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Review Article

Bile Acids: A Bridge Linking Gut Microbiota and NAFLD

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Nowadays, nonalcoholic fatty liver disease (NAFLD) becomes the most common cause of liver disease worldwide. Mounting evidence indicates that dysbiosis contributes to the pathogenesis of NAFLD. Bile acids (BAs), the molecules that are first synthesized in hepatocytes and further metabolized by gut microbes, can either circulate in enterohepatic system or be found in circulations to exert various effects. Dysbiosis brings about the dysregulated BA composition, which is also observed in the pathology of NAFLD. As important signaling molecules, BAs bind to broadly expressed bile acid receptors (BARs) and play diverse roles in biological activity. Energy metabolism, immune system, and intestinal barrier function are affected by changes in BAs and their signaling pathways, which may explain the mechanisms of how altered BA pool affect NAFLD. Several novel NAFLD treatments targeting BA signaling are under development and their challenges and limitations are also discussed in this review.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) refers to a condition characterized by excessive fat accumulation in the liver without any clear causes, such as alcohol use, being identified. NAFLD with early pathological changes and weaker disturbance on the liver function is subdivided as nonalcoholic fatty liver (NAFL). As the risk factors persist, it can gradually progress into a more critical stage classified as nonalcoholic steatohepatitis (NASH), which is typically complicated by liver inflammation and cirrhosis [1]. Additionally, NASH is not the terminal stage of the disease process but a trigger for more severe pathological changes in the liver, such as fibrosis and hepatic cell carcinoma (HCC) when not treated and controlled properly [1]. NAFLD has been estimated as the most common liver disease worldwide, affecting 25% of the global population. Researchers have also focused on a sharp increase in prevalence of NAFLD from 15% to 25% in 10 years [1], which is consistent with the surging global morbidity of obesity and type 2 diabetes mellitus (T2DM), the two major risk factors of NAFLD [2]. Furthermore, there has been plenty of evidence supporting the participation of gut microbiota in NAFLD development, and the introduction of this new factor makes the pathogenesis of NAFLD more integral [3].

The gut microbiota is an ecological community of commensal, symbiotic, and pathogenic microorganisms colonized in human gut, whose normal composition and function are of great importance to our health. Recent studies have shown that metabolic diseases such as obesity, diabetes, and the metabolic syndrome are closely related to gut dysbiosis [4]. NAFLD as well is strongly associated with microorganisms in digestive tract as shown by mounting evidence [3]. With the same embryonic origin, the gut and liver have multiple aspects of associated interdependence in terms of anatomy and function [5]. The gut-liver axis refers to the reciprocal interaction between the gut, as well as its microbiota, and the liver. This

mutual relationship is founded by the portal vein, which allows the transport of gut-derived metabolites directly into the liver, and the feedback route of bile secretion from the liver to the intestine. Theories have been raised to explain the gut-liver axis in NAFLD and the interaction with the microbiota, especially for the pathogenic role of microbiota-derived metabolites in the pathogenesis and development of NAFLD, such as trimethylamine, short-chain fatty acids, ethanol, and secondary bile acids [6]. Among a variety of potential mechanisms of how gut microbiota influences NAFLD, bile acids (BAs), which connect the gut and liver with enterohepatic circulation, have become a new focus.

BAs, molecules produced by hepatocytes, take an essential part in fat absorption, specifically in emulsifying and solubilizing processes [7]. The primary BAs like cholic acid and chenodeoxycholic acid are the hepatocyte-generated products of cholesterol oxidation [7]. Further, the conjugated BAs form when the primary BAs are combined with glycine and taurine in the hepatocytes, which are water-soluble in the duodenum to exert their effect on fat absorption [8]. In the intestine, the microbiota metabolizes the primary BAs into secondary BAs, such as deoxycholic acid, lithocholic acid, and ursodeoxycholic acid [7]. Approximately 95% of BAs are actively reabsorbed by enterocytes and transported back to the liver, which is called enterohepatic circulation of BAs. Moreover, BAs can also be found in the circulation and perform peripheral effects including energy metabolism regulation by acting as important signaling molecules [9]. It has been reported that the bile acid signaling pathway is impaired in patients with NAFLD and dysbiosis [10], which suggests that BAs are vital in linking dysbiosis and NAFLD.

In this review, we summarize the mechanisms of the interaction between gut microbiota and NAFLD from the view of BAs in detail. Hopefully, our understanding of such mechanism can pave the way for new treatment of NAFLD from the aspect of BAs and raise a new focus on the reciprocal interaction between BA and gut microbiota in the fundamental research field of NAFLD.

2. Gut Microbiota and NAFLD

The link between gut microbiota and NAFLD has long been discussed. Both studies on animals and humans have identified a significant interaction of gut microbiota with hepatic steatosis, and more specifically, reduced diversity of gut flora is commonly measured in NAFLD [10]. High-fat diet- (HFD-) fed mice have been reported to have increased *Proteobacteria* and reduced *Akkermansia* in their gut [11]. Intriguingly, fecal microbiota from normally fed or cured mice transplanted to HFD-fed ones alleviated HFD-induced hepatosteatosis, which credibly proved the impact of gut microbiota on liver fat accumulation [12]. In the intestine of human NAFLD cases, 16S rRNA sequencing has described an increase in *Firmicutes* and a reduction in *Bacteroidetes* with one accord, while an opposite trend is observed when NAFLD is suppressed [13, 14]. A study from Wong et al. comparing fecal bacteria content of 16 NASH patients with 22 controls revealed an increased abundance of *Parabacteroides* and *Allisonella*, as well as a decreased abundance of *Faecalibacterium* and *Anaerosporobacter* in NASH [15]. Yang et al. have demonstrated generally richer fecal *Lactobacillus* and *Firmicutes* but a lower level of *Ruminococcaceae*, a family of phylum *Firmicutes*, among NAFLD patients [13]. However, a consensus on microbiome signature is still to be reached on levels inferior to phyla.

On the other hand, numerous attempts aiming to alleviate NAFLD in HFD-fed mice by changing the composition of gut microbiota have been proved successful. *Lactobacillus rhamnosus* GG (LGG) treatment has been found to reduce adipose tissues in the liver, mesenteric and subcutaneous of HFD-fed mice, and reverse the diversity shifts of gut microbes caused by HFD [16]. Prebiotics, a class of substances that have clear-cut regulatory effects on gut microbiota, are also unfolding their therapeutic value. A recent study from Zhang et al. has revealed that resistant dextrin addition for HFD-fed mice improves their hepatic mitochondrial integrity and reactive oxygen species accumulation with increased abundance of *Parabacteroides*, *Blautia*, and *Dubosiella* in their guts [14]. Several other prebiotics, including Astragalus polysaccharides (APS), Lycium barbarum polysaccharides (LBP), and inulin (INU), similarly ameliorate hepatic steatosis in mice [13, 16]. INU and fructooligosaccharide (FOS) are also proved effective in clinical trials [17], manifesting as decreased nonalcoholic fatty liver activity score (NAS). In general, gut microbiota serves as a key hinge on the pathological chain of NAFLD, despite some room for discussion on the detailed mechanisms.

3. Enterohepatic Circulation of Bile Acids

3.1. Synthesis, Transport, and Metabolism of Bile Acids. Bile acids are produced in the human liver and synthesized by hepatocytes, assisted by 17 enzymes [18], among which the cytochrome P450 superfamily plays the most important role in the enzyme-catalysed reactions. Bile acids are synthesized via cytochrome P450-mediated oxidation of cholesterol, and the process can be realized through two biosynthetic pathways called "classical pathway" and "alternative pathway" [7]. Classical pathway is the major approach of BA synthesis to produce primary BAs including cholic acid (CA) and chenodeoxycholic acid (CDCA). Cholesterol 7a-hydroxylase (CYP7A1), which specifically works in classical pathway, catalyses the initial catabolic reaction of cholesterol and serves as the only rate limiting factor of bile acid synthesis regulation in the liver. Subsequently, microsomal sterol 12α-hydroxylase (CYP8B1) works in the synthesis of CA, while CDCA is synthesized through sterol 27-hydroxylase (CYP27A1) instead of CYP8B1. Alternative pathway, which takes the secondary position in BA synthesis (about 18%), is initiated by CYP27A1 and continuously catalysed by oxysterol 7α-hydroxylase (CYP7B1) to generate CDCA [7]. The digestive fluid composed of CA, CDCA, and other components is excreted to tiny bile canaliculi, which is known as the beginning of biliary passages, and then drains into common hepatic duct via the interlobular bile duct and hepatic duct. Stored in gallbladder, bile is excreted to the intestine when eating. At the lumen of terminal ileum, about 95% of BAs are actively taken up back to the liver via the superior mesenteric vein efficiently, leaving only approximately 5% (about 0.5 g/d) in the colon [19].

3.2. Regulation of Bile Acids by Gut Microbiota. Involvement of gut microbiota in regulating BA homeostasis has long been emphasized. The ability of gut microbiota to biotransform BA such as deconjugation, oxidation, dehydroxylation, and desulfation is central to the BA homeostasis. For the deconjugation, the main effective bacterial genera includes *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, *Clostridium*, and *Listeria*, which produce bile salt hydrolases (BSHs) and deconjugate taurine and glycine groups in primary BAs that are produced in the liver [7]. In terms of oxidation, bacterial genera like *Bacteroides*, *Clostridium*, *Eubacterium*, *Escherichia*, *Eggerthella*, *Peptostreptococcus*, and *Ruminococcus* produce BA hydroxysteroid dehydrogenases (HSDHs) to convert toxic BA into ursodeoxycholic acid (UDCA), which is less toxic to human cells and more water-soluble [20]. As for the dehydroxylation, *Clostridium* and *Eubacterium* of the *Firmicutes* phylum can generate BA 7α-dehydroxylase that converts primary BAs (CA and CDCA) into secondary BAs (deoxycholic acid, lithocholic acid, and UDCA) [21]. Deoxycholic acid (DCA) and lithocholic acid (LCA), the two most abundant secondary BAs in humans [22], are known to have notable biological effects on prevention of *Clostridium difficile* outgrowth [23], induction of hepatocellular carcinogenesis [24], and modulation of host metabolic and immune responses [7]. Lastly, multiple gut bacteria such as *Clostridium* sp. Strain S2 can increase the desulfation of BAs with the help of the sulfatase it produces [25]. Desulfation of BAs by gut microbiome benefits BA reabsorption and is essential for maintaining the homeostasis of the BA pool [26].

4. Bile Acid Signaling

Aside from their role in digestion and absorption, BAs have been found to act on BA receptors (BARs) as a kind of signaling molecules. The farnesoid-X receptor (FXR) and the G protein BA-activated receptor- (GPBAR-) 1 are the two most discussed BARs.

FXR belongs to the nuclear receptor superfamily of transcription factors and is mainly activated by primary BAs. CDCA is the most efficient endogenous ligand of FXR, and other natural ligands include DCA, LCA, and CA [27]. Similar to other members of the nuclear receptor superfamily, the function of FXR relies on its direct binding with DNA [28]. The activation of FXR basically induces a negative feedback on BA synthesis, through the inhibition of CYP7A1 [29]. The contribution of FXR in immunity has also gradually become apparent in recent years. Researches from Campbell et al. and Hang et al. have reported that FXR inhibits the differentiation of regulatory T (Treg) cells and counteracts macrophage effector functions, inducing the maintenance of tolerance of the hepatic immune system towards antigens and xenobiotics originating from the intestine [30, 31], and evidence has also shown that abnormal FXR activity is involved in intestine innate immunity disorder and disabled Paneth cell function in inflammatory bowel disease (IBD) cases [32]. As for metabolic regulation, FXR has been reported to attenuate lipogenesis by modulating transcriptional activity of sterol regulatory element-binding protein (SREBP) 1c and improve glucose tolerance as well [33].

GPBAR-1, also known as Takeda G-protein-coupled receptor 5 (TGR5), is a member of the G protein-coupled receptor (GPCR) superfamily. In contrast to FXR, secondary BAs including DCA and LCA are efficient endogenous ligands of GPBAR-1 [34]. GPBAR-1 has been reported to regulate metabolism by alleviating HFD-induced obesity and insulin resistance in mice [35], and its hepatoprotective effect has been gradually unraveled [36]. Furthermore, the activation of GPBAR-1 in immune cells, such as macrophages, suppresses their proinflammatory cytokine production, showing a potential solution to inflammatory diseases, including atherosclerosis, sclerosing cholangitis, and colitis [37].

There are two other members of BARs whose mechanism and function are worth a mention. The vitamin D receptor (VDR) or known as the calcitriol receptor is able to bind with LCA at relatively lower affinity [38]. VDR participates in bone metabolism and regulation of calcium and phosphate homeostasis, and its mutation and dysfunction are recognized as an important cause of hereditary vitamin D-resistant rickets [39]. The pregnane X receptor (PXR), another nuclear receptor superfamily member, is initially discovered to regulate the transcription and expression of cytochrome P450 3A4 (CYP3A4), an enzyme that removes toxins and drugs from the body [40]. Several recent studies have reported the impact of PXR on lipid metabolism and blood pressure control through the regulation on 4β-hydroxycholesterol (4βHC), indicating that the PXR-4βHC axis is a putative therapeutic target for metabolic syndrome and hypertension [41].

5. Dysbiosis, Bile Acids, and NAFLD

Dysbiosis, which refers to the disruption of the normal gut microbiota, has been implicated in a plethora of diseases including NAFLD. Evidence linking dysbiosis to the pathogenesis of NAFLD has accumulated rapidly during the past few decades, and the role of BA signaling in this course has been supported by several studies [10]. As summarized above, gut microbiota plays an important role in BA biotransformation, which is crucial for maintaining the homeostasis of BAs. Thus, the disruption of normal intestinal microbiota can impair the balance of BA pool. Hence, the BAs may be an indispensable element linking dysbiosis to NAFLD.

5.1. Dysbiosis and NAFLD. Dysbiosis can result from multiple factors including environmental, immunological, or host factors as well as alterations in bile flow, gastric pH, or intestinal dysmotility. The first study reporting the link between dysbiosis and NAFLD has found intestinal microbial overgrowth and a higher TNF-α level in NAFLD patients [42]. Further evidence also indicates a shift in microbial composition in NAFLD, which will be elaborated in the following section.

Animal experiments studying changes of microbiota provide strong evidence supporting the role of dysbiosis in the pathogenesis of NAFLD. A recent study has found that diet-induced dysbiosis in mice facilitates NASH development by damaging the gut vascular barrier (GVB) [43]. Zeng et al. have unraveled that the advanced liver steatosis is concurrent with an elevated level of secondary BA-producing bacteria (e.g., *Lactobacillaceae*/*Lachnospiraceae*) in mice with HFD [44].

Furthermore, researchers have reported that the antibiotic treatment alleviates HFD-induced glucose intolerance, hepatic steatosis, and inflammation in hamsters. Specifically, the study has found elevated CYP7B1 in the livers of antibiotic-treated hamsters, contributing to a more hydrophilic BA profile with increased tauro-β-muricholic acid (TβMCA). This study potentially determines the role of gut microbiota-mediated BA metabolism in modulating diet-induced glucose intolerance and hepatic steatosis in the hamster [45].

Evidence linking dysbiosis and NAFLD can also be found in humans. Mounting researches have reported a close relationship between gut microbiota shifts and the progression of NAFLD in patients from children to adults. A research evaluating the association between dysbiosis and severe NAFLD lesions, including NASH and fibrosis in a population of adult NAFLD, has identified *Bacteroides* as independently associated with NASH and *Ruminococcus* with significant fibrosis, showing that microbiota analysis may appear as a novel predictor of NAFLD severity [46]. In support of these findings, the metabolic products of microbiota also seem to differ between patients and the healthy, which include BAs, short-chain fatty acids, and endogenous ethanol [47, 48]. Especially the BAs, as synthesized in the liver and modified by gut microbiota, may play an intermediary role between gut microbiota and NAFLD. A study has found that higher serum and fecal BA levels are associated with advanced fibrosis in NAFLD, and the changes of the BA profile are associated with specific gut bacteria [47]. For instance, *Bacteroides* sp. and a *Ruminococcus* strain could enhance BA 7α-dehydroxylation to upregulate the production of 7α-dehydroxylated secondary bile acid DCA and LCA [49]. Likewise, researchers have also reported that circulating GCA and DCA levels are significantly higher in NAFLD patients [48]. Together, these findings confirm the role that gut microbiota and BAs play in the progression of NAFLD.

5.2. Mechanisms Linking Bile Acids to NAFLD

5.2.1. Liver Metabolism

(1) Lipid Metabolism. By binding to FXR and TGR5, BAs play a critical role in lipid metabolism. FXR activation stimulated by agonists like CDCA reduces fat synthesis through the FXR-SHP-SREBP-1 pathway [50], PPARγ receptors, and related enzymes of fatty acid synthase (FAS) and stearoyl-CoA desaturase (SCD) [51]. On the other hand, FXR activation can inhibit apolipoprotein (Apo) CIII, the gene that suppresses catabolism of triglyceride-rich lipoprotein [52], and promotes fibroblast growth factor 19 (FGF19, FGF15 in mice), the downstream factor of FXR that restrains the expression of acetyl-CoA carboxylase-β (ACCβ) to increase fatty acid oxidation in mice [53], and thus increases fatty acid decomposition. In addition, FXR can also reduce lipid absorption to control hepatic lipids (Figure 1) [54].

As mentioned above, BAs play an important role in regulating liver fat homeostasis through FXR. Thus, inhibition of FXR leads to abnormal lipid metabolism, resulting in NAFLD, which has been further proved by studies with applications of FXR agonists in NAFLD. UDCA, a natural ligand of FXR, can increase fatty acid β-oxidation and reduce SREBP-1c expression to activate sirtuin-1 and reduce hepatic steatosis, inhibiting lipid accumulation in hepatocytes to improve NAFLD [55]. WAY-362450, a potent and orally active FXR agonist, suppresses the expression of hepatic lipid droplet protein adipose differentiation-related protein (ADRP) to significantly reduce triglyceride accumulation in liver and serum triglyceride in mice [56]. Another nonsteroidal molecule GW4064 markedly represses the expression of CD36, which transports long-chain fatty acids into the adipose and hepatic tissues, reducing the levels of triglyceride, free fatty acid, and cholesterol in the liver to curb hepatic steatosis [57].

(2) Glucose Metabolism. NAFLD has been shown to be closely related to metabolic syndrome, and insulin resistance plays a critical role in it [1]. Bile acids take vital part in modulating glucose metabolism through FXR and TGR5. FXR activation induced by BAs increases the expression of the small heterodimer partner (SHP) in the liver and the levels of circulating FGF19 in the gut, which stimulates hepatic protein and glycogen synthesis, and thus affects glucose and energy homeostasis [58, 59]. Additionally, activation of TGR5 by BAs promotes intestinal L cells secreting glucagon-like peptide-1 (GLP-1), which acts on the pancreatic β cells to regulate insulin secretion stimulated by blood glucose change [60]. TGR5 signaling in L cells induces mitochondrial oxidative phosphorylation and raises the ATP/ADP ratio, then closes the ATP-dependent potassium channel (KATP), and enhances mobilization of intracellular calcium, finally resulting in secretion of GLP-1 and improvement in glucose homeostasis [61]. Further animal experiments have shown that overexpression of TGR5 in HFD-fed mice enables glucose-stimulated insulin secretion to be improved by comparison with wild-type mice, and the use of TGR5 agonists could reduce hepatic glucose production in obese and diabetic mice (Figure 1) [61].

Many researchers have used BA receptor agonists to confirm the therapeutic effect of BA regulating glucose metabolism on NAFLD. GW4064 not only curbs hepatic steatosis but also blocks insulin resistance through decreasing the transcription of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, key enzymes in gluconeogenesis, to suppress hyperinsulinemia and hyperglycemia [57]. OCA has been proved to activate FXR, mediating the increase of FGF19 and thus improving insulin sensitivity [62]. RDX8940, a novel orally administered TGR5 agonist, induces the secretion of GLP-1 to improve insulin sensitivity in a model of NAFLD and mild insulin resistance [63].

5.2.2. Immune Regulation. NASH is characterized by the activation of the immune system, in which the representative effectors are Kupffer cells and recruited macrophages. Studies have also noted that natural killer T cells are key players in macrophage recruitment, and both natural killer T cells and T cells contribute to the progressive liver disease [64]. By regulating the immune components, BAs have gathered increasing attention from studies on immune regulation [65]. In view of this, the dysfunctional BA metabolism in NAFLD patients may contribute to the dysregulated

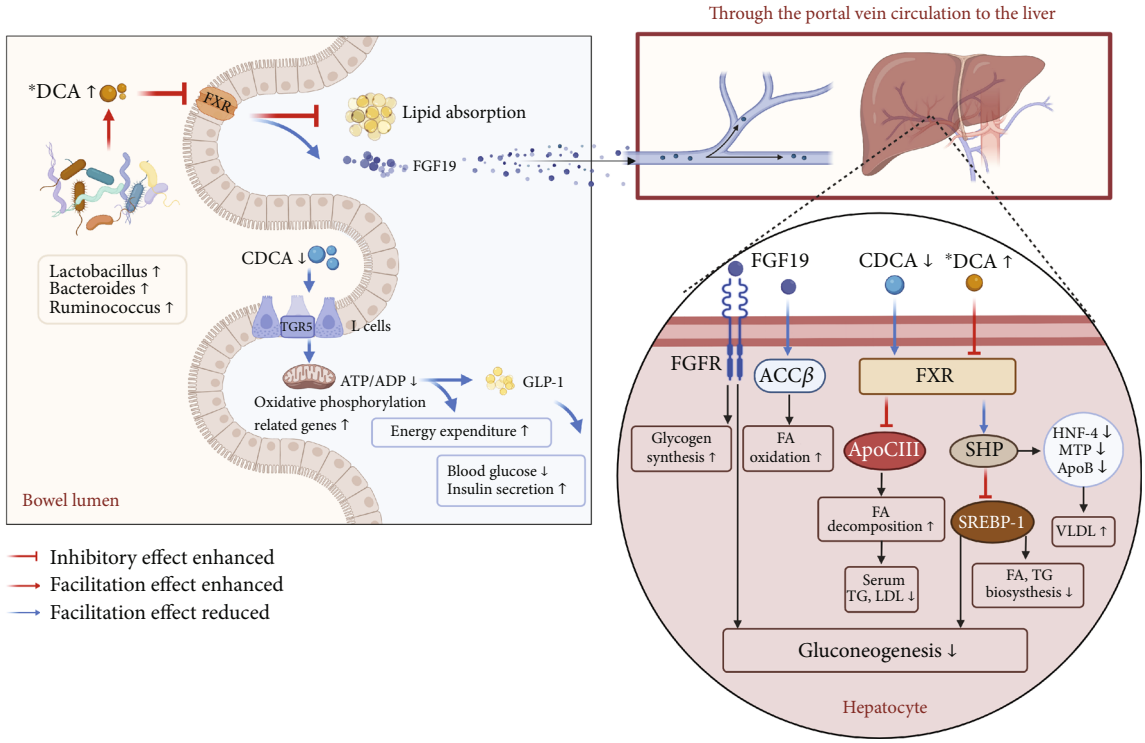

Figure 1: The mechanism of how changed BAs affected liver metabolism. The altered content of DCA and CDCA disrupted the normal liver glucose and lipid metabolism. The increased availability of a substance is depicted by an upwards arrow and the decreased availability by a downwards arrow. BA: bile acid; DCA: deoxycholic acid; CDCA: chenodeoxycholic acid; FXR: farnesoid-X receptors; TGR5: Takeda G-protein-coupled receptor 5; FGF 19: fibroblast growth factor 19; GLP-1: glucagon-like peptide-1; FA: fatty acid; TG: triglyceride; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; ACCβ: acetyl-CoA carboxylase-β; FGFR: fibroblast growth factor receptor; SHP: small heterodimer partner; SREBP: sterol regulatory element-binding protein; Apo: apolipoprotein; HNF-4: hepatocyte nuclear factor-4.

immune reaction in the liver and thus exacerbates NAFLD (Figure 2).

(1) Kupffer Cells. Kupffer cells (KCs) are tissue-resident macrophages that localize within liver sinusoids, accounting for about 10% of total liver cells [66]. The overactivation of KCs plays a central part in the development of NASH, through the recruitment of other immune cells and the excretion of proinflammatory cytokines [66]. An animal study has found that treating the mice with a combination of CDCA and CA, which are primary BAs, has a protective effect on the onset of fructose-induced hepatic steatosis. This treatment with BAs exerts its effect by modulating cytokines and proteins that are linked to inflammation, which includes normalizing the markers of KC activation [67]. In obstructive cholestasis, research has pointed out that BAs, especially the hydrophobic BAs, reversibly suppress the KC activation [68]. In NAFLD patients, researchers have reported the increased abundance of *Escherichia* and *Bilophila* in their gut microbiota, which metabolize taurine and glycine, suggesting an increase in secondary BAs. They have also found that the proportion of CDCA decreases in the BA pool of NAFLD patients [10]. Thus, in NAFLD patients, the decreased proportion of primary BAs may lead to a weakened protective effect of Kupffer cell activation, therefore playing a role in the development of NASH.

In response to diverse signals, macrophages may experience either classical M1 activation or alternative M2 activation. Classical M1 activation, stimulated by toll-like receptor ligands and interferon-γ, exerts proinflammatory effects. Oppositely, M2 activation that stimulated by IL-4/IL-13 has anti-inflammatory effects [69]. Thus, the balance of Kupffer cells M1/M2 polarization regulates inflammation in the liver [66], especially the excessive M1 KC, by releasing inflammatory mediators, contributing to the pathogenesis of liver steatosis, recruitment of inflammatory immune cells, and the activation of fibrogenesis [66]. TGR5 is expressed in KCs and thus exerts its immune regulatory effects. The activation of TGR5 in KCs potentially has anti-inflammatory effects through inhibiting ROS production, secretion of proinflammatory cytokines, and M1-predominant polarization of Kupffer cells, via suppressing NF-κB signaling and activating nuclear factor 2 (Nrf2)/HO-1 signaling [70]. Moreover, animal studies have revealed that TGR5 modulates macrophage differentiation and infiltration in both adipose tissue and liver, which in turn reduces metabolic inflammation [71].

(2) T Cells. Besides, the composition of multiple hepatic T cell subsets, i.e., T helper (Th) cells, regulatory T (Treg) cells, and cytotoxic T (Tc) cells, as well as several innate T-cell subsets, also plays a vital role in the pathogenesis of NAFLD. Studies have found that concerning NAFLD, both Th22 and

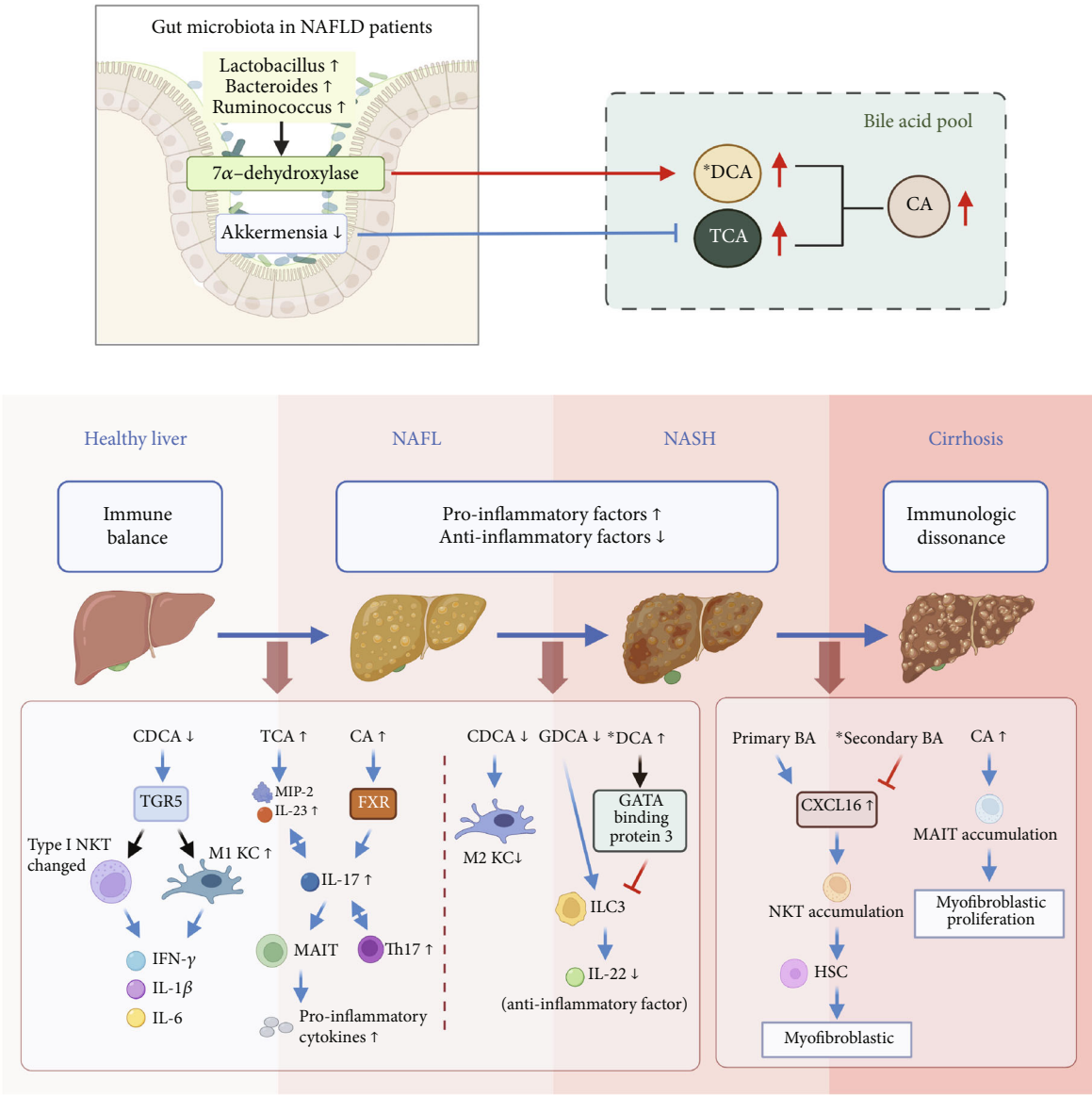

Figure 2: The mechanism of altered BA pool promoting progression of NAFLD through immune regulation. The imbalanced BA pool caused by dysbiosis in NAFLD patients disturbed the immune balance and contributed to the progression of NAFLD. BA: bile acid; TCA: taurocholic acid; CA: cholic acid; GDCA: glycodeoxycholic acid; DCA: deoxycholic acid; CDCA: chenodeoxycholic acid; FXR: farnesoid-X receptors; TGR5: Takeda G-protein-coupled receptor 5; KC: Kupffer cell; MAIT: mucosal-associated invariant T cell; Th17: T helper 17 cell; ILC: innate lymphoid cell; NKT: nature killer T cell; IFN: interferon; IL: interleukin; MIP: macrophage inflammatory protein.

Treg cells seem to have an overall tempering effect, whereas Th17 and Tc cells appear to exacerbate liver damage and fibrosis progression [72]. In particular, IL-17 that is produced by Th17 has been implicated in NAFLD pathogenesis [73], while the tight link between microbial BA metabolism and adaptive immunity has been found recently [74]. In the studies of cholestasis, BAs have been found to alter the function of T cells directly [31, 75]. During cholestasis, BAs that include taurocholic acid (TCA) are found to upregulate macrophage inflammatory protein 2 (MIP-2), IL-23, and other cytokines, as well as their interaction with IL-17A, contributing to hepatic inflammation and Th17 infiltration [75]. Accordingly, a published study has reported elevated levels of TCA after HFD in mice and found that TCA may be a serum biomarker of liver injury [76]. Moreover, two metabolites of LCA, 3-OxoLCA and isoalloLCA, are identified as T cell regulators [31]. 3-OxoLCA inhibits the Th17 differentiation by directly binding to the transcription factor retinoid-related orphan receptor-γt (RORγt) and isoalloLCA increases the Treg cell differentiation through producing mitochondrial reactive oxygen species (mitoROS), leading to an increase in forehead box P3 (FOXP3) expression, which has been further confirmed by treating mice with these two LCA metabolites [31]. This study suggests that by modulating the

balance of Th17 and Treg cells, LCA plays a critical role in immune regulation. Further, Song et al. [77] have reported that another Treg population that expresses RORγ, e.g., RORγ+ Treg cells, which play an important role in maintaining colonic immune homeostasis, is modulated by BAs, including intestinal dominant primary BAs as well as certain potential secondary BAs, e.g., LCA/3-OxoLCA, through the BA receptor VDR.

(3) Mucosal-Associated Invariant T (MAIT) Cells. MAIT cells, as an innate-like T cell population, are significantly enriched in circulation and human livers, which constitute up to 20-50% of all intrahepatic T cells and are mainly located at bile ducts in the portal tracts [78]. MAIT cells are closely related to the pathogenesis of NAFLD, while their precise effect has been reported to be controversial. In obese patients, preferentially recruited MAIT cells aggregate in adipose tissue and produce a significant amount of IL-17; thus, peripheral MAIT cells decrease [79]. In patients with NASH-related cirrhosis, although the frequency of MAIT cells in both circulation and liver is reduced, the circulating MAIT cells appear as an activated phenotype and hepatic MAIT cells accumulate in the mesenchymal space with the fibrotic septa [80]. In a study of CCl4-induced liver fibrosis, MAIT cells have been found to have profibrogenic functions by promoting the proliferation of hepatic myofibroblasts and intensifying their proinflammatory properties through the production of TNF-α [80]. However, another study has reported decreased frequency and altered function of circulating MAIT cells with increased production of IL-4 whereas the decreased production of IFN-γ and TNF-α, along with an elevated number of MAIT cells in the livers of NAFLD patients compared to healthy controls. They have also found that mice deficient in MAIT cells exhibit more severe hepatic steatosis and inflammation upon methionine- and choline-deficient diet (MCD), along with increased M1 and decreased M2 in livers, which indicates that MAIT cells protect against inflammation in NAFLD by producing regulatory and inducing anti-inflammatory macrophage polarization [81].

Bile acids, however, have been reported to have regulatory effects on the function and activation of MAIT cells. A clinical study has revealed that BA concentrations, especially of conjugated BAs, are negatively associated with the number of activated MAIT cells [82]. In hepatocyte cell line L02, CA has been found to upregulate IL-7 expression by inducing FXR binding to the IL-7 promoter, which induces signal transduction and activation of transcription 5 (STAT5) phosphorylation in MAIT cells to increasingly produce inflammatory cytokines and granzyme B [83]. Furthermore, a previous study has shown that MR1-/- knockout (KO) mice (lacking MAIT cells) harbor a unique microbiota that is resistant to antibiotic disruption. In stool samples of KO mice, increases in TCA intensity and decreases in DCA intensity are found and are associated with depletion of certain gut bacteria [84]. This study indicates potent relations among BA, gut microbiota, and MAIT cells. Together, though the effects of BAs on MAIT function and activation have been partially studied, more precise role of MAIT in the pathogenesis of NAFLD and how BAs regulate MAIT cells in NAFLD are worthy of further exploration.

(4) Nature Killer T (NKT) Cells. NKT cells, which take the major proportion of liver innate-like lymphocytes, are mainly divided into invariant NKT (iNKT) and noninvariant NKT cells. iNKT cells, also called type I NKT cells, are particularly enriched in liver of mice and humans, while their effects on the progression of NAFLD seem to be controversial. On the one hand, iNKT cells have displayed a protective effect against liver inflammation and the progression to liver fibrosis in HFD-induced NASH mouse models, but not against steatosis [85]. The high-fat or high-sucrose diet induces the apoptosis of NKT cells in the liver of mice, leading to decreased NKT cells and promoting liver inflammation through an excessive production of IFN-γ and TNF-α [86]. On the other hand, in animal models of MCD-induced NASH, NKT cells accumulate through Hedgehog (Hh) pathway activation, and NKT-derived factors stimulate hepatic stellate cells (HSC) to become myofibroblast, promoting fibrosis progression [64]. The recruitment and adhesion of NKT cells in liver are regulated by the Hh-dependent production of chemokine ligand 16 (CXCL16) and expression of vascular cell adhesion molecule 1 (VCAM-1) by immature ductular cells [64], while fibrosis is prevented in CD1d-deficient mice that lack NKT cells [64]. Moreover, in high-fat high carbohydrate- (HFHC-) induced NASH model, the absence of NKT cells also protects mice from progressive NASH [87]. Accordingly, in patients with NASH, the number of intrahepatic NKT cells has also been found to be significantly upregulated [64].

Gut microbiota-mediated BA metabolism as well as BA receptors has been reported to have effects on NKT cells. Primary BA, particularly CDCA, increases the expression of CXCL16, which is vital for NKT cell accumulation, while secondary BA glycolithocholic acid (GLCA) exhibits an opposite effect [88]. Researchers use antibiotic to deplete the bacteria that mediate primary-to-secondary BA conversion in mice and found an induced NKT cell accumulation [88]. This study shows an intimate connection between gut microbiota-mediated BA metabolism and NKT accumulation, which may explain how BAs affect the progression of NAFLD. Moreover, BAs, by activating GPBAR1, could also attenuate liver inflammation by targeting NKT cells in rodent models of immune-mediated hepatitis. In mouse models of hepatitis, GPBAR1-/- mice result to have a type I NKT cell phenotype that is biased toward a proinflammatory, IFN-γ producing NKT cell subtype, thus worsening severity of hepatitis [89].

(5) Group 3 Innate Lymphoid Cells (ILC3s). ILC3s are a group of innate lymphoid cells expressing RORγt to promote the production of cytokines IL-17A and/or IL-22 [90]. Among the studies targeting on NAFLD/NASH development, quite a few have elucidated the contribution of ILC3 subset. In a CCL4-induced mouse liver fibrosis model, ILC3s have been found to have profibrotic effects, and increased numbers of IL-17A$^+$ ILC3 and IL-22$^+$ ILC3 subsets are presented in the liver [91]. However, in another study feeding mice with HFD, mice deficient in ILC3s exhibit significant fatty liver and liver fibrosis and significantly increase palmitic acid levels in serum and liver. The results of this study indicate that ILC3s protect against the steatohepatitis by secreting IL-22 and

upregulating hepatic lipid metabolism [92]. IL-22, in another study, has been found to improve insulin resistance probably through adipose tissue browning. In a study of polycystic ovary syndrome (PCOS), gut microbiota-BA-interleukin-22 axis has been reported to play a critical role in regulating PCOS. Mechanistically, the study reveals that glycodeoxycholic acid (GDCA) induces the secretion of IL-22 by ILC3s through GATA binding protein 3 [93]. Feeding mice with long-term HFD induces an elevated level of DCA, which leads to a decrease of ILC3s in ileal mucosa, along with reduced ileal IL-22 concentration [94]. Thus, in NAFLD patients, the elevated level of DCA indicates the unbalance of GDCA and DCA, which leads to the decreased abundance of ILC3s as well as less secretion of IL-22, and thus promotes the hepatic inflammation and affects the normal lipid metabolism [95].

5.2.3. Intestinal Barrier Function

(1) Intestinal Barrier Failure and Bile Acids. There has been abundant evidence pointing to a strong association between BAs and integrity and permeability of the intestinal barrier, which is usually reflected in protecting the intestinal epithelium from bacterial invasion. In fact, altered intestinal barrier accompanied by bile excretion disorder has been recognized long before [96]. A clinical trial on the patients with obstructive jaundice showed an increased level of acute phase response and circulating antiendotoxin core antibodies, which is regarded as the assessment of the raised intestinal permeability, or "the leaky gut" [97]. The immunohistochemical results revealed the regional loss of the tight junction-associated protein occludin in the intestinal epithelium of bile duct-ligated rats [98]. In cirrhosis, bacterial translocation is regarded as a hallmark of poor liver prognosis and is closely related to impaired GVB and raised intestinal permeability. Research conducted by Lorenzo-Zúñiga et al. has found that cirrhotic rats are prone to intestinal bacterial overgrowth, bacterial translocation, and even endotoxemia [99]. Oral conjugated BAs have reduced bacterial overgrowth and bacterial translocation, eased endotoxemia, and eventually increased survival of mouse models [99]. Therefore, the dysfunction of the intestinal barrier in bile excretion disorder can be confidently attributed to the decreased levels of the BAs accepted by the gut.

For detailed mechanisms of the link between bile excretion and intestinal barrier, bile acid signaling, especially the activation of FXR, has been proven to be a critical supportive factor [100, 101]. Inagaki et al. [100] have measured increased ileal levels of bacteria among the mice lacking FXR and confirmed the critical role of FXR in limiting bacterial overgrowth and translocation. As described by Sorribas et al. [101], the bile duct-ligated mice develop the bacterial translocation similar to the cirrhosis ones, and a decreased thickness of the intestinal mucous layer as well as loss of goblet cells has been observed. The researchers then perform the alleviation of bacterial translocation and relieve the specific symptoms through the treatment of specific FXR agonist fexaramine (Fex) [101]. Another research has achieved a similar conclusion by using another FXR agonist OCA and proves that FXR downregulates the expression of key proinflammatory cytokines in colonic mucosa, suppressing IBD-related symptoms in mice, including mucous ulceration and hemorrhage [102]. The utilization of various FXR agonists provides strong support for the role of FXR in the maintenance of intestinal barrier and regulation of intestinal mucosal immunity. Moreover, a study suggests that the knockout of the mouse GPBAR1 gene may result in altered morphology of mucous cells and abnormal epithelial tight junctions in colons, and treating the colitis mice with GPBAR1 ligands reverses these changes [103], though the research focusing on whether GPBAR1 participates in intestinal barrier maintenance is not sufficient yet.

(2) Effects of Bile Acids on Intestinal Epithelial Tight Junction and Cell Proliferation. Some studies have turned their attention to the molecular and cellular structure of intestinal epithelial tight junction. As found by Yang et al., mice subjected to common bile duct ligation feature as lower expression of intestinal tight junction proteins zonula occludens-1 (ZO-1), claudin-1, and occludin compared to those that have underwent sham operation, whereas addition of bile into diet reserves the downregulation of these proteins and decreased intestinal permeability [104]. Further discovery suggests that obstructive jaundice in mice elevated the intestinal level of claudin-4 expression, which has been found to be closely related to intestinal barrier failure and endotoxemia [98].

A research has also pointed out that common bile duct ligation inhibits the proliferation of intestinal epithelial cells and leads to apoptosis in intestinal crypts and thinner mucosal thickness, preventing the host circulatory system from bacterial invasion [98]. Yamaguchi et al. [105] have confirmed that exposure to the bile salt taurodeoxycholate (TDCA) in physiological concentrations restores the proliferation of intestinal epithelial cells and attenuates the apoptosis. They have identified the mechanism as a proto-oncogene-c-myc-induced process [105], while another similar research attributes the effect of TDCA countering TNF-α-induced apoptosis to NF-κB activation and subsequent blocking of the caspase-dependent pathways [106]. Therefore, further characterization of specific mechanism by which bile salts interact with the intestinal barrier is to be settled.

6. Bile Acid-Based Promising Pharmaceutical Strategy

6.1. Natural FXR Agonist. UDCA, a natural agonist of FXR, has recently been regarded as a potential medication for NAFLD. Mueller et al. have revealed that UDCA is effective in reducing the levels of LDL cholesterol and hepatic triglyceride content [107]. They have proposed that UDCA administration enhances the synthesis of BAs and subsequently activates some key enzymes involved in cholesterol synthesis. Triglyceride accumulation can be observed when FXR is blunted [107]. A clinical trial showing the downregulation of UDCA on indicators of liver injury and steatosis agrees with this conclusion [108]. Moreover, some BA phospholipids, which include ursodeoxycholyl lysophosphatidylethanolamide (UDCA-LPE), have been proved hepatic protective [109]. UDCA-LPE has

shown its significant downregulation of inflammatory genes as well as changes in lipid metabolism [109]. Despite the increasingly profound understanding of how UDCA alters the hepatic lipid accumulation, the clearly positive impact of UDCA on NASH has not been affirmed by some other studies [110]. Thus, more and larger trials are needed to verify the actual NAFLD-therapeutic value of UDCA in humans.

6.2. Synthetic FXR Agonists. It has been mentioned above that BA signaling regulates the synthesis of BAs, improves nutrient metabolism, and is involved in the immune of the host digestive system. The treatments targeting the BA signaling, especially the utilization of FXR agonists, are gaining more attention in protecting the liver from adverse effects triggered by excessive fat accumulation.

OCA, also known as INT-747, is a semisynthetic 6α-ethyl-substituted analogue of CDCA with much stronger potence than CDCA for FXR activation [111]. According to a study from Schwabl et al., OCA significantly alleviates the pathological changes of hepatocytes and improves serum lipid mass spectrum. It has also been demonstrated that OCA, by driving β-catenin activation in endothelial cells, protects against GVB disruption and serves as preventive and therapeutic agent on NAFLD [112]. Moreover, a multicentre phase 3 trial has shown that OCA 25 mg notably ameliorates fibrosis and NASH disease activity [113]. The clinical adverse reactions are evaluated as acceptable [111]. A rise of circulation cholesterol after INT-747 treatment has been noticed and is considered to be caused by the inhibited cholesterol catabolism as FXR is activated [62, 111]. Some evidence also suggests the recovery of insulin sensitivity led by OCA [111]. Under investigation in multiple clinical trials and applied in clinical practices, OCA is one of the most promising medications for NAFLD [2, 114].

GW4064 is a specific synthetic agonist of FXR. A research has shown that the activation of FXR by GW4064 decreases the level of hepatic inflammation induced by endotoxin in NAFLD mice and reduces serum levels of hepatic enzymes as well as hepatic levels of apoptosis cytokine [115]. An earlier research has confirmed that GW4064 improves insulin insensitivity and raises hepatic glycogen synthesis and glycogen content. Ma et al. have detected the weakened insulin resistance and downregulated lipid transporter CD36 gene expression caused by GW4064 treatment in NAFLD mice [57]. These findings provide considerable confidence for GW4064 in the field of NAFLD treatment.

Cilofexor, formerly known as GS-9674, is another potent and selective agonist of FXR that has been evaluated effective for NAFLD. According to the research from Loomba et al., cilofexor combined with firsocostat performs significantly well among the cases with fibrosis induced by NASH as reflected by improved hepatic steatosis, liver function tests, and serological markers [116]. Compared with separate treatment, combination with firsocostat alleviates pruritus and is well tolerated [116]. Collectively, cilofexor combined with firsocostat could be a novel potential option for advanced NASH accompanied by fibrosis, and phase 2 clinical trials for cilofexor against NAFLD are under way [117].

There are a variety of additional synthetic FXR agonists with the potential to solve NAFLD. PX-102, also known as PX20606, is a nonsteroidal FXR agonist proven to be effective against fibrosis, vascular remodeling, and portal hypertension attributed to NASH [112]. A recent study has demonstrated that PX-104, an isomer of PX-102, improves insulin sensitivity and hepatic inflammatory stress after 4-week treatment in nondiabetic NAFLD patients [113]. EDP-305 is a potent isoxazole-type FXR agonist developed by Enanta Pharmaceuticals. Recent evidence has shown its inhibitory influence on liver injury and hepatic fibrosis and suppression on portal pressure in murine NAFLD models [118]. Tropifexor, or known as LJN-452, is a highly potent and nonsteroidal FXR agonist that has progressed into phase 2 clinical trials [119]. In the study, using a mouse NASH model, tropifexor administration significantly has reduced steatohepatitis, fibrosis, and the expression of profibrogenic gene. The efficacy of tropifexor at <1 mg/kg has been proven superior to that of OCA at 25 mg/kg in the liver. The emerging FXR agonists are providing novel options for the fight against NAFLD, though more investigations and trials are warranted.

7. Conclusions

BAs, as produced by hepatocytes and further metabolized by gut microbes, link the gut and liver with the enterohepatic circulation. Moreover, overgrowth of secondary BA-producing bacteria has been found in NAFLD patients, which leads to an abnormal BA pool. By binding to BARs including TGR5, FXR, and VDR that are widely expressed in the enterohepatic system, BAs exert multiple effects on metabolism, immune regulation, and intestinal barrier function, which may account for the vital role of dysbiosis-induced altered the BA pool in NAFLD pathogenesis. For the metabolism, elevated DCA and decreased CDCA inhibit FXR signaling and TGR5 signaling pathways, resulting in abnormal lipid and glucose metabolism (Figure 1). Furthermore, the dysfunctional BA metabolism in NAFLD induces excessive proinflammatory factors and reduced anti-inflammatory factors in the liver, facilitating the progression of NAFLD (Figure 2). The altered BA pool and its dysregulated signaling pathways also lead to impaired intestinal barrier through downregulating the expression of intestinal tight junction proteins.

Several therapeutic approaches against dysregulated BA metabolism and signaling have been used clinically or in animal experiments, in which FXR agonists are mostly studied. UDCA, a natural FXR agonist, and OCA as well as other synthetic FXR agonists that improve dysregulated BA signaling have been proven to be hepatoprotective against NAFLD. Based on multiple clinical trials and clinical practice, OCA is one of the most promising remedies in treating and preventing NAFLD with acceptable side effects. As for other novel synthetic FXR agonists, however, the quality and sample size of their clinical trials are often limited, indicating further studies are warranted.

Data Availability

No data are included in this review.

Conflicts of Interest

All authors declare no relevant conflicting interests.

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References

[1] D. Q. Huang, H. B. El-Serag, and R. Loomba, "Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention," *Nature Reviews Gastroenterology & Hepatology*, vol. 18, no. 4, pp. 223–238, 2021.

[2] Z. M. Younossi, "Non-alcoholic fatty liver disease - a global public health perspective," *Journal of Hepatology*, vol. 70, no. 3, pp. 531–544, 2019.

[3] F. Fianchi, A. Liguori, A. Gasbarrini, A. Grieco, and L. Miele, "Nonalcoholic fatty liver disease (NAFLD) as model of gut-liver axis interaction: from pathophysiology to potential target of treatment for personalized therapy," *International Journal of Molecular Sciences*, vol. 22, no. 12, 2021.

[4] C. O. Iatcu, A. Steen, and M. Covasa, "Gut microbiota and complications of type-2 diabetes," *Nutrients*, vol. 14, no. 1, 2021.

[5] P. H. Dedhia, N. Bertaux-Skeirik, Y. Zavros, and J. R. Spence, "Organoid models of human gastrointestinal development and disease," *Gastroenterology*, vol. 150, no. 5, pp. 1098–1112, 2016.

[6] A. Albillos, A. de Gottardi, and M. Rescigno, "The gut-liver axis in liver disease: pathophysiological basis for therapy," *Journal of Hepatology*, vol. 72, no. 3, pp. 558–577, 2020.

[7] A. Wahlström, S. I. Sayin, H. U. Marschall, and F. Bäckhed, "Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism," *Cell Metabolism*, vol. 24, no. 1, pp. 41–50, 2016.

[8] V. K. Ridaura, J. J. Faith, F. E. Rey et al., "Gut microbiota from twins discordant for obesity modulate metabolism in mice," *Science*, vol. 341, no. 6150, p. 1241214, 2013.

[9] O. Chávez-Talavera, A. Tailleux, P. Lefebvre, and B. Staels, "Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease," *Gastroenterology*, vol. 152, no. 7, pp. 1679–1694, 2017.

[10] N. Jiao, S. S. Baker, A. Chapa-Rodriguez et al., "Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD," *Gut*, vol. 67, no. 10, pp. 1881–1891, 2018.

[11] L.-L. Gao, J.-M. Ma, Y.-N. Fan et al., "Lycium barbarum polysaccharide combined with aerobic exercise ameliorated non-alcoholic fatty liver disease through restoring gut microbiota, intestinal barrier and inhibiting hepatic inflammation," *International Journal of Biological Macromolecules*, vol. 183, pp. 1379–1392, 2021.

[12] H.-J. Tsai, Y.-C. Tsai, W.-W. Hung, W. C. Hung, C. C. Chang, and C. Y. Dai, "Gut microbiota and non-alcoholic fatty liver disease severity in type 2 diabetes patients," *Journal of Personalized Medicine*, vol. 11, no. 3, p. 238, 2021.

[13] Y. Yang, Y. Chang, Y. Wu et al., "A homogeneous polysaccharide from Lycium barbarum: structural characterizations, anti-obesity effects and impacts on gut microbiota," *International Journal of Biological Macromolecules*, vol. 183, pp. 2074–2087, 2021.

[14] Z. Zhang, X. Chen, and B. Cui, "Modulation of the fecal microbiome and metabolome by resistant dextrin ameliorates hepatic steatosis and mitochondrial abnormalities in mice," *Food & Function*, vol. 12, no. 10, pp. 4504–4518, 2021.

[15] V. W. Wong, C. H. Tse, T. T. Lam et al., "Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis–a longitudinal study," *PLoS One*, vol. 8, no. 4, article e62885, 2013.

[16] T. Bao, F. He, X. Zhang et al., "Inulin exerts beneficial effects on non-alcoholic fatty liver disease via modulating gut microbiome and suppressing the lipopolysaccharide-toll-like receptor 4-Mψ-nuclear factor-*K*b-nod-like receptor protein 3 pathway via gut-liver axis in mice," *Frontiers in Pharmacology*, vol. 11, article 558525, 2020.

[17] C. Y. L. Chong, D. Orr, L. D. Plank, T. Vatanen, J. M. O'Sullivan, and R. Murphy, "Randomised double-blind placebo-controlled trial of inulin with metronidazole in non-alcoholic fatty liver disease (NAFLD)," *Nutrients*, vol. 12, no. 4, p. 937, 2020.

[18] D. W. Russell, "The enzymes, regulation, and genetics of bile acid synthesis," *Annual Review of Biochemistry*, vol. 72, no. 1, pp. 137–174, 2003.

[19] J. S. Cai and J. H. Chen, "The mechanism of enterohepatic circulation in the formation of gallstone disease," *The Journal of Membrane Biology*, vol. 247, no. 11, pp. 1067–1082, 2014.

[20] J. Y. Lee, H. Arai, Y. Nakamura, S. Fukiya, M. Wada, and A. Yokota, "Contribution of the 7β-hydroxysteroid dehydrogenase from Ruminococcus gnavus N53 to ursodeoxycholic acid formation in the human colon," *Journal of Lipid Research*, vol. 54, no. 11, pp. 3062–3069, 2013.

[21] H. Doden, L. A. Sallam, S. Devendran et al., "Metabolism of oxo-bile acids and characterization of recombinant 12α-hydroxysteroid dehydrogenases from bile acid 7α-dehydroxylating human gut bacteria," *Applied and Environmental Microbiology*, vol. 84, no. 10, 2018.

[22] J. P. Hamilton, G. Xie, J. P. Raufman et al., "Human cecal bile acids: concentration and spectrum," *American Journal of Physiology Gastrointestinal and Liver Physiology*, vol. 293, no. 1, pp. G256–G263, 2007.

[23] C. G. Buffie, V. Bucci, R. R. Stein et al., "Precision microbiome reconstitution restores bile acid mediated resistance to clostridium difficile," *Nature*, vol. 517, no. 7533, pp. 205–208, 2015.

[24] S. Yoshimoto, T. M. Loo, K. Atarashi et al., "Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome," *Nature*, vol. 499, no. 7456, pp. 97–101, 2013.

[25] J. Robben, G. Parmentier, and H. Eyssen, "Isolation of a rat intestinal clostridium strain producing 5 alpha- and 5 beta-bile salt 3 alpha-sulfatase activity," *Applied and Environmental Microbiology*, vol. 51, no. 1, pp. 32–38, 1986.

[26] J. Robben, P. Caenepeel, J. Van Eldere, and H. Eyssen, "Effects of intestinal microbial bile salt sulfatase activity on bile salt kinetics in gnotobiotic rats," *Gastroenterology*, vol. 94, no. 2, pp. 494–502, 1988.

[27] C. Jiang, C. Xie, Y. Lv et al., "Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction," *Nature Communications*, vol. 6, article 10166, 2015.

[28] M. Biagioli and A. Carino, "Signaling from intestine to the host: how bile acids regulate intestinal and liver immunity," *Handbook of Experimental Pharmacology*, vol. 256, 2019.

[29] J. Y. Chiang, R. Kimmel, C. Weinberger, and D. Stroup, "Farnesoid X receptor responds to bile acids and represses cholesterol 7alpha-hydroxylase gene (Cyp7a1) transcription," *The Journal of Biological Chemistry*, vol. 275, no. 15, pp. 10918–10924, 2000.

[30] C. Campbell, P. T. McKenney, D. Konstantinovsky et al., "Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells," *Nature*, vol. 581, no. 7809, pp. 475–479, 2020.

[31] S. Hang, D. Paik, L. Yao et al., "Bile acid metabolites control T_H17 and T_{reg} cell differentiation," *Nature*, vol. 576, no. 7785, pp. 143–148, 2019.

[32] T. C. Liu, J. T. Kern, U. Jain et al., "Western diet induces Paneth cell defects through microbiome alterations and farnesoid X receptor and type I interferon activation," *Cell Host & Microbe*, vol. 29, no. 6, 2021.

[33] A. Molinaro, A. Wahlström, and H.-U. Marschall, "Role of bile acids in metabolic control," *Trends in Endocrinology and Metabolism*, vol. 29, no. 1, pp. 31–41, 2018.

[34] Y. Kawamata, R. Fujii, M. Hosoya et al., "A G protein-coupled receptor responsive to bile acids," *The Journal of Biological Chemistry*, vol. 278, no. 11, pp. 9435–9440, 2003.

[35] L. Ding, Q. Yang, E. Zhang et al., "Notoginsenoside Ft1 acts as a TGR5 agonist but FXR antagonist to alleviate high fat diet-induced obesity and insulin resistance in mice," *Acta Pharmaceutica Sinica B*, vol. 11, no. 6, pp. 1541–1554, 2021.

[36] G. Merlen, V. Bidault-Jourdainne, N. Kahale et al., "Hepatoprotective impact of the bile acid receptor TGR5," *Liver International*, vol. 40, no. 5, pp. 1005–1015, 2020.

[37] M. Reich, L. Spomer, C. Klindt et al., "Downregulation of TGR5 (GPBAR1) in biliary epithelial cells contributes to the pathogenesis of sclerosing cholangitis," *Journal of Hepatology*, vol. 75, no. 3, pp. 634–646, 2021.

[38] D. D. Moore, S. Kato, W. Xie et al., "International Union of Pharmacology. Lxii. The NR1H and NR1I receptors: constitutive androstane receptor, pregnene X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor," *Pharmacological Reviews*, vol. 58, no. 4, pp. 742–759, 2006.

[39] F. A. Al-Jaberi, M. Kongsbak-Wismann, A. Aguayo-Orozco et al., "Impaired vitamin D signaling in T cells from a family with hereditary vitamin D resistant rickets," *Frontiers in Immunology*, vol. 12, article 684015, 2021.

[40] G. Bertilsson, J. Heidrich, K. Svensson et al., "Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 21, pp. 12208–12213, 1998.

[41] J. Hukkanen and J. Hakkola, "PXR and 4β-hydroxycholesterol axis and the components of metabolic syndrome," *Cell*, vol. 9, no. 11, 2020.

[42] A. J. Wigg, I. C. Roberts-Thomson, R. B. Dymock, P. McCarthy, R. H. Grose, and A. G. Cummins, "The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis," *Gut*, vol. 48, no. 2, pp. 206–211, 2001.

[43] J. Mouries, P. Brescia, A. Silvestri et al., "Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development," *Journal of Hepatology*, vol. 71, no. 6, pp. 1216–1228, 2019.

[44] H. Zeng, K. J. Larson, W. H. Cheng et al., "Advanced liver steatosis accompanies an increase in hepatic inflammation, colonic, secondary bile acids and Lactobacillaceae/Lachnospiraceae bacteria in C57bl/6 mice fed a high-fat diet," *The Journal of Nutritional Biochemistry*, vol. 78, article 108336, 2020.

[45] L. Sun, Y. Pang, X. Wang et al., "Ablation of gut microbiota alleviates obesity-induced hepatic steatosis and glucose intolerance by modulating bile acid metabolism in hamsters," *Acta Pharmaceutica Sinica B*, vol. 9, no. 4, pp. 702–710, 2019.

[46] J. Boursier, O. Mueller, M. Barret et al., "The severity of non-alcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota," *Hepatology*, vol. 63, no. 3, pp. 764–775, 2016.

[47] L. A. Adams, Z. Wang, C. Liddle et al., "Bile acids associate with specific gut microbiota, low-level alcohol consumption and liver fibrosis in patients with non-alcoholic fatty liver disease," *Liver International*, vol. 40, no. 6, pp. 1356–1365, 2020.

[48] G. Aragonès, M. Colom-Pellicer, C. Aguilar et al., "Circulating microbiota-derived metabolites: a "liquid biopsy?"," *International Journal of Obesity*, vol. 44, no. 4, pp. 875–885, 2020.

[49] S. Marion, N. Studer, L. Desharnais et al., "In *vitro* and *in vivo* characterization of *Clostridium scindens* bile acid transformations," *Gut Microbes*, vol. 10, no. 4, pp. 481–503, 2019.

[50] M. Watanabe, S. M. Houten, C. Mataki et al., "Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation," *Nature*, vol. 439, no. 7075, pp. 484–489, 2006.

[51] S. Shinohara and K. Fujimori, "Promotion of lipogenesis by PPARγ-activated FXR expression in adipocytes," *Biochemical and Biophysical Research Communications*, vol. 527, no. 1, pp. 49–55, 2020.

[52] T. Claudel, Y. Inoue, O. Barbier et al., "Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression," *Gastroenterology*, vol. 125, no. 2, pp. 544–555, 2003.

[53] S. Bhatnagar, H. A. Damron, and F. B. Hillgartner, "Fibroblast growth factor-19, a novel factor that inhibits hepatic fatty acid synthesis," *The Journal of Biological Chemistry*, vol. 284, no. 15, pp. 10023–10033, 2009.

[54] B. L. Clifford, L. R. Sedgeman, K. J. Williams et al., "FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption," *Cell Metabolism*, vol. 33, no. 8, pp. 1671–1684, 2021.

[55] Y. S. Chen, H. M. Liu, and T. Y. Lee, "Ursodeoxycholic acid regulates hepatic energy homeostasis and white adipose tissue macrophages polarization in leptin-deficiency obese mice," *Cell*, vol. 8, no. 3, 2019.

[56] X. Liu, R. Xue, L. Ji et al., "Activation of farnesoid X receptor (FXR) protects against fructose-induced liver steatosis via inflammatory inhibition and ADRP reduction," *Biochemical and Biophysical Research Communications*, vol. 450, no. 1, pp. 117–123, 2014.

[57] Y. Ma, Y. Huang, L. Yan, M. Gao, and D. Liu, "Synthetic FXR agonist GW4064 prevents diet-induced hepatic steatosis and insulin resistance," *Pharmaceutical Research*, vol. 30, no. 5, pp. 1447–1457, 2013.

[58] S. Kir, S. A. Beddow, V. T. Samuel et al., "FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis," *Science*, vol. 331, no. 6024, pp. 1621–1624, 2011.

[59] K. Ma, P. K. Saha, L. Chan, and D. D. Moore, "Farnesoid X receptor is essential for normal glucose homeostasis," *The Journal of Clinical Investigation*, vol. 116, no. 4, pp. 1102–1109, 2006.

[60] S. Katsuma, A. Hirasawa, and G. Tsujimoto, "Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1," *Biochemical and Biophysical Research Communications*, vol. 329, no. 1, pp. 386–390, 2005.

[61] C. Thomas, A. Gioiello, L. Noriega et al., "TGR5-mediated bile acid sensing controls glucose homeostasis," *Cell Metabolism*, vol. 10, no. 3, pp. 167–177, 2009.

[62] S. Mudaliar, R. R. Henry, A. J. Sanyal et al., "Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease," *Gastroenterology*, vol. 145, no. 3, pp. 574–582.e1, 2013.

[63] P. D. Finn, D. Rodriguez, J. Kohler et al., "Intestinal TGR5 agonism improves hepatic steatosis and insulin sensitivity in western diet-fed mice," *American Journal of Physiology. Gastrointestinal and Liver Physiology*, vol. 316, no. 3, pp. G412–g424, 2019.

[64] W. K. Syn, Y. Htun Oo, T. A. Pereira et al., "Accumulation of natural killer T cells in progressive nonalcoholic fatty liver disease," *Hepatology*, vol. 51, no. 6, pp. 1998–2007, 2010.

[65] I. Evangelakos, J. Heeren, E. Verkade, and F. Kuipers, "Role of bile acids in inflammatory liver diseases," *Seminars in Immunopathology*, vol. 43, no. 4, pp. 577–590, 2021.

[66] A. Sica, P. Invernizzi, and A. Mantovani, "Macrophage plasticity and polarization in liver homeostasis and pathology," *Hepatology*, vol. 59, no. 5, pp. 2034–2042, 2014.

[67] V. Volynets, A. Spruss, G. Kanuri, S. Wagnerberger, S. C. Bischoff, and I. Bergheim, "Protective effect of bile acids on the onset of fructose-induced hepatic steatosis in mice," *Journal of Lipid Research*, vol. 51, no. 12, pp. 3414–3424, 2010.

[68] J. J. Sung and M. Y. Go, "Reversible Kupffer cell suppression in biliary obstruction is caused by hydrophobic bile acids," *Journal of Hepatology*, vol. 30, no. 3, pp. 413–418, 1999.

[69] A. Mantovani, A. Sica, S. Sozzani, P. Allavena, A. Vecchi, and M. Locati, "The chemokine system in diverse forms of macrophage activation and polarization," *Trends in Immunology*, vol. 25, no. 12, pp. 677–686, 2004.

[70] H. Yang, F. Luo, Y. Wei et al., "TGR5 protects against cholestatic liver disease via suppressing the NF-*K*B pathway and activating the Nrf2/HO-1 pathway," *Annals of Translational Medicine*, vol. 9, no. 14, p. 1158, 2021.

[71] A. Perino and K. Schoonjans, "TGR5 and immunometabolism: insights from physiology and pharmacology," *Trends in Pharmacological Sciences*, vol. 36, no. 12, pp. 847–857, 2015.

[72] M. A. Van Herck, J. Weyler, W. J. Kwanten et al., "The differential roles of T cells in non-alcoholic fatty liver disease and obesity," *Frontiers in Immunology*, vol. 10, p. 82, 2019.

[73] D. A. Giles, M. E. Moreno-Fernandez, and S. Divanovic, "IL-17 axis driven inflammation in non-alcoholic fatty liver disease progression," *Current Drug Targets*, vol. 16, no. 12, pp. 1315–1323, 2015.

[74] F. Kuipers, J. F. de Boer, and B. Staels, "Microbiome modulation of the host adaptive immunity through bile acid modification," *Cell Metabolism*, vol. 31, no. 3, pp. 445–447, 2020.

[75] K. M. O'Brien, K. M. Allen, C. E. Rockwell, K. Towery, J. P. Luyendyk, and B. L. Copple, "IL-17a synergistically enhances bile acid-induced inflammation during obstructive cholestasis," *The American Journal of Pathology*, vol. 183, no. 5, pp. 1498–1507, 2013.

[76] Y. Naito, C. Ushiroda, K. Mizushima et al., "Epigallocatechin-3-gallate (EGCG) attenuates non-alcoholic fatty liver disease via modulating the interaction between gut microbiota and bile acids," *Journal of Clinical Biochemistry and Nutrition*, vol. 67, no. 1, pp. 2–9, 2020.

[77] X. Song, X. Sun, S. F. Oh et al., "Microbial bile acid metabolites modulate gut RORγ^+ regulatory T cell homeostasis," *Nature*, vol. 577, no. 7790, pp. 410–415, 2020.

[78] F. J. Bolte and B. Rehermann, "Mucosal-associated invariant T cells in chronic inflammatory liver disease," *Seminars in Liver Disease*, vol. 38, no. 1, pp. 60–65, 2018.

[79] I. Magalhaes, K. Pingris, C. Poitou et al., "Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients," *The Journal of Clinical Investigation*, vol. 125, no. 4, pp. 1752–1762, 2015.

[80] P. Hegde, E. Weiss, V. Paradis et al., "Mucosal-associated invariant T cells are a profibrogenic immune cell population in the liver," *Nature Communications*, vol. 9, no. 1, p. 2146, 2018.

[81] Y. Li, B. Huang, X. Jiang et al., "Mucosal-associated invariant T cells improve nonalcoholic fatty liver disease through regulating macrophage polarization," *Frontiers in Immunology*, vol. 9, p. 1994, 2018.

[82] A. Mendler, A. Pierzchalski, M. Bauer et al., "Mait cell activation in adolescents is impacted by bile acid concentrations and body weight," *Clinical and Experimental Immunology*, vol. 200, no. 2, pp. 199–213, 2020.

[83] X. Jiang, M. Lian, Y. Li et al., "The immunobiology of mucosal-associated invariant T cell (MAIT) function in primary biliary cholangitis: regulation by cholic acid-induced interleukin-7," *Journal of Autoimmunity*, vol. 90, pp. 64–75, 2018.

[84] J. Sun, Z. Cao, A. D. Smith et al., "Bile acid profile and its changes in response to cefoperazone treatment in MR1 deficient mice," *Metabolites*, vol. 10, no. 4, p. 127, 2020.

[85] T. Miyagi, T. Takehara, A. Uemura et al., "Absence of invariant natural killer T cells deteriorates liver inflammation and fibrosis in mice fed high-fat diet," *Journal of Gastroenterology*, vol. 45, no. 12, pp. 1247–1254, 2010.

[86] Z. Li, M. J. Soloski, and A. M. Diehl, "Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease," *Hepatology*, vol. 42, no. 4, pp. 880–885, 2005.

[87] J. Bhattacharjee, M. Kirby, S. Softic et al., "Hepatic natural killer T-cell and CD8+ T-cell signatures in mice with nonalcoholic steatohepatitis," *Hepatology Communications*, vol. 1, no. 4, pp. 299–310, 2017.

[88] C. Ma, M. Han, B. Heinrich et al., "Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells," *Science*, vol. 360, no. 6391, 2018.

[89] M. Biagioli, A. Carino, C. Fiorucci et al., "GPBAR1 functions as gatekeeper for liver NKT cells and provides

counterregulatory signals in mouse models of immune-mediated hepatitis," *Cellular and Molecular Gastroenterology and Hepatology*, vol. 8, no. 3, pp. 447–473, 2019.

[90] R. N. Germain and Y. Huang, "Ilc2s - resident lymphocytes pre-adapted to a specific tissue or migratory effectors that adapt to where they move?," *Current Opinion in Immunology*, vol. 56, pp. 76–81, 2019.

[91] S. Wang, J. Li, S. Wu et al., "Type 3 innate lymphoid cell: a new player in liver fibrosis progression," *Clinical Science (London, England)*, vol. 132, no. 24, pp. 2565–2582, 2018.

[92] M. Hamaguchi, T. Okamura, T. Fukuda et al., "Group 3 innate lymphoid cells protect steatohepatitis from high-fat diet induced toxicity," *Frontiers in Immunology*, vol. 12, article 648754, 2021.

[93] X. Qi, C. Yun, L. Sun et al., "Gut microbiota-bile acid-interleukin-22 axis orchestrates polycystic ovary syndrome," *Nature Medicine*, vol. 25, no. 8, pp. 1225–1233, 2019.

[94] J. Xu, D. Huang, X. Xu et al., "An elevated deoxycholic acid level induced by high-fat feeding damages intestinal stem cells by reducing the ileal IL-22," *Biochemical and Biophysical Research Communications*, vol. 579, pp. 153–160, 2021.

[95] E. M. Brunt, "Pathology of nonalcoholic fatty liver disease," *Nature Reviews. Gastroenterology & Hepatology*, vol. 7, no. 4, pp. 195–203, 2010.

[96] J. W. Ding, R. Andersson, V. Soltesz, R. Willén, and S. Bengmark, "The role of bile and bile acids in bacterial translocation in obstructive jaundice in rats," *European Surgical Research*, vol. 25, no. 1, pp. 11–19, 1993.

[97] H. Fukui, "Increased intestinal permeability and decreased barrier function: does it really influence the risk of inflammation?," *Inflammatory Intestinal Diseases*, vol. 1, no. 3, pp. 135–145, 2016.

[98] S. F. Assimakopoulos, C. E. Vagianos, A. S. Charonis et al., "Experimental obstructive jaundice alters claudin-4 expression in intestinal mucosa: effect of bombesin and neurotensin," *World Journal of Gastroenterology*, vol. 12, no. 21, pp. 3410–3415, 2006.

[99] V. Lorenzo-Zuñiga, R. Bartoli, R. Planas et al., "Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats," *Hepatology*, vol. 37, no. 3, pp. 551–557, 2003.

[100] T. Inagaki, A. Moschetta, Y.-K. Lee et al., "Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 10, pp. 3920–3925, 2006.

[101] M. Sorribas, M. O. Jakob, B. Yilmaz et al., "FXR modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis," *Journal of Hepatology*, vol. 71, no. 6, pp. 1126–1140, 2019.

[102] R. M. Gadaleta, K. J. van Erpecum, B. Oldenburg et al., "Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease," *Gut*, vol. 60, no. 4, pp. 463–472, 2011.

[103] S. Cipriani, A. Mencarelli, M. G. Chini et al., "The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis," *PLoS One*, vol. 6, no. 10, article e25637, 2011.

[104] R. Yang, T. Harada, J. Li et al., "Bile modulates intestinal epithelial barrier function via an extracellular signal related kinase 1/2 dependent mechanism," *Intensive Care Medicine*, vol. 31, no. 5, pp. 709–717, 2005.

[105] J. Yamaguchi, A. Toledo, B. L. Bass et al., "Taurodeoxycholate increases intestinal epithelial cell proliferation through C-Myc expression," *Surgery*, vol. 135, no. 2, pp. 215–221, 2004.

[106] A. Toledo, J. Yamaguchi, J. Y. Wang, B. L. Bass, D. J. Turner, and E. D. Strauch, "Taurodeoxycholate stimulates intestinal cell proliferation and protects against apoptotic cell death through activation of Nf-Kappab," *Digestive Diseases and Sciences*, vol. 49, no. 10, pp. 1664–1671, 2004.

[107] M. Mueller, A. Thorell, T. Claudel et al., "Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity," *Journal of Hepatology*, vol. 62, no. 6, pp. 1398–1404, 2015.

[108] J. Laurin, K. D. Lindor, J. S. Crippin et al., "Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study," *Hepatology*, vol. 23, no. 6, pp. 1464–1467, 1996.

[109] A. Pathil, G. Liebisch, J. G. Okun, W. Chamulitrat, G. Schmitz, and W. Stremmel, "Ursodeoxycholyl lysophosphatidylethanolamide modifies aberrant lipid profiles in NAFLD," *European Journal of Clinical Investigation*, vol. 45, no. 9, pp. 925–931, 2015.

[110] U. F. H. Leuschner, B. Lindenthal, G. Herrmann et al., "High-dose ursodeoxycholic acid therapy for nonalcoholic steatohepatitis: a double-blind, randomized, placebo-controlled trial," *Hepatology*, vol. 52, no. 2, pp. 472–479, 2010.

[111] B. A. Neuschwander-Tetri, R. Loomba, A. J. Sanyal et al., "Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial," *Lancet*, vol. 385, no. 9972, pp. 956–965, 2015.

[112] P. Schwabl, E. Hambruch, B. A. Seeland et al., "The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction," *Journal of Hepatology*, vol. 66, no. 4, pp. 724–733, 2017.

[113] S. Traussnigg, E. Halilbasic, H. Hofer et al., "Open-label phase ii study evaluating safety and efficacy of the non-steroidal farnesoid X receptor agonist PX-104 in non-alcoholic fatty liver disease," *Wiener Klinische Wochenschrift*, vol. 133, no. 9-10, pp. 441–451, 2021.

[114] E. F. Murphy, P. D. Cotter, A. Hogan et al., "Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity," *Gut*, vol. 62, no. 2, pp. 220–226, 2013.

[115] J. Yao, C. S. Zhou, X. Ma et al., "FXR agonist GW4064 alleviates endotoxin-induced hepatic inflammation by repressing macrophage activation," *World Journal of Gastroenterology*, vol. 20, no. 39, pp. 14430–14441, 2014.

[116] R. Loomba, M. Noureddin, K. V. Kowdley et al., "Combination therapies including cilofexor and firsocostat for bridging fibrosis and cirrhosis attributable to NASH," *Hepatology*, vol. 73, no. 2, pp. 625–643, 2021.

[117] K. Patel, S. A. Harrison, M. Elkhashab et al., "Cilofexor, a nonsteroidal FXR agonist, in patients with noncirrhotic NASH: a phase 2 randomized controlled trial," *Hepatology*, vol. 72, no. 1, pp. 58–71, 2020.

[118] P. An, G. Wei, P. Huang et al., "A novel non-bile acid FXR agonist EDP-305 potently suppresses liver injury and fibrosis without worsening of ductular reaction," *Liver International*, vol. 40, no. 7, pp. 1655–1669, 2020.

[119] L. Jiang, D. Xiao, Y. Li et al., "Structural basis of tropifexor as a potent and selective agonist of farnesoid X receptor," *Biochemical and Biophysical Research Communications*, vol. 534, pp. 1047–1052, 2021.