Nuclear Receptors and Liver Disease: Summary of the 2017 Basic Research Symposium

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The nuclear receptor superfamily contains important transcriptional regulators that play pleiotropic roles in cell differentiation, development, proliferation, and metabolic processes to govern liver physiology and pathology. Many nuclear receptors are ligand-activated transcription factors that regulate the expression of their target genes by modulating transcriptional activities and epigenetic changes. Additionally, the protein complex associated with nuclear receptors consists of a multitude of coregulators, corepressors, and noncoding RNAs. Therefore, acquiring new information on nuclear receptors may provide invaluable insight into novel therapies and shed light on new interventions to reduce the burden and incidence of liver diseases. (Hepatology Communications 2018;2:765-777)

The Nuclear Receptor Superfamily

Humans have a total of 48 nuclear receptors while mice have 49.(1) These nuclear receptors can be categorized into seven different subfamilies and are classified as NR0 to NR6 (Table 1). The classical steroid hormone receptor family includes the estrogen receptor (ER), progesterone receptor, androgen receptor, glucocorticoid receptor (GR), mineralocorticoid receptor, thyroid receptor, vitamin D receptor (VDR), and retinoic acid receptor (RARα/β/γ). The adopted orphan receptor family includes farnesoid X receptor (FXR), liver X receptor (LXR), pregnane X receptor (PXR), peroxisome proliferator-activated receptors (PPARα/γ/δ), and retinoid X receptor (RXRα/β/γ). Another class of nuclear receptors is known as enigmatic orphan receptors, which have identified ligands, but ligand-dependent regulation has not been firmly established. Members of these orphan receptor class include constitutive androstane receptor (CAR), estrogen-related receptor (ERRα/β/γ), hepatocyte nuclear factor (HNFα/c), liver receptor homolog 1 (LRH-1), and RAR-related orphan receptors (RORα/β/γ).{(1-4)

Abbreviations: ABCB, adenosine triphosphate-binding cassette subfamily B; BA, bile acid; BCL2, B-cell lymphoma 2; BSEP, bile salt export pump; CAR, constitutive androstane receptor; CREB1, cyclic adenosine monophosphate responsive element-binding protein 1; Cyp7A1, cholesterol 7-alpha hydroxylase; DBD, DNA-binding domain; DEX, dexamethasone; ER, estrogen receptor; FAO, fatty acid oxidation; FXR, farnesoid X receptor; Gly-MCA, glycylic-β-muricholic acid; GR, glucocorticoid receptor; HFD, high-fat diet; HNF, hepatocyte nuclear factor; Insig2, insulin-induced gene 2; LBD, ligand-binding domain; LDL, low-density lipoprotein; lncRNA, long noncoding RNA; Lpaat3, lyophosphatidylcholine acyltransferase 3; LRH-1, liver receptor homolog 1; LXR, liver X receptor; MEG3, maternally expressed gene 3; miR, microRNA; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ob, obese; OCA, obeticholic acid; P1, P2, promoter 1 or 2; PC, phosphatidylcholine; Pkc, protein kinase C; PPAR, peroxisome proliferator-activated receptor; PTBP1, polypyrimidine tract-binding protein 1; PXR, pregnane X receptor; RAR, retinoic acid receptor; ROR, retinoic acid receptor-related orphan receptor; SETDB2, SET domain bifurcated 2; SHP, small heterodimer partner; SREBP, sterol regulatory element-binding protein; TGR5, G protein-coupled bile acid receptor 5; VDR, vitamin D receptor; VSG, vertical sleeve gastrectomy; WT, wild-type.

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The proteins of the nuclear receptor superfamily all share a common structure and contain the following domains: i) an N-terminal domain that contains the activation function 1 for interaction with cofactors; ii) a highly conserved DNA-binding domain (DBD) that binds to specific sequences of DNA, called hormone response elements; iii) a variable hinge region that connects the DBD with the ligand-binding domain (LBD); and iv) an LBD that is unique for each nuclear receptor. The LBD also contains activation function 2, which is dependent on the presence of bound ligand and v) a C-terminal domain(1) (Fig. 1).

Basic Mechanisms of Nuclear Receptor Function

Nonalcoholic fatty liver disease (NAFLD) is associated with altered nuclear receptor function and perturbations along the gut–liver axis. These perturbations include obesity, increased intestinal permeability with systemic inflammation, abnormal hepatic lipid metabolism, and insulin resistance.(4) PPARα is activated in the liver in the fasted state and has roles in coordinating fatty acid oxidation.(5) In contrast, FXR is activated under the fed state by bile acids (BAs) to maintain BA homeostasis. FXR also has direct effects on suppression of gluconeogenesis and lipogenesis.(6) The study by Lee et al.(7) demonstrated that treatment with a PPARα agonist activated autophagy in wild-type (WT) mice under fed conditions; this was absent in Pparα knockout mice. In contrast, pharmacological activation of FXR suppressed autophagy in the fasted state, which was not observed in Fxr knockout mice. The study suggests that these receptors have mutually antagonistic impacts on autophagy and that these effects were induced by PPARα but suppressed by FXR. Furthermore, the authors identified hepatic secretome as another target for the regulation of liver energy balance. FXR can also activate the secretion of complement and coagulation factors in human hepatocytes,(8) while PPARα can transcriptionally repress the secretome.(7) Therefore, PPARα and FXR have opposing roles that act in concert to regulate liver energy balance through the regulation of autophagy and protein secretion.

Accumulative evidence has implicated LXR as an important regulator of lipid metabolism and inflammation. LXR participates in cholesterol and fatty acid metabolism in liver and exerts both positive and negative control of a wide range of metabolic and inflammatory genes.(9) Phospholipids are important lipid molecules that have been implicated in determining the biophysical properties of the membrane. Lyso- phosphatidylcholine acyltransferase 3 (Lpcat3) is an LXR-responsive phospholipid that regulates fatty acid and cholesterol absorption in the liver. Loss of Lpcat3 decreases phosphatidylcholine (PC) in liver membrane to reduce membrane fluidity and impair very low-density lipoprotein secretion.(10,11) Mice lacking the Lpcat3 gene in the intestine show impaired fatty acid transport in enterocytes as well as reduced chylomicron production.(12) Furthermore, mice on the high-fat diet (HFD) had increased production of gut hormones but decreased food intake despite starvation, indicating the importance of membrane phospholipid acyl chain composition in dietary lipid intake.

LXRs are oxysterol-activated transcription factors that up-regulate a number of genes involved in cholesterol and lipid homeostasis.(8) Hepatic activation of LXR can increase plasma triglyceride levels, which are under the control of LXRα activation.(13,14) LXR also mediates sterol regulatory element-binding protein (Srebp)1c transcriptional activity in response to increased hepatic sterol levels. Conversely, SREBP-1c expression and lipogenesis were decreased in mice...
lacking LXRα and LXRβ. LXR can modulate Srebp1c processing independently of transcription through the induction of Lpcat3. Furthermore, loss of Lpcat3 expression can reduce lipogenesis in mice. Lpcat3 activity also contributes to the abundance of polyunsaturated PC species in obese (ob)/ob liver ER. In response to feeding, the abundance of ER polyunsaturated PC is increased in the absence of changes in Lpcat3 messenger RNA (mRNA).(12) The ER phospholipids, linoleoyl and arachidonoyl PCs, are stimulators of SREPB maturation, levels of which are regulated during feeding and in diet-induced obesity. Therefore, phospholipid composition could be used as a regulatory strategy to facilitate lipid transport and metabolic homeostasis in response to nutrient availability.

The transcriptional response is highly cell specific and can be achieved on multiple levels with chromatin structure and accessibility implicated as a key step. Although many advances have been made in recent years, the role of chromatin structure in the regulation of genes by nuclear receptors is only beginning to be understood. The liver is the major site of metabolism that can respond to nutritional cues. In response to fasting, many transcription factors are activated to regulate various genes involved in metabolic pathways to restore normal homeostasis. There are four major transcription factors that regulate the fasting response: GR, cyclic adenosine monophosphate responsive element-binding protein 1 (CREB1), PPARα, and CCAAT/enhancer binding protein beta. CREB and GR, are activated by glucagon and glucocorticoids, while PPARα is activated by fatty acids.(15)

Two transcription-regulating modules were identified following an ex vivo approach to examine fasting-induced genes.(15) During early fasting, blood glucose levels decrease, leading to a reduction in insulin secretion and a rise in glucagon secretion and the activation of CREB1.(16) Upon subsequent secretion of cortisol, GR is activated and augments CREB1-dependent gluconeogenesis. However, with prolonged fasting, GR induces PPARα expression for activation of fatty acid oxidation (FAO)/ketogenesis gene expression. Therefore, two modules operate sequentially during the fasting response in the liver: 1) GR–CREB1 in the gluconeogenic module and 2) GR–PPARα in the FAO/ketogenic module. The FAO/ketogenic module operates through a transcription factor cascade whereby GR induces PPARα gene expression.(17) In contrast, the gluconeogenic module functions through a rapid and dynamic loading mechanism. This model demonstrates that the binding of the transcription

![FIG. 1. Protein domains and activation of nuclear receptors. (A, top) Schematic amino acid sequence of a nuclear receptor. N-terminal domain (A/B), DBD (C), hinge region (D), LBD (E), and C-terminal domain (F). (A, bottom) Schematic diagram for nuclear receptor dimerization and DNA binding. (B) Schematic diagram of ligand-dependent activation of nuclear receptors. Without ligands, nuclear receptors interact with the corepressor complex and there is limited gene expression. With ligand binding, nuclear receptors kick off the corepressor complex and recruit the coactivator complex to activate gene expression. Abbreviation: AF, activation function;](image-url)
factor is not randomly distributed but rather through specific binding spots. The fasting-related transcription factors tend to bind in the same region and assist each other’s loading onto DNA to promote binding of a second factor.\textsuperscript{(17,18)} Therefore, using specific tools to monitor chromatin structure and transcription binding can dissect out the intricate regulation of fuel production by hormonal signals.

The PPAR subfamily members are major regulators of lipid metabolism.\textsuperscript{(19)} Hepatic expression of PPARz mRNA is normally low in humans and in murine models of steatosis and NAFLD, and its activation improves steatosis, inflammation, and fibrosis in rodent models of nonalcoholic steatohepatitis (NASH).\textsuperscript{(20)} On the other hand, PPAR\gamma has roles in adipose tissue and is the master regulator of adipogenesis.\textsuperscript{(21)} Genetic variations in the noncoding regulatory DNA can cause different phenotypic differences among individuals, leading to differences in NAFLD progression. Raymond Soccio’s group identified genome-wide binding regions for PPARz in liver from two strains of mice, C57BL/6J and 129S1/SvImJ, which had different genetic susceptibility to obesity. The group stimulated PPARz activity in mice by fasting or with the agonist drug fenofibrate, and strain-dependent gene expression patterns were observed in the liver. Fenofibrate selectively induced two main classes of genes in one strain. The first class of genes had basal expression that did not differ between strains in the absence of fenofibrate. This suggests that strain-selective PPARz sites were found to be enriched near the transcription start site of the strain with the higher ligand-activated expression as if these regulatory sites were driving the ligand response. The second class of genes had differences in basal expression such that the ligand selectively induced expression in the strain with lower starting levels. These findings demonstrate the different signatures of regulatory genetic variation between different mouse strains.

Nuclear Receptors in Hepatic Lipid Metabolism

LRH-1 has diverse roles in hepatic cholesterol and glucose metabolism. Liver-specific \textit{Lrb-1} knockout mice have reduced glycolytic flux and lipogenesis as a result of impaired glucokinase activity.\textsuperscript{(23)} 1,2-Dilauroyl-sn-glycero-3-phosphorylcholine, a phospholipid that binds to and induces LRH-1 activity, can improve glucose tolerance and insulin resistance without any overt effects on body weight in diabetic mouse models.\textsuperscript{(24)} On the other hand, mice heterozygous for \textit{Lrb-1} had a mild but significant increase in body weight in response to an HFD but did not display changes in glucose homeostasis or lipid metabolism.\textsuperscript{(25)} These studies suggest that receptor activation plays a pertinent role in the metabolic effects reported as opposed to loss of LRH-1. However, the direct link between LRH-1 and fatty liver disease is not well understood. A recent study by Holly Ingraham’s group\textsuperscript{(26)} demonstrated the use of an adeno-associated virus vector 8–thyroid hormone-binding globulin viral system to delete LRH-1 in adult livers of mice. The group demonstrated increased hepatic lipid accumulation in adeno-associated virus vector-8 LRH-1 treated mice while liver injury and altered glucose metabolism were further enhanced with an HFD. Furthermore, replacing mouse with human LRH-1 prevents the development of steatosis and improves glucose homeostasis in HFD-fed mice. This study suggests a role for LRH-1 and phospholipids in fatty liver disease, and further studies will be required to develop a targeted and safe approach that could be useful to treat these hepatic disorders.

HNF4z is a highly conserved member of the nuclear receptor superfamily. The expression of the gene is driven by two promoters and gives rise to many splice variants/isoforms. The promoter (P) 1 is active in the adult liver driving expression of splice variant HNF4z1-6 and gives rise to two major isoforms, HNF4z1 and HNF4z2. Meanwhile, P2 drives expression of splice variant HNF4z7-9 but is only expressed in fetal livers and in hepatocarcinoma. Many target genes that are associated with xenobiotics, drug metabolism, and glucose homeostasis have been identified for HNF4z. HNF4z also regulates hepatic gluconeogenesis and lipid metabolism where the proximal P1 predominates.\textsuperscript{(27)}

In the study by Frances Sladek’s group,\textsuperscript{(28)} the effect of HNF4z isoforms on liver function was examined in exon swap mice that expressed either P1- or P2-HNF4z in liver. RNA sequencing and HNF4z chromatin immunoprecipitation sequencing analyses were used to identify isoform-specific target genes. The
group found that HNF4α was one of the most abundantly expressed transcription factors in the liver and that P2-HNF4α yielded transcriptomes that were similar to fetal livers and hepatocellular carcinoma. Thus, the use of genome wide assays, such as protein-binding microarrays, provide a powerful approach to identify direct targets of HNF4α.

A number of animal and human models have demonstrated that intestinal microbiota contribute to the development of NAFLD. Transplantation studies demonstrate that microbiota from lean humans led to mice becoming resistant to obesity whereas mice receiving microbiota from obese humans were prone to obesity. Thus, the gut microbiota may significantly influence the pathogenesis of metabolic diseases. FXR regulation has been proposed as a link to the liver–gut axis in modulating NAFLD. FXR is highly expressed in the liver and intestine and is activated by primary human and murine BAs, chenodeoxycholic acid, and cholic acid, while the rodent–specific primary BA tauroursodeoxycholic acid antagonizes FXR activation. Furthermore, activation of FXR by BAs induces fibroblast growth factor 15/fibroblast growth factor receptor 4 signaling to repress cholesterol 7-alpha hydroxylase (Cyp7A1) expression. Additionally, antagonizing FXR stimulation increased insulin sensitivity, attenuating the expression of lipogenic genes, and suppressing inflammation. 

A recent study demonstrated the use of a BA derivative, glycine-β-muricholic acid (Gly-MCA), as a potential therapeutic application due to its resistance property in gut bacterial bile salt hydrolase. In mice, oral treatment with Gly-MCA prevents the development of diet-induced obesity and steatosis and improves glucose tolerance and insulin sensitivity. Gly-MCA was found to selectively inhibit the intestinal FXR signal and regulate BA composition and ceramide metabolism. The authors then evaluated the role of the intestinal FXR–ceramide axis in response to Gly-MCA treatment. Gly-MCA treatment prevents intestine-specific FXR-null mice from HFD-induced obesity, metabolic dysregulation, and improved beige fat biogenesis through the inhibition of the intestinal FXR–ceramide axis. Furthermore, ceramide treatment reversed the action of Gly-MCA in HFD-fed mice. These results suggest that Gly-MCA may be a candidate for the treatment of metabolic disorders. Therefore, targeting FXR in the intestine could make it a promising strategy for the treatment of metabolic diseases, such as NAFLD.

**Nuclear Receptor Crosstalk With Noncoding RNAs**

Small heterodimer partner (SHP) is an orphan nuclear receptor that contains the dimerization and LBD similar to other family members but lacks the conserved DBD. SHP has been shown to be involved in BA synthesis, lipid and cholesterol metabolism, glucose homeostasis, apoptosis, and cell invasion. SHP has also been identified as a mediating factor in the metabolic circadian clock. In addition, SHP acts as a transcriptional repressor of microRNA expression and function through interaction with other nuclear receptors. Accumulative evidence demonstrates the involvement of long noncoding RNAs (lncRNAs) in lipid metabolism, microbial susceptibility, nuclear reprogramming, and epigenetics.

The anti-apoptotic protein B-cell lymphoma 2 (BCL2) was highly induced in cholestatic liver injury. Overexpression of BCL2 in mice was reported to disrupt BA metabolism and resulted in cholestatic liver fibrosis and up-regulated lncRNA H19 expression. Another lncRNA, maternally expressed gene 3 (MEG3), has been reported to be a potential tumor suppressor, with loss of function observed in cancer studies. RNA immunoprecipitation demonstrated that RNA-binding protein polypyrimidine tract-binding protein 1 (PTBP1) interacts with MEG3. Interestingly, putative binding sites for PTBP1 within the coding region of SHP have also been reported. Expression of MEG3 in hepatocellular carcinoma cells...
regulation of lipopolysaccharides and interferon-viral and anti-inflammatory response through negative with gene silencing and has linked SETDB2 to an anti-suppressor of variegation 3-9 homolog 1 (SUV39H1), domain-containing lysine methyltransferases that include (SETDB2) is a member of the KMT1 subfamily of SET G9a, and SETDB1. H3K9 methylation is associated homeostasis. SETDB2 was also found to induce expression in a feedback regulatory fashion. These under fasting conditions, nuclear accumulation. Because INSIG2a is localized at the endoplasmic reticulum, it traps the SREBP chaperone–SREBP complex in the endoplasmic reticulum preventing SREBP translocation, thereby preventing the activation of lipogenic genes. Therefore, induction of Insig2a by GR-SETDB2 contributes to the negative regulation of lipogenesis during fasting. SETDB2 was also found to induce expression in response to DEX challenge during the refeeding cycle and in the livers of ob/ob mice. Levels of SREBP were also significantly elevated, which promote lipogenesis. The glucocorticoid-dependent increase in Insig2a also reduced nuclear accumulation of SREBPs. The results from this study link glucocorticoids and GR directly to lipid metabolism by crosstalk of SETDB2 and INSIG2. This indicates that SETDB2 could be a potential target to modulate glucocorticoid action in metabolic diseases associated with altered glucocorticoid sensitivity or in patients undergoing chronic glucocorticoid treatment.

BA receptors FXR and G protein-coupled bile acid receptor 5 (TGR5) regulate gene expression involved in the synthesis and transport of BAs and thus are major modulators of BA homeostasis and enterohepatic circulation. In addition, FXR has beneficial roles in triglyceride and cholesterol homeostasis and glucose metabolism. Bariatric surgeries, including vertical sleeve gastrectomy (VSG), has been proposed as an effective method for treatment of obesity and diabetes and can significantly improve the development of NAFLD and NASH. In particular, obese mice that received VSG had reduced hepatic steatosis. However, the molecular mechanism for the improved effects in response to VSG is not known but may suggest a potential role for BA receptors FXR and TGR5. Du et al. performed a genome-wide profiling of chromatin accessibility and gene expression in C57BL/6J mice on an HFD after VSG or sham surgery. VSG mice had significant weight loss following surgery; this was associated with reduced hepatic triglyceride levels and improved steatosis. Furthermore, there were changes in inflammatory and metabolic genes in livers after VSG. Several other studies have reported changes in BA composition following VSG. In Fxr null mice, the beneficial effects of VSG were absent following surgery. Furthermore, TGR5 was identified as a molecular target of VSG and was necessary for VSG-induced weight loss and improvements of fatty liver disease. Interestingly, Shp knockout mice that underwent VSG lost weight but developed hepatic inflammation.

Interestingly, the microRNA miR-26a has been identified as one of the downstream targets of TGR5 that may provide beneficial metabolic effects. Recent studies have shown that miR-26 controls glucose and lipid metabolism. Global and liver-specific transgene of miR-26a in mice prevented obesity-induced insulin resistance, reduced lipid accumulation, and elevated hepatic glucose production. Furthermore, miR-26a target genes are associated with insulin signaling (glycogen synthase kinase 3β [Gsk3β], protein kinase C δ [Pkcδ], and Pkcθ), fatty acid metabolism (acylcoenzyme A synthetase long chain family member 3
Acsl3 and Acsl4), and gluconeogenesis (phosphoenolpyruvate carboxykinase 1 [Pck1] and transcription factor 7-like 2 [Tcf7l2]). These findings suggest that activating TGR5 by small molecules to regulate liver metabolism may have potential therapeutic applications for NAFLD. 

Nuclear Receptors as Therapeutic Targets

Nuclear receptors are at the cross roads of metabolism, inflammation, and regeneration and represent attractive therapeutic targets. They control a broad range of processes, including BA metabolism and transport (relevant for cholestasis disease), inflammation, microbiota, innate and adaptive immunity (relevant for autoimmune liver and inflammatory bowel diseases), cell proliferation (relevant for liver regeneration and cancer), glucose homeostasis and lipid metabolism (relevant for obesity and type 2 diabetes), and drug metabolism (impact on drug hepatotoxicity). Importantly, the rapidly progressing prevalence of liver disease will require novel therapeutics to prevent or treat disease progression (Table 2).

A wealth of data over the last 2 decades has shown that BAs can function as signaling molecules to regulate their own synthesis as well as other metabolic processes. The regulatory action of BAs are mediated by specific BA receptors, including FXRs, VDRs, PXR, CAR, and members of the G-protein coupled receptor superfamily. Activation of these receptors results in the repression of BA synthesis, induction of phase I and II BA hydroxylation, and conjugation as well as stimulation of alternative BA export while limiting BA import. In addition, FXR also has beneficial roles in triglyceride and cholesterol homeostasis and glucose metabolism. As a consequence, FXR ligands have been identified as new therapeutic options in a wide range of diseases related to metabolic, inflammatory, and immune-modulated disorders. An FXR agonist has been shown to be beneficial in several preclinical models of NASH/NAFLD; this has been attributed to its metabolic actions using natural ligands, semisynthetic modified BAs, or synthetic nonsteroidal molecules. Human studies are ongoing with the BA-derivative agonist obeticholic acid (OCA), which improves insulin resistance and decreases liver fibrosis markers. OCA has also been approved for treatment of primary biliary cholangitis and is in late clinical studies for NASH.

Some nuclear receptors have been pharmacologically targeted in metabolic disorders, including those from the PPAR family. Fibrates have been used clinically for treatment of dyslipidemia; however, fibrates differ in their relative affinities for PPAR isoforms. Treatment with known PPARα agonists (fibrates) have been shown to induce fatty acid metabolizing enzymes
to improve NAFLD, whereas PPARγ agonists (glitazones) promote adipose fat storage, decrease circulating free fatty acids and improves insulin sensitivity. Furthermore, treatment with glitazones can improve NASH features in WT mice fed a methionine- and choline-deficient diet. New selective PPAR modulators have been developed to overcome the limitations of the current fibrates. These include the dual PPARα/γ agonist aleglitazar and the dual PPARα/δ agonist elafibranor (GFT505). These dual agonists can improve insulin resistance and fasting hypertriglyceremia and low-density lipoprotein (LDL) cholesterol levels as well as liver enzymes in obese and insulin-resistant males and in rodent NASH and fibrosis models.

Other nuclear receptors may also have therapeutic applications, including VDR. Vitamin D knockout mice spontaneously developed liver fibrosis, while mice fed a vitamin D-deficient diet developed severe hepatic steatosis and inflammation, demonstrating that NAFLD may be associated with vitamin D deficiency. However, clinical studies on vitamin D supplementation on NAFLD pathogenesis have been limited; thus, more studies are needed to provide conclusive results. On the other hand, studies have shown that PXR can activate de novo lipogenesis in an SREBP1c-dependent manner. Clinical studies showed that the anti-epileptic drug phenobarbital can activate PXR/CAR. Phenobarbital improved insulin resistance by suppressing gluconeogenesis through CAR, whereas a PXR agonist might impair glucose tolerance in healthy volunteers receiving rifampicin. However, separating out the beneficial metabolic effects from the adverse effects is challenging. The RORs gamma 2 (RORγt) and REV-ERB α/β, which are regulators of the circadian clock and lipid metabolism, may also provide therapeutic benefits. To date, no clear clinical data have shown promising results on targeting these nuclear receptors; thus, the need to do additional testing may provide alternative means for nuclear receptor-related therapies in liver disease.

The human gastrointestinal tract harbors a community of approximately $10^{14}$ microorganisms living within the human host. Interaction with the host/microbiome and disease entities, such as NAFLD, obesity, and diabetes, can provide insight into developing new therapies to target metabolic diseases. Current research has demonstrated that BAs can modulate pathways associated with inflammation, cholesterol, and lipid homeostasis, and the microbiota may play a role in orchestrating these events.

BAs are synthesized in the liver as primary BAs and are transformed by the intestinal microbiota into a variety of metabolically active metabolites. The primary and secondary BAs act as agonists or antagonists for nuclear receptors, including FXR, CAR, PXR, LXR, VDR and G-protein-coupled receptors. Hepatic expression of Cyp7A1 and Cyp8b1 is regulated by FXR, which is highly expressed in both ileum and liver. Intestinal FXR also regulates hepatic Cyp7A1 through fibroblast growth factor 15-dependent mechanisms. Therefore, the gut microbiota modulates FXR signaling in gut by repressing activity of Cyp7a1 in liver. A wide range of studies in microbiome research has offered insight into the multifactorial mechanisms through which metabolic pathologies arise; these may provide new prospects for development of novel therapeutics and targets within human and mouse models. Therefore, modulating BA composition may be the key to the microbiome–host health pathway in the development of metabolic disorders.

Recent studies have shed light on the use of pharmacological targets for treatment of cholestatic liver disease. These agents affect BA signaling, the enterohepatic circulation of BAs. Several agents function as specific agonists for various nuclear receptors to control BA homeostasis. In particular, the FXR agonist obeticholic acid has been shown to improve cholestasis in patients with primary biliary cirrhosis who were non-responders or intolerant to ursodeoxycholic acid; obeticholic acid is now approved for use by the U.S. Food and Drug Administration.

Furthermore, genetic defects in specific canalicular transport proteins resulting from mutations in adenosine triphosphate 8B1 (an aminophospholipid flippase
important for maintaining asymmetry of the plasma membrane), adenosine triphosphate-binding cassette subfamily B member 11 (ABCB11; encoding the bile salt export pump [BSEP]), and ABCB4 (encodes the multidrug resistance protein 3 for biliary secretion of phospholipids) can disrupt hepatic bile flow. These defects are usually present in infancy with cholestasis and associated problems, like pruritus, and progressive cholestasis can eventually lead to liver failure. A number of drugs have now been tested, including 4-phenylbutyrate, an approved pharmacological chaperone that has been shown to retarget some mislocalized BSEP mutants at the plasma membrane and partially correct BSEP mutant targeting and improve pathologic liver injuries. Additionally, loss of ABCB4 function can be rescued by the clinically approved potentiator ivacaftor; however, there have been reports of worsening of liver disease when used in combination with other agents. The potential use of chaperone therapy in progressive familial intrahepatic cholestasis may hold great promise in targeting cholestatic liver diseases; however, more research is warranted to better understand the risks and benefits of using such therapy.

PXR and CAR are recognized as xenobiotic receptors that transcriptionally regulate expression of phase I and phase II drug/xenobiotic enzymes and transporters. PXR and CAR share the same ligands and regulate an overlapping set of target genes and associated pathways. Aside from their involvement in drug metabolism, PXR and CAR can also regulate other signal transduction pathways and diseases, including cancer, diabetes, and inflammatory and liver diseases.

Activation of CAR can suppress or reverse adiposity in various mouse models of obesity by suppressing lipogenic genes, reducing triglyceride levels, and improving hepatic steatosis. This is due to decreased SREBP expression as a result of CAR activation by inducing target gene Insig-1. Other studies also show that CAR activation can decrease LDL cholesterol levels in LDL-receptor null mice and inhibit atherosclerotic lesions. Treatment with CAR agonist 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) attenuated diet-induced obesity and diabetes in animal models through decreased hepatic gluconeogenesis.

In contrast to CAR, activation of PXR appears to promote steatosis, obesity, and insulin resistance due to increased fatty acid uptake, synthesis, and decreased β-oxidation in a SREBP-independent manner. In PXR-humanized mice, obesity and glucose intolerance were reported when compared to WT mice fed an HFD. Furthermore, long-term stimulation of humanized PXR by rifaximin, a nonabsorbed antibiotic PXR ligand in humans, resulted in hepatic steatosis due to up-regulation of intestinal fatty acid binding protein and clusters of differentiation (CD)36 expression. In WT mice, PXR activation increased very low-density lipoprotein and LDL levels in a PXR-dependent manner, with increased atherosclerosis in apolipoprotein E null mice. On the contrary, Per deficiency decreased atherosclerosis in apolipoprotein E mice; this was associated with decreased CD36 expression and oxidized LDL uptake in peritoneal macrophages.

CAR activation ameliorates the hallmarks of metabolic syndrome, conferring the properties of CAR as a promising therapeutic target for metabolic disease. However, CAR activation also promotes liver hyperplasia and carcinogenesis. On the other hand, CAR activation by phenobarbital or its derivative TCPOBOP in mice has been shown to mediate hepatic toxicity. Thus, separating out the metabolic benefits of CAR from its adverse effects may be beneficial to treat metabolic diseases. Alternatively, herbal medicines may also activate CAR without any adverse effects. PXR activation exerts anti-inflammatory effects so PXR agonists may also ameliorate metabolic disease associated with chronic immune activation. Natural PXR agonists from herbal medicine and marine species could also serve as a potential therapeutic agent for development of novel PXR modulators, but further characterization is warranted. Thus, the continuing search for selective PXR and CAR modulators may provide novel therapeutic tools for the management of metabolic diseases.

**Concluding Remarks**

Nuclear receptors are essential in understanding the physiology and pathobiology of liver diseases. The dysregulation of nuclear receptor signaling contributes to the pathogenesis of NAFLD/NASH by impacting the integrated control of energy/nutrient metabolism through the gut–liver–adipose axis and inflammatory signaling, placing nuclear receptors at the forefront of therapeutic interventions. Therefore, pharmacological modulation of nuclear receptors is expected to attenuate or reduce hepatic steatosis, inflammation, fibrosis, insulin resistance, and obesity. However, some nuclear receptor ligands often exhibit paradoxical effects. The development of more potent and more stable single
agonist or antagonist and the possible combination of therapies may provide an alternative solution. Improvement of organ/tissue specificity should also be considered for efficacy of drugs to stimulate/inhibit nuclear receptors. Furthermore, strategies targeting epigenetic and posttranscriptional modulations and stimulation of nuclear receptor counterparts, such as co-activators, might also provide promising therapeutics in the future.

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