Critical roles of bile acids in regulating intestinal mucosal immune responses

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***Abstract*:** Bile acids are a class of cholesterol derivatives that have been known for a long time for their critical roles in facilitating the digestion and absorption of lipid from the daily diet. The transformation of primary bile acids produced by the liver to secondary bile acids appears under the action of microbiota in the intestine, greatly expanding the molecular diversity of the intestinal environment. With the discovery of several new receptors of bile acids and signaling pathways, bile acids are considered as a family of important metabolites that play pleiotropic roles in regulating many aspects of human overall health, especially in the maintenance of the microbiota homeostasis and the balance of the mucosal immune system in the intestine. Accordingly, disruption of the process involved in the metabolism or circulation of bile acids is implicated in many disorders that mainly affect the intestine, such as inflammatory bowel disease and colon cancer. In this review, we discuss the different metabolism profiles in diseases associated with the intestinal mucosa and the diverse roles of bile acids in regulating the intestinal immune system. Furthermore, we also summarize recent advances in the field of new drugs that target bile acid signaling and highlight the importance of bile acids as a new target for disease intervention.

Keywords: bile acids, colorectal cancer, inflammatory bowel disease, microbiota, mucosal immunity, therapeutic intervention

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Introduction

Owing to continuous exposure to different foreign antigens and a wide variety of metabolites, intestinal immune cells are faced with the challenges of detecting and eliminating detrimental pathogenic microorganisms, regulating intestinal metabolite pool, and maintaining intestinal homeostasis.[1] In addition to interacting with each other, immune cells also play critical roles in interacting with intestinal epithelia, microorganisms, and various metabolites. Therefore, the balance of network connecting immune cells with other components in the intestine is extremely important for intestinal homeostasis and even overall health.[2] Impairment of the balance could precipitate many chronic inflammatory diseases in the intestine, such as inflammatory bowel disease (IBD).[3] As an important class of metabolites, up to now, bile acids have been known for their roles in not only facilitating digestion and absorption but also regulating intestinal mucosal immune responses, owing to the increasing understanding of bile acids from recent basic studies. Bile acids regulate intestinal mucosal homeostasis and inflammation through interaction with bile acid receptors and signaling.[4] On the one hand, bile acids contribute to shaping the microbiota community. On the other hand, bile acids, especially secondary bile acids, are also metabolized by many intestinal microorganisms.[5] *Via* different receptors and respective signalings, such as farsenoid X receptor (FXR) and G-protein bile acid-activated receptor 1 (GPBAR1), bile acids regulate intestinal mucosal homeostasis and inflammation.[6] In this paper, we provide an overview of the roles of bile acids in regulating metabolism and immune responses, and discuss the possibilities that allow us to

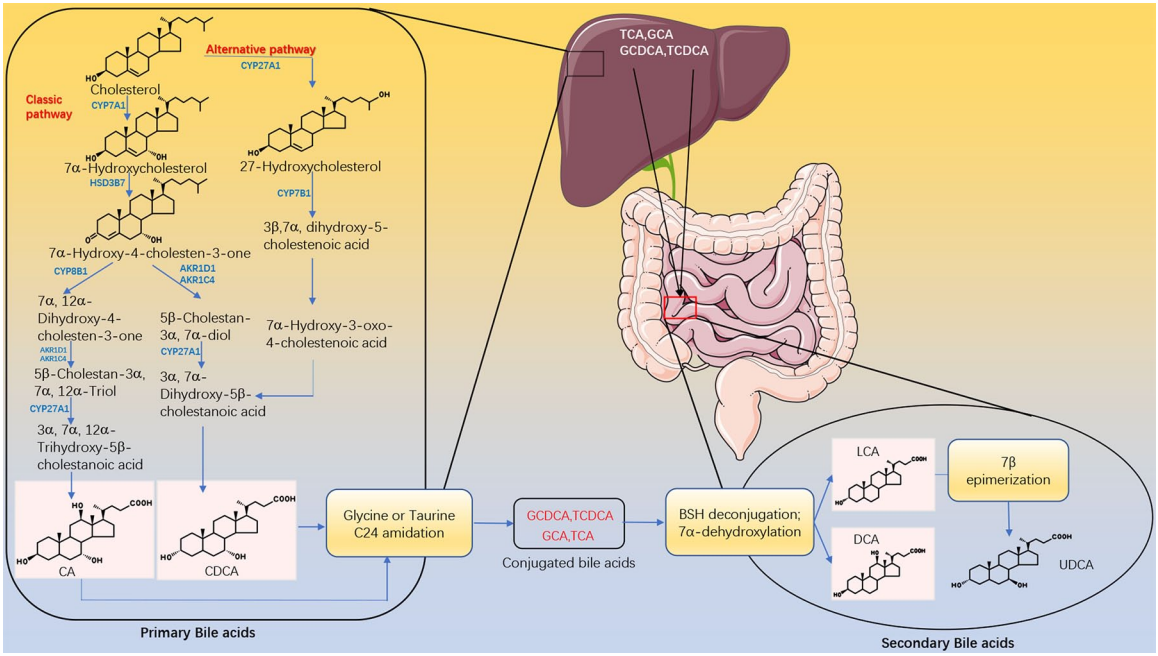

Figure 1. Bile acid biosynthetic pathways. In liver, cholesterol 7α-hydroxylase (CYP7A1) and mitochondrial sterol 27-hydroxylase (CYP27A1) are the key enzymes which initiate the classic pathway and the alternative pathway, respectively. Through the classic pathway, CYP7A1 converts cholesterol to 7α-hydroxycholesterol, which is then converted to 7α-hydroxy-4-cholesten-3-one by 3β-hydroxysteroid dehydrogenase (HSD3B7). Sterol 12α-hydroxylase (CYP8B1) catalyzes 7α-hydroxy-4-cholesten-3-one into 7α,12α-dihydroxy-4-cholesten-3-one, which successively transforms into 7α,12α-dihydroxy-4-cholesten-3-one and 5β-cholestan-3α,7α,12α-triol under the effect of aldos-keto reductase 1D1 (AKR1D1) and AKR1C4 and sterol 27-hydroxylase (CYP27A1), respectively, finally leading to the generation of 3α,7α,12α-trihydroxy-5β-cholestanoic acid (CA). Also, 7α-hydroxy-4-cholesten-3-one can be converted to 5β-cholestan-3α,7α-diol by AKR1D1 and AKR1C4, and finally transformed into 3α,7α-dihydroxy-5β-cholestanoic acid (CDCA) by CYP27A1. In the alternative pathway, cholesterol can be directly converted to 27-hydroxycholesterol by CYP27A1, which is finally transformed into CDCA following the catalyzation of oxysterol 7α-hydroxylase (CYP7B1) and other enzymes. Then CA and CDCA are amidated with glycine or taurine in the liver to form the conjugated bile salts (GCA and GCDCA, TCA and TCDCA). When secreted into the intestine, conjugated bile acids are converted to secondary bile acids [lithocholic acid (LCA) and deoxycholic acid (DCA)] after the deamination performed by bile salt hydrolases (BSHs) and subsequent 7α-dihydroxylation by bacterial 7α-dehydroxylase. The 7β epimerization of CDCA leads to the formation of ursodeoxycholic acid (UDCA), which is a secondary bile acid in humans.

transform the advances of basic data to application in clinical use.

Bile acid metabolism

Bile acids are metabolic products of cholesterol. The human liver synthesizes about 200–600 mg of bile acids every day and excretes them into the feces (Figure 1). Hepatocytes use cholesterol to synthesize primary bile acids through multiple steps, which is the main way the liver clears cholesterol.[4,7,8] The conversion of cholesterol into bile acids involves 17 distinct enzymes located in different cellular architectures such as cytosol, endoplasmic reticulum, and peroxisomes, of which cholesterol 7α-hydroxylase is the key enzyme.[9–11] Bile acids synthesized directly from hepatocytes using cholesterol are called primary bile acids, containing cholic acid (CA) and chenodeoxycholic acid (CDCA). CA and CDCA are amidated with glycine or taurine in the liver to form the conjugated bile salts. Secondary bile acids, including lithocholic acid (LCA) and deoxycholic acid (DCA), are produced through the 7-α dehydroxylation of primary bile acids under the action of intestinal bacteria after the deamination of conjugated bile acids performed by bile salt hydrolases (BSHs).[12] The structural difference between CA and CDCA is the number of hydroxyl groups: three hydroxyl groups (3α, 7α, 12α) and two hydroxyl groups (3α, 7α), respectively. There are no hydroxyl groups on the C-7

position of DCA and LCA.[11,13] The primary bile acids that flow into the intestine tract assist digestion and absorption of lipid substances and food, and a part of them is hydrolyzed to remove the 7α-hydroxyl group and then converted into secondary bile acids by bacteria in the distal intestine.[14] An amount of ursodeoxycholic acid (UDCA) can be produced along with the synthesis of secondary bile acids, which have the same function of dissolving gallstones as CDCA.

On average, 95% of the various bile acids in the intestine are reabsorbed by the intestinal wall, and the rest is excreted into feces. There are two main ways to reabsorb bile acids: (1) conjugated bile acids are actively reabsorbed at the ileal site and (2) deconjugated bile acids are passively reabsorbed in different parts of the small intestine and large intestine. The reabsorption of bile acids mainly depends on active reabsorption. Most LCA exists in free form without being reabsorbed. Reabsorbed bile acids in the intestine, including primary and secondary bile acids, conjugated and deconjugated bile acids, enter the liver through the portal vein, where deconjugated bile acids are converted into conjugated bile acids by hepatic enzymes, then secreted and recirculated to the gallbladder for storage. This process is called "enterohepatic circulation of bile acids". The physiological significance of enterohepatic circulation of bile acids is (1) to regulate bile acid synthesis by feedback inhibition and (2) to absorb and transport cholesterol, fats, and nutrients to the liver for distribution to other tissues/organs.[15] In humans, a total bile acids pool of 3–5 g is not enough to facilitate lipid digestion and absorption, which can be solved by the enterohepatic circulation of bile acids. After each meal, enterohepatic circulation can be completed approximately two to four times so that the limited bile acids can exert the maximum emulsification to maintain digestion and absorption of lipid food.[16] Once enterohepatic circulation is disrupted, such as by severe diarrhea or large ileal resection, digestion and absorption of lipid food are impaired, leading to increased incidence of gallstone owing to the accumulation of cholesterol.[17] As amphipathic molecules, bile acids contain both hydroxyl and carboxyl or sulfonic acid groups that are hydrophilic, and hydrocarbon cores as well as methyl groups that are hydrophobic. These groups with adverse properties located on different sides of cyclopentane poly hydro phenanthrene nucleus make bile acid surfactant, thus decreasing the surface tension between oil and water as well as increasing the emulsification of lipids. In addition, amphipathic properties of bile acids contribute to expanding contact surface between lipase and substrates, which can accelerate digestion of lipids.

The effects of bile acids and receptors on the intestinal mucosal immune system

Crosstalk of bile acid receptors plays a significant regulatory role in the intestinal immune system (Figure 2). The roles of bile acids and their receptors, such as GPBAR1 and FXR, in regulating intestinal immunity and homeostasis have been investigated by many studies (Table 1). As typical for ligand-bound nuclear receptors, FXR undergoes a conformational change such that corepressors are released and coactivator are recruited, thus activating FXR, the first described nuclear receptor for bile acids. FXR can be activated by endogenous CDCA > DCA > LCA > CA with decreasing affinity.[18,19] In addition to the inhibition of apical sodium-dependent bile acid transporter (ASBT) expression and promotion of ileal bile acid-binding protein and bile acid transporters OST α/β to enforce efficient bile acid transcellular export,[20,21] bile acid-dependent FXR activation is also reported to be crucial for mucosal immune homeostasis, which is often decreased during intestinal inflammation. In several mouse colitis models, including dextran sulfate sodium (DSS)- and 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, mucosal inflammation is decreased in the presence of FXR agonist treatment while increased in FXR-deficient mice.[22] The expression of pro-inflammatory cytokine (e.g. IL-1β, IL-6) and chemokine (e.g. CCL2) is found to be diminished in murine colitis treated with INT-747, an agonist of FXR, which is the original name of obeticholic acid (OCA). These phenotypes have been associated with the strengthened intestinal barrier function and increased antimicrobial peptide production that contributes to the limitation of bacterial translocation across the intestinal epithelial barrier in the presence of FXR activation.[22] In human $CD14^+$ monocytes and DCs (dendritic cell) cultured *in vitro*, the expression of inflammatory cytokine and chemokine is restricted by INT-747-dependent FXR activation.[22] Similarly, the expression of TLR4-mediated pro-inflammatory genes is also repressed by FXR activation with INT-747 in intestinal epithelial cells (IECs).[23] FXR activation has been reported to repress NF-κB activity by preventing nuclear coreceptor clearance

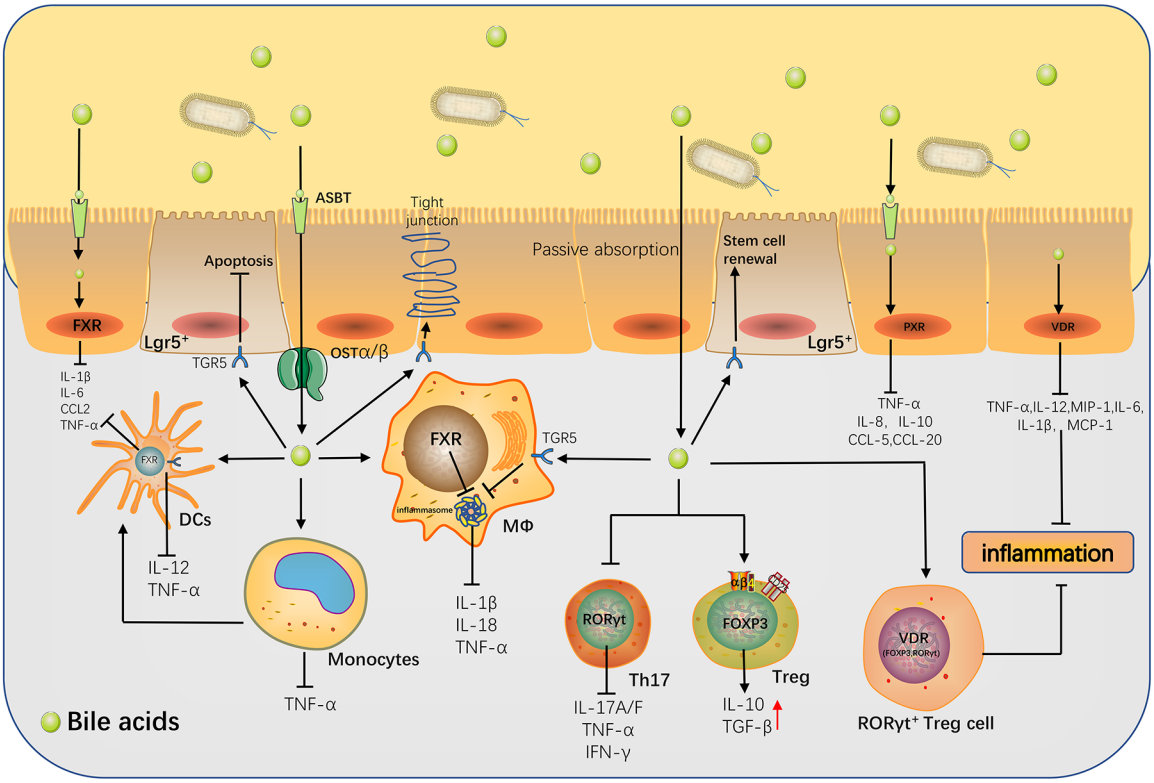

Figure 2. Potential roles of bile acids in regulating immune response in intestinal mucosa. Bile acids in the intestine are actively reabsorbed into lamina propria through sodium-dependent bile acid transporter (ASBT) and organic solute transporter (OST)-α/β complex or by passive absorption, where they interact with a variety of mucosal immune cells. The activation of farsenoid X receptor (FXR) and TGR5 commonly inhibits inflammasome assembly and reduces the associated pro-inflammatory cytokine expression in macrophages and DC (dendritic cell). Bile acids suppress pro-inflammatory ability of monocytes and concomitantly facilitate monocytes to differentiate into DC with poor production of IL-12 and TNF-α. The differentiation of Th17 cells is decreased while regulatory T-cell (Treg cell) differentiation is increased by bile acids, characterized by down-regulated pro-inflammatory cytokines such as IL-17A/F and TNF-α and up-regulated anti-inflammatory factors such as IL-10 and TGF-β, respectively. Strikingly, *via* vitamin D receptor (VDR), colon-resident Foxp3+ Treg cells simultaneously expressing RORγt can be modulated by bile acids, thus ameliorating mucosal inflammation. TGR5 activation in intestinal epithelial cells strengthens tight junction and protects intestinal barrier integrity. In Lgr5+ crypt stem cells, TGR5 activation promotes stem cell renewal and inhibits apoptosis, thus maintaining intestinal epithelial homeostasis. The activation of FXR and pregnane X receptor (PXR) by bile acids in intestinal epithelium can also exhibit an anti-inflammatory effect with decreased levels of pro-inflammatory cytokines.

from NF-κB-binding sites in the *Tnf* and *Il1b* loci.[24,25] Also, FXR activation displays an anti-inflammatory effect, resulting in elevated levels of serum IL-10, the persistence of spleen DCs, and augmented numbers of regulatory T-cells (Treg cells).[26] A recent study has shown that assembly of NLRP3 inflammasomes can be suppressed by FXR, which physically interacts with NLRP3 and caspase-1.[27] Interestingly, this study showed that bile acids promoted NLRP3 activation as a damage-associated molecular pattern and the inhibition of inflammasome by FXR occurs without binding of bile acids.[27]

GPBAR1, also called TGR5, discovered as a membrane receptor for bile acids in 2002, belongs to the G protein-coupled receptor (GPCR) superfamily.[43,44] The expression of GPBAR1 is detected on the membrane of most cells covering the intestinal and biliary tracts, such as epithelial cells, immune cells, and enteric nerves. GPBAR1 can be activated by LCA > DCA > CDCA > UDCA > CA according

Table 1. Functional regulation of bile acids on immune cells and epithelial cells in gut mucosa.

Cell type	Bile acid receptor	Bile acid ligands	Synthetic ligands	Regulated factors	Function and effect	Disorder	References
IEC	FXR	CDCA > DCA > LCA > CA[a]	GW4064, obeticholic acid, BAR502, fexaramine, Px-104, tropifexor, LMB763	Down: ASBT, IL-1β, IL-6, TNF-α, CCL2 Up: IBABP, iNOs, cathelicidin, Ang1, MUC2, occluding, claudin-1	Anti-inflammatory effect, strengthened intestinal barrier integrity, strengthened mucosal barrier, limited translocation of bacteria, maintaining bile acid metabolism	IBD, CRC	Dawson *et al.*,[20] Ding *et al.*,[21] Gadaleta *et al.*,[22] Vavassori *et al.*,[23] Wang *et al.*[24]
	TGR5	LCA > DCA > CDCA > UDCA > CA	INT-767, INT-777, BAR501, BAR502	Down: IL-1β, IL-6, IL-8, TNF-α, IFN-g Up: cAMP, protein kinase A, tight junction protein, ZO-1, occluding, IL-10, resolvin, lipoxin	Anti-inflammatory effect, strengthened intestinal epithelial barrier, inhibition of apoptosis, promotion of proliferation	IBD	Cipriani *et al.*,[28] Ichikawa *et al.*,[29] McMahan *et al.*,[30] Biagioli *et al.*[31]
	PXR	LCA, 3-keto-LCA, CDCA, DCA, CA		Down: TNF-α, IL-8, CCL5, CCL20, TLR-4 Up: TGF-β, IL-10	Anti-inflammatory effect, acceleration of mucosal wound repairing	IBD, CRC	Venkatesh *et al.*,[32] Huang *et al.*,[33] Shah *et al.*,[34] Terc *et al.*,[35] Cheng *et al.*[36]
T-cells	FXR	CDCA > DCA > LCA > CA	GW4064, obeticholic acid, BAR502, fexaramine, Px-104, tropifexor, LMB763	Down: TNF-α, IL-6, IL-1β Up: IL-10, Foxp3	Augmented number of Treg cells, anti-inflammatory effect	IBD	Massafra *et al.*,[26] Song *et al.*,[37] Korn *et al.*[38]
	VDR	3-oxoLCA, isoalloLCA, LCA		Down: RORγt Up: Foxp3	Inhibited differentiation of Th17 increased differentiation of Treg	IBD	Korn *et al.*,[38] Hang *et al.*[39]
Macrophage	FXR	CDCA > DCA > LCA > CA	GW4064, obeticholic acid, BAR502, fexaramine, Px-104, tropifexor, LMB763	Down: IL-1β, IL-8, TNF-α, NLRP-3, caspase-1	Decreased level of pro-inflammatory cytokines, anti-inflammatory effect	IBD	Garcia-Irigoyen and Moschetta[27]
	TGR5	LCA > DCA > CDCA > UDCA > CA	INT-767, INT-777, BAR501, BAR502	Down: IL-1β, IL-8, TNF-α, NLRP3, caspase-1	Anti-inflammatory effect, polarization from M1 to M2 phenotype	IBD	Pols *et al.*,[40] Yoneno *et al.*,[41] Biagioli *et al.*,[31] Guo *et al.*[42]
DC	FXR	CDCA > DCA > LCA > CA	GW4064, obeticholic acid, BAR502, fexaramine, Px-104, tropifexor, LMB763	Down: IL-6, IL-1β, TNF-α	Anti-inflammatory effect	IBD	Gadaleta *et al.*,[22] Massafra *et al.*[26]
	TGR5	LCA > DCA > CDCA > UDCA > CA	INT-767, INT-777, BAR501, BAR502	Down: IL-12, TNF-α	Anti-inflammatory effect	IBD	Ichikawa *et al.*[29]
Monocyte	FXR	CDCA > DCA > LCA > CA	GW4064, obeticholic acid, BAR502, fexaramine, Px-104, tropifexor, LMB763	Down: TNF-α	Anti-inflammatory effect	IBD	Gadaleta *et al.*[22]

Bile acid receptors in different immune cells can be activated by endogenous agonists such as CDCA and LCA, and by synthetic ligands such as GW4064, obeticholic acid, and BAR502. When immune cells are stimulated with these ligands, several biological processes and events are altered and consequently regulated, including down-regulated pro-inflammatory cytokines (TNF-α, IL-6), chemokines (CCL2, CCL5) and inflammation-associated complex (NLRP3 inflammasome, RORγt), as well as up-regulated structure proteins (ZO-1, claudin-1), anti-inflammatory cytokines (IL-10, TGF-β) and associated transcription factors (Foxp3). Such alteration and regulation lead to strengthened intestinal mucosal barrier integrity, limited translocation of bacteria, and decreased levels of pro-inflammatory cytokines. Finally, bile acids and their receptor signaling exert their anti-inflammatory effects on the intestinal mucosal immune system and maintaining the homeostasis in gut.
[a]>, higher affinity than.
Ang1, angiopoietin 1; ASBT, apical sodium dependent bile acid transporter; CA, cholic acid; CCL, chemokine (C-C motif) ligand; CDCA, chenodeoxycholic acid; CRC, colorectal cancer; DC, dendritic cell; DCA, deoxycholic acid; Foxp3, forkhead box P3; FXR, farsenoid X receptor; IBABP, ileal bile acid-binding protein; IBD, inflammatory bowel disease; IEC, intestinal epithelial cell; IFN-g, interferon g; IL, interleukin; iNOs, inducible nitric oxide synthase; LCA, lithocholic acid; MUC2, mucin 2; NLRP3, NOD-, LRR- and pyrin domain-containing protein 3; PXR, pregnane X receptor; RORγt, retinoic acid-related orphan receptor gamma t; TGF-β, transforming growth factor β; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; Treg, regulatory T-cell; UDCA, ursodeoxycholic acid.

to the affinity. LCA is the strongest natural agonist of GPBAR1, but GPBAR1 also responds to (un)conjugated DCA, CDCA, UDCA, and CA.[45–50] As a new target in the treatment of liver, cardiovascular, and metabolic diseases, GPBAR1 is receiving a great deal of attention and interest at present.[51–55] *In vivo* experimental results have demonstrated that *Gpbar1* deficiency results in the destroyed architecture of epithelial tight junctions in the intestine and abnormal distribution of zonulin-1.[28] Particularly, GPBAR1 expressed in enteric neurons is currently known for the necessary roles in the regulation of intestinal motility[56] while GPBAR1 in innate immune cells delivers suppressive signals to inflammatory responses.[29,30] Endogenous bile acids or the synthetic GPBAR1 agonist 6α-ethyl-23(S)-methylcholic acid (S-EMCA/INT-777) can activate GPBAR1 and suppress expression of inflammatory cytokines induced by LPS (lipopolysaccharide), whereas bile acids or GPBAR1 agonist could not exert such an anti-inflammatory function in *Gpbar1*-deficient macrophages.[40,41] *In vivo* activation of GPBAR1 by the steroidal ligand BAR501, a small molecule agonist, attenuates inflammation in murine models of colitis by contributing to the polarization of mucosa-associated macrophages from M1 to M2.[31] Bile acids are also reported to inhibit the activation of NLRP3 inflammasome *via* the GPBAR1–cAMP–PKA (cyclic adenosine monophosphate-protein kinase A) axis, indicating that bile acids function not only in modulating the metabolic system but also in fine-tuning inflammatory responses.[42] Furthermore, bile acid-dependent GPBAR1 activation is found to induce the differentiation of human monocytes into IL-12 and TNF-α hypo-producing DC *via* the GPBAR1–cAMP pathway.[29] Collectively, FXR and GPBAR1, as two classical regulators of bile acid metabolism, generally play an anti-inflammatory role in the intestinal mucosal immune system.

In addition to FXR and GPBAR1, bile acids also play important roles through other receptors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR), and vitamin D receptor (VDR). Diet antigens or bacteria-derived metabolites such as xenobiotics can activate these nuclear receptors, whereby they act on the intestinal mucosal immunity and homeostasis.[32,57,58] It is notable that expression of PXR, VDR, and CAR target genes, which commonly promote bile acid detoxification and protect tissue damage from bile acids,[31] is decreased in mucosal biopsies from IBD patients.[59–61] There has been a historical study on the roles of PXR as a sensor of LCA in coordinately regulating the expression of genes that reduce the concentrations of LCA to avoid toxic damage to the host.[62] Evidence has shown that a distinct intestinal pathology with destroyed epithelium structure is present in *Pxr*-deficient mice[32] and PXR signaling in non-hematopoietic compartments is indispensable to the maintenance of the barrier functions and the balance of intestinal inflammatory signaling network.[32] Interestingly, the expression of toll-like receptor 4 (TLR4) is up-regulated in IECs of *Pxr*-deficient mice,[32] which is consistent with previous results showing that TLR4 mRNA stability is decreased in the presence of PXR activation dependent on LCA.[33] Recently, a critical mechanism has been elucidated: PXR activation in the colon can repress the expression of NF-κB target genes, thus decreasing the susceptibility to colitis induced by DSS in mice.[34] The activity of p38 MAP kinase and IEC motility can also be stimulated by PXR activation, thereby accelerating the mucosal wound repairing and improving the level of TGF-β, which limits expression of several inflammatory cytokines and chemokines, including TNF, IL-8, CCL5, and CCL20.[35,36] Considerable advances have been made in the understanding of PXR during recent years, and in addition to its detoxifying roles, it also exerts potent cytoprotective and anti-inflammatory effects on intestinal epithelial cells.[63]

Bile acids control many aspects of physiological processes, including cell differentiation and inflammatory responses by VDR, which is another nuclear receptor of bile acids and is expressed throughout the body.[64–66] As a risk factor for IBD, vitamin D deficiency and reduced expression of VDR commonly occur in patients with IBD.[59] In several chemically-induced colitis models, VDR-deficient mice display impaired production of antimicrobial peptides, increased epithelium permeability, and gut dysbiosis.[67,68] In different experimental colitis models, mice with transgenic human VDR in IECs exhibit high resistance to colitis, demonstrating that the activation of epithelial VDR signaling provides protection to the mucosal barrier that inhibits colitis, whereas activation of non-epithelial immune VDR has no such an effect.[69] Recently, two bile acid metabolites, 3-oxoLCA and isoalloLCA, have been identified as important regulators for T-cells by VDR in mice.[37] In addition to VDR, RORγt had been shown to be able to act as oxo-bile acid receptor, such as 3-oxoLCA, a derivative from LCA.[37] For a long time, RORγt was recognized as a critical transcriptional factor that drives Th17

Table 2. Dynamic changes of bile acids in sera and stool in different gut disorders.

Disease	Sub-disease	Sera	Stool	References
IBD	Active IBD	Reduced secondary bile acids*	Reduced secondary bile acids** Increased conjugated bile acids* Increased sulfated bile acids**	Lloyd-Price *et al.*[80]
	IBD in remission	Reduced secondary bile acids*	Reduced secondary bile acids* Increased conjugated bile acids*	Lloyd-Price *et al.*[80]
CRC		Increased DCA	Increased DCA in MP Increased glycocholate and taurocholate in S0	Sakanaka *et al.*[88]
IBS		Increased primary bile acids and amino-conjugated bile acids in IBS-D and IBS-C	Increased total bile acids, sulfated bile acids, conjugated bile acids, and UDCA in IBS-D	Fryer *et al.*[89]

*$p < 0.05$.
**$p < 0.01$.
CRC, colorectal cancer; DCA, deoxycholic acid; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IBS-C, constipation-predominant IBS; IBS-D, diarrhea-predominant IBS; MP, multiple polypoid adenomas with low-grade dysplasia; S0, stage 0 intramucosal carcinoma [polypoid adenoma(s) with high-grade dysplasia]; UDCA, ursodeoxycholic acid.

differentiation in several inflammatory diseases, while a recent study revealed a new role different from previous understanding.[38] The differentiation of Th17 cells has been observed to be reduced while the Treg cell differentiation is markedly increased in the intestinal lamina propria of mice under the effects of 3-oxoLCA and isoalloLCA, respectively. Mechanistically, the physical binding of 3-oxoLCA to the RORγt, the key transcription factor that drives the differentiation of Th17 cells, can inhibit the activity of RORγt, leading to a lower proportion of Th17 cells, and the increased differentiation of Treg cells is due to the isoalloLCA-induced up-regulation of mitochondrial reactive oxygen species, which increases the expression of transcription factor Foxp3, suggesting that bile acids play a critical role in immune responses by skewing the differentiation of Th17/Treg cells.[39] Consistent with these results, another study has reported that the gut bile acid pool is significantly influenced by daily diets and microbial factors, and that *via* bile acid receptors the population of colon-resident Foxp3$^+$ Treg cells simultaneously expressing RORγ can be modulated by bile acid metabolites, which contributes to the regulation of intestinal inflammation.[37] Interestingly, a recent study has identified the secondary bile acid 3β-hydroxydeoxycholic acid (isoDCA) as a potent regulator of the differentiation of peripheral Treg cells for its ability to induce increased expression of Foxp3 by inhibiting the immunostimulatory properties of DCs. By using the approach of engineered *Bacteroides* strains that specifically produce isoDCA when colonized with *Clostridium scindens* in mice, the increased population of Treg cells expressing RORγt in the colon is found to be associated with the isoDCA-producing consortia, which can enhance extrathymic differentiation dependent on non-coding sequence 1.[70] Altogether, these results indicate that bile acids and their signaling play a protective role in maintaining intestinal homeostasis. Understanding how such a complex mechanism between bile acid metabolites and microbiota ultimately impacts the intestinal epithelial immune system should be an important hot topic of intestinal mucosal immunity in the coming years.

Characteristics of bile acid metabolism in gut-associated diseases

IBD, including Crohn's disease (CD) and ulcerative colitis, is a chronic, recurrent, and multifactorial disease, involving the environment, genetics, metabolism disorders, and immunity.[71–76] Bile acids are important metabolites in humans which play roles in maintaining the intestinal homeostasis and immune environment, besides their pivotal roles in dietary lipid absorption and

cholesterol metabolism (Table 2). Of note, impaired metabolism of bile acids has been implicated in the pathogenesis and development of IBD.[77–80] A study on bile acid metabolism in IBD patients has demonstrated an increased proportion of conjugated bile acids and a reduced proportion of secondary bile acids in the feces of IBD patients, especially during the active period, even though the total concentrations of fecal bile acids are not significantly different between IBD patients and healthy controls. Furthermore, a much higher proportion of 3-OH-sulfated bile acids has been reported in the feces of patients with active IBD compared with IBD patients in remission and healthy controls. Nevertheless, any other significant differences in serum bile acid concentrations are not observed between IBD patients and healthy controls except that the concentration of secondary bile acid is reduced in the sera of IBD patients, suggesting an impaired luminal bacterial bile acid metabolism in IBD patients.[81] Primary sclerosing cholangitis (PSC) is highly associated with IBD and is a risk factor for colon cancer.[82,83] Several studies have demonstrated a reduced total bile acid pool in PSC-associated IBD patients and a decreased proportion of secondary bile acids in the stool of active IBD patients.[81,84] A pilot study of fecal bile acids and microbiota in patients with IBD and PSC has illustrated that PSC-associated IBD patients present a higher proportion of conjugated bile acids in stool, although statistical significances are not observed. Furthermore, DCA, a secondary bile acid, is also observed to be elevated in stool from PSC-associated IBD, while the proportion of UDCA in stool is not different between PSC-associated IBD and IBD alone.[84] In addition to the significantly reduced total stool bile acid pool in PSC-associated IBD, the serum bile acid pool is increased in these patients as compared with IBD alone. Correspondingly, patients with PSC-associated IBD demonstrate enrichment in bacteria from the *Fusobacterium* and *Ruminococcus* taxa, and a decrease in bacteria from the genera *Veillonella*, *Dorea*, *Blautia*, *Lachnospira*, and *Roseburia*.[84] These results align with previous findings, displaying an increase of *Fusobacterium* and *Ruminococcus* in stools from patients with PSC-IBD.[85] However, a recent study on the fecal bile acid pool in patients with PSC-associated IBD revealed that there is no substantial difference in the fecal bile acid profiles of patients with IBD-associated PSC compared with IBD alone or healthy controls. However, microbiota diversity is significantly decreased in those with PSC-IBD compared with IBD alone or healthy controls.[86] This discrepancy between the two studies may be associated with the different methods of fecal collection or bile acid analysis. The enterohepatic circulation of bile acids principally depends on the absorption of bile acid in the terminal ileum and colon, which could be disturbed in IBD. A study using a real-time polymerase chain reaction method to detect the expression of bile acid transporter in mucosal biopsy specimens from patients with CD or ulcerative colitis demonstrates an altered mRNA expression of important intestinal bile acid transporters.[87] The most striking observation in CD patients is the down-regulation of ASBT mRNA, which may be associated with altered bile acid profiles in IBD patients.

In IBD, the inflammation of the intestinal wall is an important contributing factor to the etiopathogenesis of diarrhea, causing the impaired ability of solute and water reabsorption,[90] destruction of epithelium integrity, disturbance of the intestinal microorganism homeostasis, and deficiency of specific transport mechanisms in the gut.[91] Actually, the diarrhea is in consequence of the impaired capacity of absorptive fluid, which is physiologically estimated to be 4.5–5 l/day. Bile acid malabsorption (BAM) is a symptom that occurs frequently in patients with IBD, especially in patients with ileal CD. It has been reported that patients with only colon disease have markedly decreased ileal bile acid absorption.[92] These results are also supported by a study in pediatric IBD, showing that 86% of CD patients with persistent diarrhea have no or only mild disease activity.[93] Interestingly, another study found that expression of apical sodium/bile acid cotransporting polypeptide responsible for ileal bile acid reabsorption in ileal biopsies from the non-inflammatory site of CD patients was significantly reduced,[94] suggesting that the diarrhea may be a potential protective mechanism whereby the accumulated toxic bile acids are diluted and excreted outside the body in time that epithelial integrity and function can be protected from the damage of toxicity of bile acids. This idea is supported by the observation that, despite elevated levels of colonic bile acids, bile acid-induced diarrhea is not associated with significant alterations in mucosal histology. Also, bile acids have been shown to impair the intestinal epithelium integrity, causing increased intestinal permeability.

This observation has gained further new support from the latest findings showing that GPBAR1 is very crucial for the integrity of the intestinal mucosa and plays important roles in the development of experimental colitis.[28] In fact, increased mucosal permeability with destroyed epithelial tight junction induced by the accumulation of bile acids in the intestine can also be present in asymptomatic CD patients.[95,96] Bacterial overgrowth is a frequent phenotype in patients with active IBD,[97] which may have a dramatic influence on bile acid metabolism. Steatorrhea often occurs due to the impaired ability of unconjugated bile acids to form micelles once the colonic bacteria transit into the proximal parts of the small intestine, leading to premature and increased deconjugation of bile acid conjugates. In addition, kidney stone disease has a high clinical correlation with CD,[98] and its pathogenesis is closely related to the damaged intestinal bile acid metabolism because DCA increases oxalate absorption.[99] In patients with CD, malabsorbed bile acids and fatty acids bind calcium ions in the intestine, thereby preventing the formation of oxalate. Reabsorbed oxalic acid is increased and secreted into the urine, promoting the formation of hyperoxaluria and kidney stones. Pigment gallstone disease in patients with CD has the same mechanism. Because of BAM, enhanced reabsorption of unconjugated bilirubin from the intestinal lumen leads to an acceleration of its enterohepatic circulation with biliary hypersecretion of bilirubin.[100,101]

Bile acids affect the intestinal environment by controlling the growth and maintenance of commensal microbiota, maintaining barrier integrity, and regulating the immune system,[102,103] which plays an important role in the development of colorectal cancer (CRC). All of these risk factors, including high-fat diet, unhealthy lifestyles such as long-term sedentariness, obesity, diabetes, and accumulation of toxic bile acids in sera, contribute to the development of CRC.[104–109] A previous study has demonstrated that tauro-β-muricholic acid and DCA could induce the abnormal proliferation and irreversible DNA damage in $Lgr5^+$ cells, while selective activation of intestinal FXR could suppress $Lgr5^+$ cell cancerous growth and curb CRC progression in mice,[110] which may be one of the reasons that a high-fat diet easily induces CRC. DNA replication errors during intestinal stem cell divisions with a high rate usually occur, which is implicated in the incidence of CRC.[111,112] In fact, the continuous renewal and persistent differentiation of intestinal stem cells at the bottom of crypts are required for the maintenance of intestinal epithelial barrier, and this process is tightly regulated by the Wnt-dependent signaling pathway. Once the intestinal crypt structure is damaged, the Wnt signaling pathway is disrupted and the $Lgr5^+$ stem cells at the bottom of the crypt are easily exposed to antigen cues in the intestinal lumen, thereby increasing the possibility of malignant transformation, which might be one reason why chronic inflammation in the intestine, such as in IBD, easily induces CRC. Furthermore, another study about liver cancer also demonstrated that bile acids can be used as a messenger signaling molecule to control the accumulation of NKT cells and antitumor immunity in the liver.[113] According to data from epidemiological and experimental studies, the development of CRC is involved in a high-fat diet.[114] More importantly, high-fat diet-related CRC has been reported to be associated with excessive bile acids, which play a critical role in the development of CRC. Using a mouse model of spontaneous tumors, increasing lines of evidence have shown that DCA can accelerate the transition from intestinal adenoma to adenocarcinoma,[115] and that DCA can cause the dysbiosis and play important contributing roles in carcinogenesis. Further studies on the effects of DCA have shown that the carcinogenic effect was mediated by DCA-induced alteration of the microbial community.[116] The pathogenesis of CRC is usually considered to be a multi-step process which involves cell mutation from adenoma to adenocarcinoma. The most common mutant genes in CRC are *APC*, *KRASras*, *p53*, *PI3K*, and *TGFb*.[117] In the normal intestinal tissue of mice, mRNA expression of FXR is the highest in fully differentiated epithelial cells, and FXR-deficient mice have damaged intestinal barrier with increased intestinal immune cell infiltration.[118] The expression of FXR is reduced in colon tumor tissues, where *APC* mutation is frequently observed.[119] A recent study has demonstrated that the loss of APC function in mouse colon mucosa and human colon cells silences the expression of FXR through *Fxr* gene CpG methylation and decreases the expression of downstream targets genes involved in the metabolic balance of bile acids such as small heterodimer partner (*Shp*) and *Ibabp*, while increasing the levels of pro-inflammatory or oncogenic factors such as COX-2 and c-MYC.[120] Taken together, the

carcinogenic effects of bile acids on the development and progression of CRC may be comprehensive, involving gut dysbiosis, stem cell renewal, apoptosis of epithelial cells, and genetic susceptibilities, and more efforts are needed to elucidate the underlying mechanisms of bile acid regulation of the carcinogenesis.

Therapeutic targeting of bile acids in intestinal diseases

Based on pleiotropic roles of bile acids in the regulation of intestinal physiology and immune responses, new therapeutic interventions targeting bile acids and their receptors or signaling have been developed recently. Either using pharmaceutical targeting bile acid transporter and receptors or, alternatively, indirectly changing the signature of the bile acid pool can be exploited as the target therapy for a variety of diseases. Gut dysbiosis is usually associated with the development of IBD.[121] The risk of CRC in patients with ulcerative colitis is six times higher than in the general population.[122] Recently, several studies have confirmed that manipulating the signature of the luminal bile acid pool to prevent or treat diseases can be achieved by the application of probiotics, which can significantly contribute to the normalization of gut microbiota and the improvement of mucosal barrier function.[123,124] Studies in animal models also demonstrate that such an approach may also be useful for the treatment of intestinal diseases. For example, under the effect of the BSHs of *Lactobacillus johnsonii* La1, the Giardia growth can be prevented by the production of secondary bile acids that show powerful toxicity to the parasite.[125] Similarly, a bile acid signature characterized by inhibition of *Clostridium difficile* infection has been created under the 7-dehydroxylating activity of *C. scindens*.[126] The dedicated bile acid receptors FXR and GBPAR1 have been prime targets for drug development. To date, some specific agonists have emerged, including PX-102, Ec001, LJN452, and GW4064 etc. Some published FXR agonists that have reached Phase I human clinical testing at least included OCA, EDP-305, cilofexor (GS-9674 or Px-201), tropifexor (LJN452), TERN-101 (LY2562175), Px-102/104, nidufexor (LMB763), EYP001(PXL007), AGN-242266 (AKN-083), WAY-450, and MET409. WAY-450 and Px-102/104 have been abandoned for undisclosed reasons. GW4064 was discovered to be the first synthetic FXR ligand in 2000. Administration of GW4064 to mice led to abrogated bacterial overgrowth in small intestine and decreased intestinal permeability and inflammation induced by bile duct ligation.[127,128] While extensively used as an experimental tool molecule for its selectivity toward FXR over many years, GW4064 never proceeded to a drug because of its low plasma bioavailability, hepatocellular toxicity, and poor pharmacokinetic properties.[129] LJN452, also called tropifexor, as a new safe drug candidate, could activate FXR with favorable properties, and it has progressed into clinical trials for the treatment of primary biliary sclerosis (PBC) and non-alcoholic steatohepatitis (NASH).[130,131]

BAR502 was a dual FXR/GPBAR1 agonist, representing a promising hit compound in treatment of NASH. Moreover, BAR502 displayed the abilities of modulating the expression of canonical FXR genes, increasing survival, and attenuating the level of alkaline phosphatase in serum without inducing pruritus in mouse model of cholestasis.[132,133] In addition, another representative dual FXR/GPBAR1 agonist, INT-767, the corresponding sulfated derivative of BAR502, was proved effective to alleviate liver damage, restore lipid and glucose metabolism, and reduce insulin resistance and pro-inflammatory response in rat model of NASH.[134] INT-767 had also been characterized in different animal models by decreasing inflammation and improving metabolism, in which INT-767 was effective to reduce ethanol-induced inflammation and steatosis in mice.[135–137] INT-777 was a potent and selective GPBAR1 agonist. In mouse model, GPBAR1 activation by INT-777 could stimulate GLP-1 release from enteroendocrine L-cells and increase energy expenditure, preventing obesity and diabetes.[138,139] BAR501, a GPBAR selective ligand, was derived from modification of UDCA and shown to exert a potent anti-inflammatory effect in mouse NASH model.[140,141] Furthermore, BAR501 was reported to regulate activation of intestinal macrophage, rescuing mice from colitis. Particularly, GPBAR1 activation by BAR501 could shift the polarization of macrophage from pro-inflammatory M1 phenotype to anti-inflammatory M2 phenotype, which reversed the colonic inflammation in response to TNBS while not influencing the ratio of resident versus inflammatory monocytes.[31] Likewise, administration of BAR501 to mice treated with a high-fat diet revealed ameliorated steatosis and fibrosis as well as attenuated fat liver deposition.[133,142] Recently, GPBAR1 agonism by BAR501 was reported to regulate the severity of liver injury by modulating

the expression of CCL2 and CCR2 in mouse model of acetaminophen-induced liver toxicity.[143] The GPBAR1 selective agonist BAR501 has been affirmed as a promising compound in IBD because of its properties of attenuating inflammation and regulating immune response by shifting macrophage in colon from M1 phenotype to M2 phenotype.[31] Moreover, other GPBAR1 agonists include but are not limited to 3-aryl-4-isoxazolecarboxamide, betulinic acid, oleanoic acid, and BIX02694 and they were reported to attenuate the severity of colitis and production of pro-inflammatory cytokines in mice.[28,31,41,88,89] In the last two decades, GPBAR1 non-steroidal agonists with improved selectivity had been developed and could be classified as follows: 3-aryl-4-isoxazolecarboxamides, 3-aminomethylquinolines, 2-phenoxynicotinamides, 4-phenylpyridines and pyrimidines, 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazoles, nipecotamide derivatives, oximes, and diazepine.[144]

OCA, the first synthetic bile acid receptor modulator approved by the FDA for treatment of PBC, was used for patients in 2016.[145] In mice, OCA was able to protect mice against DSS-induced injury, alleviate disease severity and maintain the integrity of intestinal epithelial barrier.[21,22] TC-100, a potent and selective FXR agonist produced from the introduction of a hydroxyl group, was endowed with improved physicochemical profiles, thus providing a novel therapeutic agent for enterohepatic disorders such as IBD.[146] FXR selectivity can also be targeted by BAR701 and BAR704. Noteworthy, BAR704 can also weakly antagonize GPBAR1, which is in contrast to GPBAR1 transactivation of OCA. Similarly, BAR704 treatment in mouse fibrosis model turned out to shift liver macrophage from M1 to M2 phenotype.[147] So far, however, only OCA has been put into human clinical use. Indeed, severe and adverse drug side effects emerged, such as pruritus, gastrointestinal problems, increased risk of acute liver decompensation, and increased low-density lipoprotein cholesterol levels that was related to increasing cardiovascular risk.[148–150] Similarly, application of GPBAR1 agonists for drug development has been hindered by side effects including inhibition of gallbladder emptying, diarrhea, itching, and other syndrome.[151] One of the potential mechanisms causing pruritus during chronic OCA administration could be imputable to OCA residual activity toward GPBAR1.[146] Therefore, the intestinal selective FXR agonists have gained substantial interest because of their beneficial effects without activation of liver FXR. As one of the earliest synthetic FXR agonists,[152] fexaramine has become a typical intestine-restricted FXR agonist with potent beneficial metabolic effects while avoiding the side effects that come with liver FXR activation because it was poorly absorbed by intestine.[153] Further on, chronic administration of fexaramine to diet-induced obesity mice could increase expression of mucosal defensin and reduce intestinal permeability, stabilizing the gut barrier,[153] which might provide the possibility of fexaramine treatment in IBD. Concerning intestinal diseases, results from preclinical and experimental studies of intestinal inflammation in the DSS-induced mouse colitis model suggest that several FXR agonists could be excellent candidate drugs that display favorable properties in the intervention and treatment of IBD.[22,154] Similarly, DSS-induced intestinal inflammation was aggravated in *Fxr*$^{-/-}$ mice, and genetic variation of FXR was reported to be associated with human IBD, implicating the critical role of FXR in IBD.[155] FXR activation alleviated inflammation and preserved the integrity of the intestinal epithelial barrier,[21,22] which was destroyed in IBD. Mice lacking GPBAR1 often develop a severe intestinal inflammation when challenged with DSS or TNBS due to inability to produce enough anti-inflammatory cytokines.[31] All of these results suggest that directly or indirectly FXR and GPBAR1 play very critical roles in IBD. Due to the function of limiting fluid secretion into the gut,[156] FXR agonists may also play a positive role in diarrheal treatment. In addition to intestinal immune responses regulated by GPBAR1 described above, secretion and motility are both regulated by GPBAR1 and its signaling,[28,50,56,88,157,158] indicating that targeting GPBAR1 could be a promising method to treat some disorders in intestine. GPBAR1 appears to play different roles in coordinating intestinal responses in different areas of the gastrointestinal (GI) tract. For instance, in small intestine, activation of GPBAR1 on L-cells resulted in decreased motility and slowing of gastric emptying, while GPBAR1 activation on epithelial cells enhanced secretion of 5-hydroxytroptamine, therefore intensifying peristalsis.[151] Specifically, evidence from mice showed that effects of bile acid on colonic motility were mediated by GPBAR1, and deficiency of GPBAR1 led to constipation in mice.[56] Another study in rat revealed that activation of GPBAR1 in colonic epithelium and cholinergic

enteric neurons by GPBAR1 agonist such as INT777 could inhibit colonocytes and cholinergic submucosal neurons and therefore reduce basal and stimulated chloride secretion, indicating colonic GPBAR1 as a potential target against secretory diarrhea-associated GI disorders.[158] As hydrophilic secondary bile acid with minute amounts in human, UDCA has historically been used to treat cholestatic disorders such as biliary atresia for many years because of its ability to stimulate bile flow and prevent contact between hepatocytes and the toxic bile acids such as LCA.[159–161] The promising roles of UDCA in the treatment of intestinal disorders, including IBD, have been indicated in several mouse and cell studies.[162] Experiments with different animal models of IBD have revealed that UDCA and its derivatives exhibit pleiotropic properties in regulating the intestinal homeostasis, including attenuating cytokine levels, inhibiting the production of antimicrobial peptides, and preventing cell apoptosis.[163–166] The effect of UDCA on intestinal mucosal immune cells curbs the activation of immune cells and the production of pro-inflammatory cytokines.[167] Very recently, UDCA has formally been recognized as a GPBAR1 agonist despite its weak GPBAR1 agonistic effect and was reported to treat mouse colitis, and experimental data characterized by the limitation of pro-inflammatory cytokines and the alleviation of colon inflammation suggested a potential effective candidate for UDCA in treating IBD.[149,168–170] Worth mentioning is the FXR antagonistic effect of UDCA since study from patients with non-alcoholic fatty liver disease showed that short-term treatment with UDCA increased bile acid generation by blunting FXR activity.[171] Intriguingly, recent studies have demonstrated that LCA exhibits an absolutely distinct role in contrast to its typical characteristic of toxicity to organs, in fact, which is necessary to fully exert the protection of UDCA on gut during inflammation.[169] Recently, 3-oxoLCA and isoalloLCA, two derivatives of LCA from gut-residing bacteria, have been found to modulate Th17 and Treg cell differentiation in the intestine, which might represent a promising new idea for treatment for IBD.[39] In addition, isoDCA can also increase Foxp3 induction and enhance the generation of Treg cells, suggesting that this secondary bile acid contributes to immunological balance in the colon and has the possibility to serve as a novel drug targeting IBD.[70] There have been exciting advances in the last decade to better understand how bile acid signaling regulates intestinal homeostasis; however, areas exist where there are knowledge gaps in humans. It is extremely important and equally challenging to understand the complex roles of bile acids as signaling intermediates between host and microbes in our intestine. Understanding this dialogue will provide great potential and opportunity to develop more specific and effective drugs.

Conclusions

Bile acids are receiving a great deal of attention and interest as critical regulators of the intestinal immune system and microbiota. In this review, we describe the current understanding of the importance of bile acids in health and diseases and some emerging advances that have been made during recent years with respect to our knowledge of bile acids' roles in the intestinal mucosal immune system. The greater appreciation of bile acids in the treatment of gut-associated diseases will lift the discovery of new drugs to target bile acid signaling to new heights, which will assure the importance of this area in the future. However, the unwanted side effects of bile acids and derivative (OCA) and non-steroidal FXR agonists are pruritus and hepatotoxicity, and these problems cannot be ignored. Furthermore, a lot of effort is needed to identify which bile acids function as a specific target drug and to elucidate the mechanism. In addition, although many concepts based on *in vitro* experiments or mice have been proposed, the transformation from basic studies to clinical application must be achieved as soon as possible.

Author contributions

ZL was responsible for conception, literature review, and revising the manuscript. RS, CX, and BF drafted the manuscript and interpreted the results. All authors agreed to the final version.

Conflict of interest statement

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References

1. Amoroso C, Perillo F, Strati F, *et al.* The role of gut microbiota biomodulators on mucosal immunity and intestinal inflammation. *Cells* 2020; 9: 1234.
2. Belkaid Y and Segre JA. Dialogue between skin microbiota and immunity. *Science* 2014; 346: 954–959.
3. Maloy KJ and Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* 2011; 474: 298–306.
4. Fiorucci S and Distrutti E. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. *Trends Mol Med* 2015; 21: 702–714.
5. Chen ML, Takeda K and Sundrud MS. Emerging roles of bile acids in mucosal immunity and inflammation. *Mucosal Immunol* 2019; 12: 851–861.
6. Fiorucci S, Biagioli M, Zampella A, *et al.* Bile acids activated receptors regulate innate immunity. *Front Immunol* 2018; 9: 1853.
7. Ridlon JM, Kang D-J and Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* 2006; 47: 241–259.
8. Chiang JY. Bile acids: regulation of synthesis. *J Lipid Res* 2009; 50: 1955–1966.
9. Chiang JY. Bile acid metabolism and signaling. *Compr Physiol* 2013; 3: 1191–1212.
10. Norlin M and Wikvall K. Enzymes in the conversion of cholesterol into bile acids. *Curr Mol Med* 2007; 7: 199–218.
11. Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; 72: 137–174.
12. Jones BV, Begley M, Hill C, *et al.* Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci U S A* 2008; 105: 13580–13585.
13. Hofmann AF, Hagey LR and Krasowski MD. Bile salts of vertebrates: structural variation and possible evolutionary significance. *J Lipid Res* 2010; 51: 226–246.
14. Hofmann AF. Detoxification of lithocholic acid, a toxic bile acid: relevance to drug hepatotoxicity. *Drug Metab Rev* 2004; 36: 703–722.
15. Hofmann AF. The enterohepatic circulation of bile acids in mammals: form and functions. *Front Biosci (Landmark Ed)* 2009; 14: 2584–2598.
16. Dawson PA and Karpen SJ. Intestinal transport and metabolism of bile acids. *J Lipid Res* 2015; 56: 1085–1099.
17. Hegyi P, Pandol S, Venglovecz V, *et al.* The acinar-ductal tango in the pathogenesis of acute pancreatitis. *Gut* 2011; 60: 544–552.
18. Steinmetz AC, Renaud JP and Moras D. Binding of ligands and activation of transcription by nuclear receptors. *Annu Rev Biophys Biomol Struct* 2001; 30: 329–359.
19. Glass CK and Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* 2000; 14: 121–141.
20. Dawson PA, Lan T and Rao A. Bile acid transporters. *J Lipid Res* 2009; 50: 2340–2357.
21. Ding L, Yang L, Wang Z, *et al.* Bile acid nuclear receptor FXR and digestive system diseases. *Acta Pharm Sin B* 2015; 5: 135–144.
22. Gadaleta RM, van Erpecum KJ, Oldenburg B, *et al.* Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* 2011; 60: 463–472.
23. Vavassori P, Mencarelli A, Renga B, *et al.* The bile acid receptor FXR is a modulator of intestinal innate immunity. *J Immunol* 2009; 183: 6251–6261.
24. Wang YD, Chen WD, Wang M, *et al.* Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology* 2008; 48: 1632–1643
25. Gadaleta RM, Oldenburg B, Willemsen EC, *et al.* Activation of bile salt nuclear receptor FXR is repressed by pro-inflammatory cytokines activating NF-κB signaling in the intestine. *Biochim Biophys Acta* 2011; 1812: 851–858.
26. Massafra V, Ijssennagger N, Plantinga M, *et al.* Splenic dendritic cell involvement in FXR-mediated amelioration of DSS colitis. *Biochim Biophys Acta* 2016; 1862: 166–173.
27. Garcia-Irigoyen O and Moschetta A. A novel protective role for FXR against inflammasome activation and endotoxemia. *Cell Metab* 2017; 25: 763–764.

28. Cipriani S, Mencarelli A, Chini MG, *et al*. The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis. *PLoS One* 2011; 6: e25637.

29. Ichikawa R, Takayama T, Yoneno K, *et al*. Bile acids induce monocyte differentiation toward interleukin-12 hypo-producing dendritic cells via a TGR5-dependent pathway. *Immunology* 2012; 136: 153–162.

30. McMahan RH, Wang XX, Cheng LL, *et al*. Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem* 2013; 288: 11761–11770.

31. Biagioli M, Carino A, Cipriani S, *et al*. The bile acid receptor GPBAR1 regulates the M1/M2 phenotype of intestinal macrophages and activation of GPBAR1 rescues mice from murine colitis. *J Immunol* 2017; 199: 718–733.

32. Venkatesh M, Mukherjee S, Wang H, *et al*. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor.4 *Immunity* 2014; 41: 296–310.

33. Huang K, Mukherjee S, DesMarais V, *et al*. Targeting the PXR-TLR4 signaling pathway to reduce intestinal inflammation in an experimental model of necrotizing enterocolitis. *Pediatr Res* 2018; 83: 1031–1040.

34. Shah YM, Ma X, Morimura K, *et al*. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-kappaB target gene expression. *Am J Physiol Gastrointest Liver Physiol* 2007; 292: G1114–G1122.

35. Terc J, Hansen A, Alston L, *et al*. Pregnane X receptor agonists enhance intestinal epithelial wound healing and repair of the intestinal barrier following the induction of experimental colitis. *Eur J Pharm Sci* 2014; 55: 12–19.

36. Cheng J, Shah YM and Gonzalez FJ. Pregnane X receptor as a target for treatment of inflammatory bowel disorders. *Trends Pharmacol Sci* 2012; 33: 323–330.

37. Song X, Sun X, Oh SF, *et al*. Microbial bile acid metabolites modulate gut RORgamma^{+} regulatory T cell homeostasis. *Nature* 2020; 577: 410–415.

38. Korn T, Bettelli E, Oukka M, *et al*. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; 27: 485–517.

39. Hang S, Paik D, Yao L, *et al*. Bile acid metabolites control TH17 and Treg cell differentiation. *Nature* 2019; 576: 143–148.

40. Pols TW, Nomura M, Harach T, *et al*. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metab* 2011; 14: 747–757.

41. Yoneno K, Hisamatsu T, Shimamura K, *et al*. TGR 5 signalling inhibits the production of pro-inflammatory cytokines by in vitro differentiated inflammatory and intestinal macrophages in Crohn's disease. *Immunology* 2013; 139: 1 9–29.

42. Guo C, Xie S, Chi Z, *et al*. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. *Immunity* 2016; 45: 802–816.

43. Kawamata Y, Fujii R, Hosoya M, *et al*. A G protein-coupled receptor responsive to bile acids. *J Biol Chem* 2003; 278: 9435–9440.

44. Maruyama T, Miyamoto Y, Nakamura T, *et al*. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun* 2002; 298: 714–719.

45. Brighton CA, Rievaj J, Kuhre RE, *et al*. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein–coupled bile acid receptors. *Endocrinology* 2015; 156: 3961–3970.

46. Hong J, Behar J, Wands J, *et al*. Bile acid reflux contributes to development of esophageal adenocarcinoma via activation of phosphatidylinositol-specific phospholipase Cgamma2 and NADPH oxidase NOX5-S. *Cancer Res* 2010; 70: 1247–1255.

47. Keitel V, Cupisti K, Ullmer C, *et al*. The membrane-bound bile acid receptor TGR5 is localized in the epithelium of human gallbladders. *Hepatology* 2009; 50: 861–870.

48. Keitel V and Häussinger D. TGR5 in the biliary tree. *Dig Dis* 2011; 29: 45–47.

49. Kowal JM, Haanes KA, Christensen NM, *et al*. Bile acid effects are mediated by ATP release and purinergic signalling in exocrine pancreatic cells. *Cell Commun Signal* 2015; 13: 28.

50. Ward JB, Mroz MS and Keely SJ. The bile acid receptor, TGR5, regulates basal and cholinergic-induced secretory responses in rat colon. *Neurogastroenterol Motil* 2013; 25: 708–711.

51. Duboc H, Taché Y and Hofmann AF. The bile acid TGR5 membrane receptor: from basic

research to clinical application. *Dig Liver Dis* 2014; 46: 302–312.

52. Kuipers F, Bloks VW and Groen AK. Beyond intestinal soap–bile acids in metabolic control. *Nat Rev Endocrinol* 2014; 10: 488–498.
53. Perino A and Schoonjans K. TGR5 and immunometabolism: insights from physiology and pharmacology. *Trends Pharmacol Sci* 2015; 36: 847–857.
54. Porez G, Prawitt J, Gross B, *et al*. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease thematic review series: new lipid and lipoprotein targets for the treatment of cardiometabolic diseases. *J Lipid Res* 2012; 53: 1723–1737.
55. Schaap FG, Trauner M and Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol* 2014; 11: 55–67.
56. Alemi F, Poole DP, Chiu J, *et al*. The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. *Gastroenterology* 2013; 144: 145–154.
57. Halilbasic E, Claudel T and Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *J Hepatol* 2013; 58: 155–168.
58. Lundin A, Bok CM, Aronsson L, *et al*. Gut flora, Toll-like receptors and nuclear receptors: a tripartite communication that tunes innate immunity in large intestine. *Cell Microbiol* 2008; 10: 1093–1103.
59. Simmons JD, Mullighan C, Welsh KI, *et al*. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. *Gut* 2000; 47: 211–214.
60. Dring MM, Goulding CA, Trimble VI, *et al*. The pregnane X receptor locus is associated with susceptibility to inflammatory bowel disease. *Gastroenterology* 2006; 130: 341–348.
61. Liu JZ, van Sommeren S, Huang H, *et al*. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* 2015; 47: 979–986.
62. Staudinger JL, Goodwin B, Jones SA, *et al*. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc Natl Acad Sci U S A* 2001; 98: 3369–3374.
63. Mencarelli A, Renga B, Palladino G, *et al*. Inhibition of NF-κB by a PXR-dependent pathway mediates counter-regulatory activities of rifaximin on innate immunity in intestinal epithelial cells. *Eur J Pharmacol* 2011; 668: 317–324.
64. Christakos S, Dhawan P, Verstuyf A, *et al*. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* 2016; 96: 365–408.
65. Li YC, Chen Y and Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. *J Steroid Biochem Mol Biol* 2015; 148: 179–183.
66. Ryan JW, Anderson PH and Morris HA. Pleiotropic activities of vitamin D receptors – adequate activation for multiple health outcomes. *Clin Biochem Rev* 2015; 36: 53–61.
67. Kim J-H, Yamaori S, Tanabe T, *et al*. Implication of intestinal VDR deficiency in inflammatory bowel disease. *Biochim Biophys Acta* 2013; 1830: 2118–2128.
68. Kong J, Zhang Z, Musch MW, *et al*. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G208–G216.
69. Liu W, Chen Y, Golan MA, *et al*. Intestinal epithelial vitamin D receptor signaling inhibits experimental colitis. *J Clin Invest* 2013; 123: 3983–3996.
70. Campbell C, McKenney PT, Konstantinovsky D, *et al*. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* 2020; 581: 475–479.
71. Morgan XC, Tickle TL, Sokol H, *et al*. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012; 13: R79.
72. Gevers D, Kugathasan S, Denson LA, *et al*. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014; 15: 382–392.
73. Lewis JD, Chen EZ, Baldassano RN, *et al*. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. *Cell Host Microbe* 2015; 18: 489–500.
74. Ni J, Wu GD, Albenberg L, *et al*. Gut microbiota and IBD: causation or correlation? *Nat Rev Gastroenterol Hepatol* 2017; 14: 573–584.
75. de Souza HS and Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol* 2016; 13: 13–27.

76. Wang MH and Achkar JP. Gene-environment interactions in inflammatory bowel disease pathogenesis. *Curr Opin Gastroenterol* 2015; 31: 277–282.

77. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, *et al*. Gut microbiome structure and metabolic activity in inflammatory bowel disease. *Nat Microbiol* 2019; 4: 293–305.

78. Jansson J, Willing B, Lucio M, *et al*. Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLoS One* 2009; 4: e6386.

79. Jacobs JP, Goudarzi M, Singh N, *et al*. A disease-associated microbial and metabolomics state in relatives of pediatric inflammatory bowel disease patients. *Cell Mol Gastroenterol Hepatol* 2016; 2: 750–766.

80. Lloyd-Price J, Arze C, Ananthakrishnan AN, *et al*. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* 2019; 569: 655–662.

81. Duboc H, Rajca S, Rainteau D, *et al*. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. *Gut* 2013; 62: 531–539.

82. Torres J, Pineton de Chambrun G, Itzkowitz S, *et al*. Review article: colorectal neoplasia in patients with primary sclerosing cholangitis and inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; 34: 497–508.

83. Goldstone R, Itzkowitz S, Harpaz N, *et al*. Dysplasia is more common in the distal than proximal colon in ulcerative colitis surveillance. *Inflamm Bowel Dis* 2012; 18: 832–837.

84. Torres J, Palmela C, Brito H, *et al*. The gut microbiota, bile acids and their correlation in primary sclerosing cholangitis associated with inflammatory bowel disease. *United European Gastroenterol J* 2018; 6: 112–122.

85. Sabino J, Vieira-Silva S, Machiels K, *et al*. Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD. *Gut* 2016; 65: 1681–1689.

86. Vaughn BP, Kaiser T, Staley C, *et al*. A pilot study of fecal bile acid and microbiota profiles in inflammatory bowel disease and primary sclerosing cholangitis. *Clin Exp Gastroenterol* 2019; 12: 9–19.

87. Jahnel J, Fickert P, Hauer AC, *et al*. Inflammatory bowel disease alters intestinal bile acid transporter expression. *Drug Metab Dispos* 2014; 42: 1423–1431.

88. Sakanaka T, Inoue T, Yorifuji N, *et al*. The effects of a TGR5 agonist and a dipeptidyl peptidase IV inhibitor on dextran sulfate sodium-induced colitis in mice. *J Gastroenterol Hepatol* 2015; 30(Suppl. 1): 60–65.

89. Fryer RM, Ng KJ, Nodop Mazurek SG, *et al*. G protein-coupled bile acid receptor 1 stimulation mediates arterial vasodilation through a K(Ca)1.1 (BK(Ca))-dependent mechanism. *J Pharmacol Exp Ther* 2014; 348: 421–431.

90. Markossian S and Kreydiyyeh SI. TNF-alpha down-regulates the Na^+-K^+ ATPase and the Na^+-K^+-$2Cl^-$cotransporter in the rat colon via PGE_2. *Cytokine* 2005; 30: 319–327.

91. Binder HJ. Mechanisms of diarrhea in inflammatory bowel diseases. *Ann N Y Acad Sci* 2009; 1165: 285–293.

92. Lenicek M, Duricova D, Komarek V, *et al*. Bile acid malabsorption in inflammatory bowel disease: assessment by serum markers. *Inflamm Bowel Dis* 2011; 17: 1322–1327.

93. Gothe F, Beigel F, Rust C, *et al*. Bile acid malabsorption assessed by 7 alpha-hydroxy-4-cholesten-3-one in pediatric inflammatory bowel disease: correlation to clinical and laboratory findings. *J Crohns Colitis* 2014; 8: 1072–1078.

94. Jung D, Fantin A, Scheurer U, *et al*. Human ileal bile acid transporter gene ASBT (SLC10A2) is transactivated by the glucocorticoid receptor. *Gut* 2004; 53: 78–84.

95. Raimondi F, Santoro P, Barone MV, *et al*. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G906–G913.

96. Zeissig S, Bergann T, Fromm A, *et al*. Altered ENaC expression leads to impaired sodium absorption in the noninflamed intestine in Crohn's disease. *Gastroenterology* 2008; 134: 1436–1447.

97. Rana SV, Sharma S, Malik A, *et al*. Small intestinal bacterial overgrowth and orocecal transit time in patients of inflammatory bowel disease. *Dig Dis Sci* 2013; 58: 2594–2598.

98. Hueppelshaeuser R, von Unruh GE, Habbig S, *et al*. Enteric hyperoxaluria, recurrent urolithiasis, and systemic oxalosis in patients with Crohn's disease. *Pediatr Nephrol* 2012; 27: 1103–1109.

99. Dobbins JW and Binder HJ. Effect of bile salts and fatty acids on the colonic absorption of oxalate. *Gastroenterology* 1976; 70: 1096–1100.

100. Vitek L and Carey MC. Enterohepatic cycling of bilirubin as a cause of 'black' pigment gallstones in adult life. *Eur J Clin Invest* 2003; 33: 799–810.

101. Vitek L and Carey MC. New pathophysiological concepts underlying pathogenesis of pigment gallstones. *Clin Res Hepatol Gastroenterol* 2012; 36: 122–129.

102. Shapiro H, Thaiss CA, Levy M, *et al*. The cross talk between microbiota and the immune system: metabolites take center stage. *Curr Opin Immunol* 2014; 30: 54–62.

103. Stenman LK, Holma R, Eggert A, *et al*. A novel mechanism for gut barrier dysfunction by dietary fat: epithelial disruption by hydrophobic bile acids. *Am J Physiol Gastrointest Liver Physiol* 2013; 304: G227–G234.

104. de Aguiar Vallim TQ, Tarling EJ and Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metab* 2013; 17: 657–669.

105. Degirolamo C, Modica S, Palasciano G, *et al*. Bile acids and colon cancer: solving the puzzle with nuclear receptors. *Trends Mol Med* 2011; 17: 564–572.

106. Downes M and Liddle C. Look who's talking: nuclear receptors in the liver and gastrointestinal tract. *Cell Metab* 2008; 7: 195–199.

107. Font-Burgada J, Sun B and Karin M. Obesity and cancer: the oil that feeds the flame. *Cell Metab* 2016; 23: 48–62.

108. Kuipers EJ, Grady WM, Lieberman D, *et al*. Colorectal cancer. *Nat Rev Dis Primers* 2015; 1: 15065.

109. Thomas C, Pellicciari R, Pruzanski M, *et al*. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* 2008; 7: 678–693.

110. Fu T, Coulter S, Yoshihara E, *et al*. FXR regulates intestinal cancer stem cell proliferation. *Cell* 2019; 176: 1098–1112.e18.

111. Tomasetti C, Li L and Vogelstein B. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. *Science* 2017; 355: 1330–1334.

112. Tomasetti C and Vogelstein B. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* 2015; 347: 78–81.

113. Ma C, Han M, Heinrich B, *et al*. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* 2018; 360: 6391.

114. Beyaz S, Mana MD, Roper J, *et al*. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* 2016; 531: 53–58.

115. Cao H, Luo S, Xu M, *et al*. The secondary bile acid, deoxycholate accelerates intestinal adenoma–adenocarcinoma sequence in Apc (min/+) mice through enhancing Wnt signaling. *Fam Cancer* 2014; 13: 563–571.

116. Cao H, Xu M, Dong W, *et al*. Secondary bile acid-induced dysbiosis promotes intestinal carcinogenesis. *Int J Cancer* 2017; 140: 2545–2556.

117. Armaghany T, Wilson JD, Chu Q, *et al*. Genetic alterations in colorectal cancer. *Gastrointest Cancer Res* 2012; 5: 19–27.

118. De Gottardi A, Touri F, Maurer CA, *et al*. The bile acid nuclear receptor FXR and the bile acid binding protein IBABP are differently expressed in colon cancer. *Dig Dis Sci* 2004; 49: 982–989.

119. Bailey AM, Zhan L, Maru D, *et al*. FXR silencing in human colon cancer by DNA methylation and KRAS signaling. *Am J Physiol Gastrointest Liver Physiol* 2014; 306: G48–G58.

120. Selmin OI, Fang C, Lyon AM, *et al*. Inactivation of adenomatous polyposis coli reduces bile acid/farnesoid X receptor expression through Fxr gene CpG methylation in mouse colon tumors and human colon cancer cells. *J Nutr* 2016; 146: 236–242.

121. Hold GL, Smith M, Grange C, *et al*. Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? *World J Gastroenterol* 2014; 20: 1192–1210.

122. Mattar MC, Lough D, Pishvaian MJ, *et al*. Current management of inflammatory bowel disease and colorectal cancer. *Gastrointest Cancer Res* 2011; 4: 53–61.

123. Morelli L and Capurso L. FAO/WHO guidelines on probiotics: 10 years later. *J Clin Gastroenterol* 2012; 46(Suppl.): S1–S2.

124. Louis P, Hold GL and Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014; 12: 661–672.

125. Travers MA, Sow C, Zirah S, *et al*. Deconjugated bile salts produced by extracellular bile-salt hydrolase-like activities from the probiotic *Lactobacillus johnsonii* La1 inhibit *Giardia duodenalis in vitro* growth. *Front Microbiol* 2016; 7: 1453.

126. Buffie CG, Bucci V, Stein RR, *et al*. Precision microbiome reconstitution restores bile acid

mediated resistance to Clostridium difficile. *Nature* 2015; 517: 205–208.

127. Inagaki T, Moschetta A, Lee YK, *et al.* Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci U S A* 2006; 103: 3920–3925.

128. Verbeke L, Farre R, Verbinnen B, *et al.* The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am J Pathol* 2015; 185: 409–419.

129. Crawley ML. Farnesoid X receptor modulators: a patent review. *Expert Opin Ther Pat* 2010; 20: 1047–1057.

130. Bahar R, Wong KA, Liu CH, *et al.* Update on new drugs and those in development for the treatment of primary biliary cholangitis. *Gastroenterol Hepatol (N Y)* 2018; 14: 154–163.

131. Massafra V, Pellicciari R, Gioiello A, *et al.* Progress and challenges of selective Farnesoid X receptor modulation. *Pharmacol Ther* 2018; 191: 162–177.

132. Cipriani S, Renga B, D'Amore C, *et al.* Impaired itching perception in murine models of cholestasis is supported by dysregulation of GPBAR1 signaling. *PLoS One* 2015; 10: e0129866.

133. Carino A, Cipriani S, Marchiano S, *et al.* BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. *Sci Rep* 2017; 7: 42801.

134. Hu YB, Liu XY and Zhan W. Farnesoid X receptor agonist INT-767 attenuates liver steatosis and inflammation in rat model of nonalcoholic steatohepatitis. *Drug Des Devel Ther* 2018; 12: 2213–2221.

135. Moris D, Giaginis C, Tsourouflis G, *et al.* Farnesoid-X receptor (FXR) as a promising pharmaceutical target in atherosclerosis. *Curr Med Chem* 2017; 24: 1147–1157.

136. Roth JD, Feigh M, Veidal SS, *et al.* INT-767 improves histopathological features in a diet-induced *ob*/*ob* mouse model of biopsy-confirmed non-alcoholic steatohepatitis. *World J Gastroenterol* 2018; 24: 195–210.

137. Iracheta-Vellve A, Calenda CD, Petrasek J, *et al.* FXR and TGR5 agonists ameliorate liver injury, steatosis, and inflammation after binge or prolonged alcohol feeding in mice. *Hepatol Commun* 2018; 2: 1379–1391.

138. Thomas C, Gioiello A, Noriega L, *et al.* TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab* 2009; 10: 167–177.

139. Yu DD, Sousa KM, Mattern DL, *et al.* Stereoselective synthesis, biological evaluation, and modeling of novel bile acid-derived G-protein coupled bile acid receptor 1 (GP-BAR1, TGR5) agonists. *Bioorg Med Chem* 2015; 23: 1613–1628.

140. Carino A, Marchiano S, Biagioli M, *et al.* Agonism for the bile acid receptor GPBAR1 reverses liver and vascular damage in a mouse model of steatohepatitis. *FASEB J* 2019; 33: 2809–2822.

141. Kida T, Omori K, Hori M, *et al.* Stimulation of G protein-coupled bile acid receptor enhances vascular endothelial barrier function via activation of protein kinase A and Rac1. *J Pharmacol Exp Ther* 2014; 348: 125–130.

142. Ramirez-Perez O, Cruz-Ramon V, Chinchilla-Lopez P, *et al.* The role of the gut microbiota in bile acid metabolism. *Ann Hepatol* 2017; 16(Suppl. 1): S21–S26.

143. Biagioli M, Carino A, Fiorucci C, *et al.* The bile acid receptor GPBAR1 modulates CCL2/CCR2 signaling at the liver sinusoidal/macrophage interface and reverses acetaminophen-induced liver toxicity. *J Immunol* 2020; 204: 2535–2551.

144. Xu Y. Recent progress on bile acid receptor modulators for treatment of metabolic diseases. *J Med Chem* 2016; 59: 6553–6579.

145. Hegade VS, Speight RA, Etherington RE, *et al.* Novel bile acid therapeutics for the treatment of chronic liver diseases. *Therap Adv Gastroenterol* 2016; 9: 376–391.

146. Pellicciari R, Passeri D, De Franco F, *et al.* Discovery of 3α, 7α, 11β-trihydroxy-6α-ethyl-5β-cholan-24-oic acid (TC-100), a novel bile acid as potent and highly selective FXR agonist for enterohepatic disorders. *J Med Chem* 2016; 59: 9201–9214.

147. Carino A, Biagioli M, Marchiano S, *et al.* Disruption of TFGbeta-SMAD3 pathway by the nuclear receptor SHP mediates the antifibrotic activities of BAR704, a novel highly selective FXR ligand. *Pharmacol Res* 2018; 131: 17–31.

148. Han CY. Update on FXR biology: promising therapeutic target? *Int J Mol Sci* 2018; 19: 2069.

149. Carino A, Biagioli M, Marchianò S, *et al.* Ursodeoxycholic acid is a GPBAR1 agonist and resets liver/intestinal FXR signaling in a model of diet-induced dysbiosis and NASH. *Biochim*

Biophys Acta Mol Cell Biol Lipids 2019; 1864: 1422–1437.

150. Pate J, Gutierrez JA, Frenette CT, *et al.* Practical strategies for pruritus management in the obeticholic acid-treated patient with PBC: proceedings from the 2018 expert panel. *BMJ Open Gastroenterol* 2019; 6: e000256.
151. Ticho AL, Malhotra P, Dudeja PK, *et al.* Bile acid receptors and gastrointestinal functions. *Liver Res* 2019; 3: 31–39.
152. Downes M, Verdecia MA, Roecker AJ, *et al.* A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. *Mol Cell* 2003; 11: 1079–1092.
153. Fang S, Suh JM, Reilly SM, *et al.* Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat Med* 2015; 21: 159–165.
154. Stojancevic M, Stankov K and Mikov M. The impact of Farnesoid X receptor activation on intestinal permeability in inflammatory bowel disease. *Can J Gastroenterol* 2012; 26: 631–637.
155. Attinkara R, Mwinyi J, Truninger K, *et al.* Association of genetic variation in the NR1H4 gene, encoding the nuclear bile acid receptor FXR, with inflammatory bowel disease. *BMC Res Notes* 2012; 5: 461.
156. Mroz MS, Keating N, Ward JB, *et al.* Farnesoid X receptor agonists attenuate colonic epithelial secretory function and prevent experimental diarrhoea in vivo. *Gut* 2014; 63: 808–817.
157. Bunnett NW. Neuro-humoral signalling by bile acids and the TGR5 receptor in the gastrointestinal tract. *J Physiol* 2014; 592: 2943–2950.
158. Duboc H, Tolstanova G, Yuan PQ, *et al.* Reduction of epithelial secretion in male rat distal colonic mucosa by bile acid receptor TGR5 agonist, INT-777: role of submucosal neurons. *Neurogastroenterol Motil* 2016; 28: 1663–1676.
159. Paumgartner G. Medical treatment of cholestatic liver diseases: from pathobiology to pharmacological targets. *World J Gastroenterol* 2006; 12: 4445.
160. Paumgartner G and Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. *Hepatology* 2002; 36: 525–531.
161. Beuers U, Boyer JL and Paumgartner G. Ursodeoxycholic acid in cholestasis: potential mechanisms of action and therapeutic applications. *Hepatology* 1998; 28: 1449–1453.
162. Vang S, Longley K, Steer CJ, *et al.* The unexpected uses of urso-and tauroursodeoxycholic acid in the treatment of non-liver diseases. *Glob Adv Health Med* 2014; 3: 58–69.
163. Laukens D, Devisscher L, Van den Bossche L, *et al.* Tauroursodeoxycholic acid inhibits experimental colitis by preventing early intestinal epithelial cell death. *Lab Invest* 2014; 94: 1419–1430.
164. Martínez-Moya P, Romero-Calvo I, Requena P, *et al.* Dose-dependent antiinflammatory effect of ursodeoxycholic acid in experimental colitis. *Int Immunopharmacol* 2013; 15: 372–380.
165. Van den Bossche L, Borsboom D, Devriese S, *et al.* Tauroursodeoxycholic acid protects bile acid homeostasis under inflammatory conditions and dampens Crohn's disease-like ileitis. *Lab Invest* 2017; 97: 519–529.
166. Yang Y, He J, Suo Y, *et al.* Tauroursodeoxycholate improves,2,4 6-trinitrobenzenesulfonic acid-induced experimental acute ulcerative colitis in mice. *Int Immunopharmacol* 2016; 36: 271–276.
167. O'Dwyer AM, Lajczak NK, Keyes JA, *et al.* Ursodeoxycholic acid inhibits TNFα-induced IL-8 release from monocytes. *Am J Physiol Gastrointest Liver Physiol* 2016; 311: G334–G341.
168. Diakonov I, Gorelik J, Swift T, *et al.* Bile acids and their respective conjugates elicit different responses in neonatal cardiomyocytes: role of Gi protein, muscarinic receptors and TGR5. *Sci Rep* 2018; 8: 7110.
169. Ward JBJ, Lajczak NK, Kelly OB, *et al.* Ursodeoxycholic acid and lithocholic acid exert anti-inflammatory actions in the colon. *Am J Physiol Gastrointest Liver Physiol* 2017; 312: G550–G558.
170. Fiorucci S, Carino A, Baldoni M, *et al.* Bile acid signaling in inflammatory bowel diseases. *Dig Dis Sci* 2021; 66: 674–693.
171. Mueller M, Thorell A, Claudel T, *et al.* Ursodeoxycholic acid exerts Farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. *J Hepatol* 2015; 62: 1398–1404.