonal and portal hepatocyte FXR should be further studied.[103,106] However, the formation of this complex may be transient, depending on injury caused by experimental cholestasis model, hepatocyte zonation, or ligand activation.

G Protein Pathway Suppressor 2

G protein pathway suppressor 2 (GPS2) is an epigenetic modifier and is considered one of the core subunits, along with silencing mediator of retinoid and thyroid receptors and nuclear receptor corepressor (NCOR), of the chromatin corepressor complex.[114,115] The role of GPS2 is critical in regulating transcription, e.g., the regulation of macrophage plasticity is conducted by tightly regulated chromatin remodeling and transcription regulation via the chromatin corepressor complex containing GPS2.[116] In murine models of nonalcoholic steatohepatitis (NASH), GPS2 has been shown to promote steatosis by antagonizing peroxisome proliferator-activated receptor α (PPARα) transcriptional activity with the corepressor, NCOR.[114] It has also been shown that hepatocyte GPS2 is required for hepatitis C virus replication in Huh-7 cell lines.[117]

For bile acid regulation, GPS2 manifested a gene-specific regulation of *CYP7A1* and *CYP8B1* expression. Where functions to enhance SHP-mediated suppression of *CYP7A1* gene transcription, GPS2 can recruit P300/CREB binding protein complex to the HNF4α response element and interact with FXR to form an enhancer/promoter loop for increased expression of *CYP8B1* in HepG2 cells.[118] While little is known about the role of hepatic GPS2 in cholestasis, further investigation of the FXR/GPS2 complex may provide insight into its regulation of FXR activity.

Glucocorticoid Receptor

Glucocorticoid receptor (GR), a member of the nuclear receptor superfamily, is activated by glucocorticoids in the cytoplasm and translocates to the nucleus to activate various transcriptional pathways.[119] GR activation promotes anti-inflammatory signaling but can lead to cholestasis and insulin resistance.[120] The formation of FXR/GR complex prevents FXR-directed SHP expression through recruitment of C-terminal binding protein to the *SHP* promoter in HepG2 cells.[120] Hepatic GR activation increases autocrine regulation of *Cyp7a1* through FGF21 secretion[121] and activation of FXR increases glucocorticoid secretion in WT mice.[122] More investigation is required to understand the FXR and GR interaction during FXR activation.

Recent Advances of the FXR Proteome

Utilization of global FXR agonists in primary biliary cholangitis and NASH patients remains controversial due to severe adverse effects such as pruritus, fatigue, and increased serum low-density lipoprotein.[123–125] In preclinical settings, inhibition of mast cell FXR reduces serum histamine levels and prevents bile duct damage in a murine model of mast cell-induced cholestasis.[58] In a murine model of nonalcoholic fatty liver disease, caffeic acid phenethyl ester treatment reduces steatosis through decreased bacterial bile salt hydrolase activity and increased tauro-β-muricholic acid, an endogenous FXR antagonist.[126] To prevent off-target effects of FXR agonism, the field must turn to understanding the tissue- and cell-specific roles of FXR.

Various research groups have explored the FXR interactome through chromatin immunoprecipitation (ChIP) with a greater focus on hepatic[15,127,128] than intestinal FXR.[129] Below we briefly describe seminal studies on the FXR proteome.

ChIP Insights

In humans, the dominance of FXR isoforms in the liver affects FXR activation responses.[15] Diseased livers from patients with NASH, cirrhosis, and HCC have increased FXRα1 isoform expression with preferential binding to the IR-1 DNA motif.[15] IR-1 binding by FXRα1 regulates bile acid metabolism/transport and inflammatory signaling. Patients with healthy or steatotic livers express increased FXRα2 with increased binding to everted repeat spaced by 2 nucleotides (ER-2) binding motif, shown pictorially in ►**Fig. 1**.[15] In vitro exploration of HepG2 cells overexpressing FXRα1 or FXRα2 confirms preferential binding to IR-1 or ER-2 regulatory regions, respectively.

In mice, FXR binds IR-1 motifs at intergenic and intron regions, with additional clusters of FXR binding within 1–2 kb of transcription start sites.[128–130] FXR Re-ChIP analysis demonstrates that FXR/RXR co-occupancy of the SHP promoter is unchanged following FXR activation, despite a marked increase in SHP mRNA expression.[128] In normal and obese mice treated with GW4064, activated FXR represses a large amount of binding motifs identified by ChIP sequencing (ChIP-seq), which challenges previous understanding that SHP represses genes following FXR activation.[128] In vitro, FXR/RXR transcriptional activity increases with LRH-1 transfection and FXR/LRH-1 complex has been detected following co-immunoprecipitation.[130] Based on these findings, FXR transcriptional activation may depend on isoform expression, cofactor interaction, disease setting, and ligand binding.

A recent study of the hepatic FXR proteome demonstrates that cistrome, epigenetic, and protein forces regulate the specific biological pathways studied in various disease models.[127] Based on analysis of publicly available databases, LRH-1, retinoic acid receptor α (RARα), and GA-binding protein (GABPA) interact with FXR to direct its intracellular protein trafficking, protein metabolism, and cell cycle functions.[127] Conversely, Foxa1/2, nuclear factor interleukin 3 (NFIL3), RAR-related orphan receptor α (RORα), GR, NCOR1, and HNF1α interact with FXR to regulate lipid and steroid, amino acid, and carbohydrate metabolism.[127] It is important to recognize that many transcriptional regulators are shared between these two sets of FXR functions. In the WT mouse liver, only complexes with CCAAT/enhancer-binding protein

β (CEBP), GATA binding protein 4 (GATA4), HNF1α, GR, and RXRα are confirmed to interact with FXR following rapid immunoprecipitation mass spectrometry of endogenous proteins, also called RIME.[127]

One of the greatest unmet needs in the field is understanding the regulation of intestinal versus hepatic FXR function. Enterohepatic ChIP-seq reveals that only 11% of total FXR DNA binding sites are shared between the liver and intestine accounting for 1,713 genes.[129] Moreover, FXR binds 4,248 unique genes in the liver and 3,406 unique genes in the intestine.[129] The most enriched liver transcription pathways include metabolic and biosynthetic processes while the intestine is enriched for catalytic activity and oxidoreductase activity following FXR activation in WT mice. It has been found that mouse livers contain IR-1 DNA motifs while intestine presents with both IR-1 and ER-2.[129] These results suggest an organ-specific transcriptome is dependent on DNA regulatory element motifs. Further investigation of FXR proteome formation, duration, and ligand dependency, in liver and intestine, will allow researchers to develop targeted therapeutics to enhance specific FXR functions.

Discussion

We have outlined the concerted efforts of transcriptional regulators in the diverse functions of FXR activation in the liver and intestine, summarized in ►Tables 1 and 2, and highlighted known binding partners of tissue-specific FXR isoforms, summarized in ►Fig. 1. However, to the best of our knowledge, few intestinal FXR proteome studies have been published to date. Increased focus on defining the intestinal FXR proteome may assist in identifying FXR protein complexes for therapeutic functions due to DNA binding heterogeneity and potential unique protein interactions. Similarly, there is little knowledge of the FXR proteome in key bile acid facing cells like cholangiocytes, endothelial cells, and renal cells. Understanding FXR function through its binding partners in these few but impactful cells will help researchers attenuate adverse effects of global FXR agonism. Continued practice of open-access ChIP-seq datasets, as done with the FXR super-signaling atlas that combines multiple single datasets into an interactive platform,[131] can inspire researchers to solve the FXR proteome puzzle.

Table 1 Posttranslational modifications of FXR

Enzyme, modification	Modification target	Function	Reference
PMRT1, methylation	Promoter region	Increases BSEP and SHP mRNA expression Increases FXR transcriptional activity Increases conjugated bile acids (liver)	88,94
p300, acetylation	Promoter region, FXR lysine 157 and lysine 217	Increases SHP expression Prevents FXR/RXRα dimerization	66,89
SIRT-1, deacetylation	FXR	Increases FXR transcriptional activity Promotes FXR/RXRα dimerization	66
SUMO1, SUMOylation	FXR	Decreases FXR binding to BSEP and SHP promoters	98
Src kinase, phosphorylation	FXR tyrosine 67	Increases FXR transcriptional activity	95
O-linked-N-acetylglucosamine transferase, O-GlcNAc	FXR serine 62 or 72, isoform-dependent	Increases glycolytic and lipogenic gene expression (in absence of FXR ligands)	79,93,101

Abbreviation: FXR, farnesoid X receptor.

Table 2 FXR binding partners

Binding partners	Detection method	Function	Reference
RXRα	EMSA, ChIP, co-IP, ALPHA	Increases FXR transcriptional activity	65,67,70
HNF4α	ChIP-Seq	Unknown biological significance	77
β-catenin	ChIP	Inhibits FXR transcription through inhibitory complex formation	104,113
GPS-2	Yeast two-hybrid interaction screening	Increases *Cyp7a1* and *Cyp8b1* expression	118
GR	ChIP, Co-IP	Represses FXR transcriptional activity and reduced hepatic gluconeogenesis	120,121
SRC1	Protein crystallization	SRC1 binds FXR ligand binding domain in FXR/RXRα complex	100

Abbreviations: ALPHA, amplified luminescence proximity homogenous assay; ChIP, chromatin immunoprecipitation; ChIP-seq, ChIP sequencing; Co-IP, co-immunoprecipitation; EMSA, electrophoretic mobility shift assay; FXR, farnesoid X receptor.

Conclusion

While we believe that deciphering tissue-specific FXR proteomes is the key to understanding the tissue-specific FXR function, the role of chromatin structure, FXR isoform expression, hepatocyte liver zonation, and DNA binding affinity cannot be ignored. The recruitment of FXR activators results in histone modification and chromatin remodeling, beyond the initial euchromatin opening by tissue-specific pioneer factors, to allow the expression of target genes. Moreover, FXR isoform expression and their protein and DNA binding affinity also impact FXR transcriptional activity and ligand activation. Liver zonation may influence FXR function through cofactor expression, ligand secretion, and downstream FXR gene expression. Effort must be made to combine research in chromatin environment, DNA binding motifs, and proteome analysis to push the field of nuclear receptor biology forward.

Conflict of Interest
None declared.

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