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Bile acid homeostasis paradigm and its connotation with cholestatic liver diseases

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Bile acid (BA) has an important role in signal transduction, and has clinical applicability as an early biomarker for the diagnosis and prevention of cholestatic liver disease, which has a close relationship with BA homeostasis. Understanding the guarantee factors, function, and regulation of BA homeostasis under physiological conditions and in cholestatic liver diseases could provide novel therapeutic approaches for treating cholestatic liver injury. Here, we review potential biomarkers of BA, and new therapeutic approaches and the latest therapeutic drugs for cholestasis. We believe that the molecular mechanisms of cholestasis and the identification of key regulatory mechanisms of the enterohepatic circulation of BA could be pharmacologically targeted to cholestatic liver diseases.

Introduction

BA homeostasis involves the feedback regulatory mechanisms of BA synthesis, detoxification, and transport throughout the gut–liver axis. BA is a sensitive index of liver injury, and early disruption of the BA profile could serve as a sensitive early preclinical biomarker for the diagnosis and prevention of cholestatic liver injury [1]. When cholestatic liver injury occurs, the accumulated BA causes hepatotoxicity and induces inflammation and oxidative stress, which lead to fibrosis and cirrhosis [2]. Therefore, BA homeostasis must be strictly monitored and regulated for normal physiological functioning of the organism. Recent studies focused on farnesoid X receptor (FXR)-fibroblast growth factor 15/19 (FGF15/19) have provided mechanistic insights into the role of BA homeostasis in cholestatic disease [3].

Hepatocytes, the major epithelial cells in the liver, similar to other epithelia, are polarised at the interface between the circulatory system and the liver, separating sinusoidal blood from canalicular bile [4,5]. The normal membrane polarity of hepatocytes is a guarantee of BA homeostasis, and its mechanisms are complex, including intracellular and membrane trafficking

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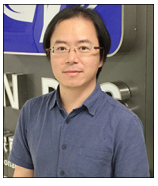

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systems, cytoskeleton, tight junctions (TJs), and intracellular trafficking components [6–9]. The loss of polarity causes the disruption of BA homeostasis and acts as a trigger for liver injury [10]. The functional destruction of BA transporters and hepatocyte TJs in cholestasis has been researched for many years, but its underlying molecular mechanisms remain poorly understood. Moreover, studies revealed that TJs are more important during early cholestasis and that sphingosine-1-phosphate receptor1 (S1PR1) could be a potential therapeutic target for cholestatic liver injury [11]. However, no studies have reported the inherent relationships among BA homeostasis, TJs, and cholestatic disease and, therefore, the molecular regulatory mechanisms of TJs based on this association require further investigation.

In addition to hepatocyte polarity, recent studies on BA homeostasis showed that it can also be regulated by intestinal microbial flora, the homeostasis of which can be influenced by BA, nutritional components, antibiotics, and disease. BA-dependent homeostasis of the gut–liver axis is essential for the normal physiological functioning of the liver [12], although the exact mechanisms of how disrupted BA homeostasis and gut dysbiosis promote cholestatic liver injury remain poorly defined and are likely to require further understanding of bacterially mediated BA dysmetabolism and cholestatic liver injury.

The importance of BA homeostasis has directed research toward the identification of signalling molecules with a role in BA transportation, as well as its association with TJs and gut microbiota that accumulatively maintain BA homeostasis in the gut–liver axis. Here, we review the molecular mechanisms of cholestasis and identify key regulatory mechanisms of BA homeostasis that could serve as potential pharmacological targets against cholestatic liver diseases.

Hepatocyte polarity-mediated BA homeostasis

BA homeostasis

Hepatocytes secrete BAs into bile canaliculi following their synthesis from cholesterol in the liver. They are then stored in the gall bladder and then reabsorbed by passive diffusion, before being transported from intestinal epithelial cells back to the liver following the enterohepatic circulation. Previous studies revealed that dynamic changes in the composition of the BA profile are reflected throughout its enterohepatic circulation, whereas individual BAs have different dynamic homeostasis in this circulatory system [13]. This circulation involves multiple organs; the structural and functional integrity of these organs is the basis for the maintenance of BA homeostasis, and any deficit in this link could affect the existing homeostasis.

Involving the modification of the backbone structure of cholesterol, the key steps of BA synthesis are oxidation and shortening of the side chain, as well as the conjugation of BA with relevant amino acids. BA synthesis is the key pathway for cholesterol metabolism [14]. Cholesterol conversion into BAs occurs via two main pathways: the classical (or neutral) pathway and the alternative (or acidic) pathway (Fig. 1). Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the two main end products of these synthetic pathways, whereas CYP7A1 and CYP27A1 have an important part in the production of BAs. Moreover, BA-CoA, amino acid *N*-acyltransferase (BAAT), and BA-CoA synthase (BACS) are important enzymes that are involved in the conjugation of the BAs. The initial biosynthesis steps occur in hepatocytes, with secondary BAs then generated from primary BAs by the enzymatic action of gut flora in the intestine through deconjugates, oxidates, and dehydroxylates. Under normal conditions, tertiary Bas, processed from secondary BAs, represent a small group of BA species. This synthetic process is strictly regulated to ensure that sufficient amounts of cholesterol and BA are catabolised to maintain physiological homeostasis. Secreted as glycine, taurine, or sulfate conjugates, BAs are excreted in bile and then transported to the intestinal tract, where they can be deconjugated by gut microbiota [15]. As shown in Table 1, intestinal bacteria generate secondary BAs by microbial BSH and HSDH enzymes through deconjugation, oxidation, and epimerisation, as well as by dehydroxylation via 7α-dehydroxylation activity in the large intestine [15–18]. These microorganisms have a central role as microbial bile salt transformational agents.

Hepatocyte polarity

Hepatocyte polarity is a key factor of BA homeostasis. Polarised at the interface between the intracellular environment and the outside of cells, hepatocytes regulate the directional transport of molecules out of and into cells. The mechanism of hepatocyte polarisation regulation is complex and involves cytoskeletal components, TJs, intracellular trafficking components, and transport systems. Many innate and acquired liver diseases involve hepatocyte polarisation [5]. The enterohepatic circulation of BAs involves the participation of multiple organs. As shown in Fig. 2 [19–21], this circulatory process comprises the biosynthesis of hepatocytes, secretion into bile canaliculi, storage in the gall bladder, release into the intestinal lumen and re-absorption in the intestinal epithelial cells (jejunal enterocytes, ileocytes, colonocytes, and renal proximal tubule cells).

TJs are important in maintaining the mucosal epithelial barrier and permeability. Structurally, TJs comprise many proteins that are anchored to the cytoskeleton. Four types of TJ integral proteins [occludin, claudins, and tricellulin (excluding junctional adhesion molecules)] are all tetraspan proteins. A variety of soluble proteins, such as ZO-1, ZO-2, ZO-3, alpha actinin 4 (7H6), and symplekin, interact with the intracellular domains of TJ integral proteins [22]. The normal functioning and structural integrity of TJs are a guarantee of physiological function. The integrity of TJs can be altered by the regulation of interactions among TJ proteins. Thus, the integrity of TJs needs to be strictly regulated to maintain the sequential diffusion. TJs are size and charge selective, and they regulate the selective diffusion of ions and solutes through the paracellular pathway [23]. Thus, TJ barriers must be established to prevent nonspecific diffusion of solutes. A pathway must also be assembled to allow for the selective diffusion of solutes along concentration gradients [31]. Compared with portal blood, bile salts are concentrated more than 1000-fold in canaliculi and are controlled by TJs [25]. The primary intercellular barrier that prevents the back diffusion of bile is the barrier formed by TJs between adjacent hepatocytes [26]. Hepatocyte TJs are essential for bile secretion, which is one of the most differentiated functions of the liver. With the function of intercellular communication, TJs enable the ordered concentration of bile canaculi from centrizonal to periportal hepatocytes [27]. Intrahepatic cholestasis is normally accompanied by impaired intercellular communication and leaking of TJs.

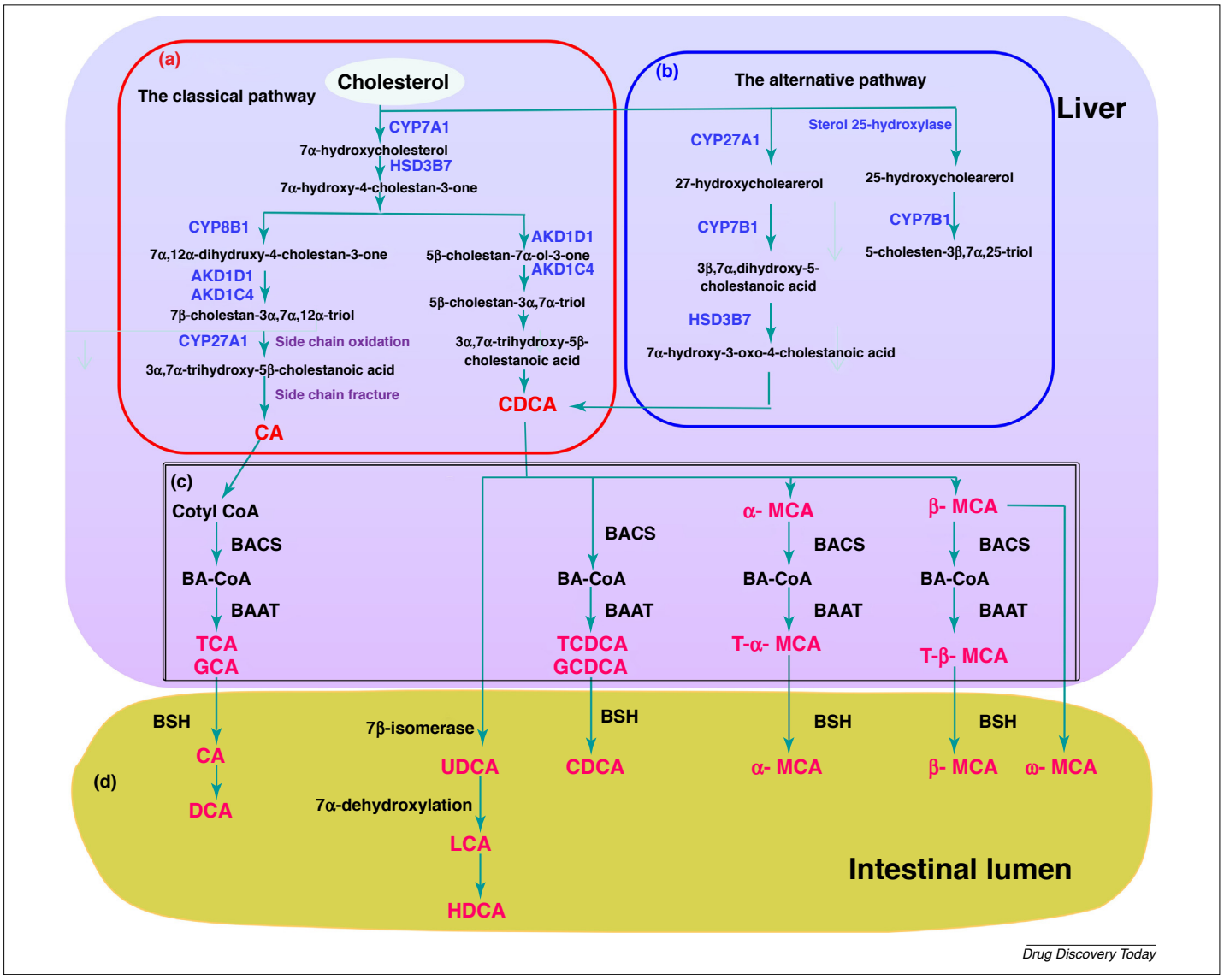

FIGURE 1

Bile acid (BA) synthesis pathways. Two major BA biosynthetic pathways are shown: (a) the classical pathway is the main BA synthesis pathway in liver, where cholesterol is converted to 7α-hydroxycholesterol (7α-HOC) by the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1), leading to the synthesis of CA or CDCA with AKD1D1 and AKD1C4; (b) the alternative pathway involves CYP27A1, which converts cholesterol to 27-hydroxycholesterol, which eventually is converted to CDCA; (c) BAAT and BACS conjugate CA or CDCA with certain amino acids in the liver; (d) in the intestinal lumen, gut microbial flora biotransform the hepatically produced BAs into DCA, LCA, CDCA, MCA, and HDCA by deconjugation, oxidation, epimerisation, and dehydroxylation with BSH, HSDH, and 7α-dehydroxylation.

The regulation of BA homeostasis

BA homeostasis can be disrupted in cholestasis. As shown in Fig. 3, earlier studies have shown that many routes can lead to cholestasis. Thus, controlled regulation of the enterohepatic circulation is needed, including BA synthesis and transport, the gut microbiome–BA axis, and the integrity of the TJ structure.

BA synthesis and transport

The body promotes the secretion of cholesterol from the liver by exploiting the biosynthetic steps that result in the transformation of cholesterol into BAs. Efflux pumps are distributed mainly over two polar surface domains of hepatocytes: bile ductular transporters and transporters of intestinal epithelial cells. The expression of selected synthetase in the BA pathway and BA transporters is tightly regulated by many related nuclear receptors (NRs) and other regulatory factors, all of which ensure a moderate supply of BAs in this dynamic metabolic environment [28]. Along with the enterohepatic circulation, BA concentrations are accorded the status of 'check points' for hepatocytes and enterocytes [29].

Drug- or metabolite-mediated disruption of hepatobiliary transporter systems and metabolism disorders of BA can bring about drug-induced cholestatic liver injury, which is always accompanied by interruption of BA homeostasis. There are many drugs that are substrates of organic anion-transporting polypeptides (OATPs), such as fexofenadine, opioid peptides, digoxin, pravastatin, enalapril, and methotrexate [30]. Furthermore, mediated by OATP1B1 and OATP1B3, the hepatotoxins phalloidin and microcystin undergo uptake into the liver. At the same time, hepatic failure

TABLE 1

Microbial biotransformations of BAs in the intestine

Microbial biotransformation	Reaction catalysed	Enzymes involved	Purified from microbiome	Refs
Deconjugation	Removal of amino acid side chain	BSHs	*Bacteroides fragilis*, *Bacteroides vulgatus*, *Clostridium perfringens*, *Listeria monocytogenes*, and several species of *Lactobacillus* and *Bifidobacterium*	[15–17]
Oxidation and epimerization	Removal of H2; 3-,7-, and 12-hydroxylation	3α/β-HSDHs	*Firmicutes* phylum, intra-species 3-hydroxy epimerization (*Peptostreptococcus productus*, *C. perfringens*, and *Eggerthella lenta*)	[18]
		7α-HSDHs	*Clostridium*, *Eubacterium*, *Bacteroides*, or *Escherichia*, intra-species 7-hydroxy epimerization (*Clostridium*, *Eubacterium*, and *Ruminococcus*)	[16]
Dehydroxylation	Replacement of hydroxyl group with hydrogen	7α/β-dehydroxylation	*Firmicutes* phylum (*Clostridium* and *Eubacterium*)	[18]

caused by uptake of the most dangerous natural toxin, amanitin, is mediated by OATP1B3 [31–34]. BSEP inhibitors and *ABCB11* (or *BSEP*) gene knockout can cause the accumulation of BA in intracellular spaces, which results in the reduction of BA biosynthesis and alteration of BA disposition [35]. All of these findings suggest the functional impairment of BSEP as a potential underlying mechanism of drug-induced cholestatic liver injury. Current studies showed that not only the inhibition of BSEP, but also the basal

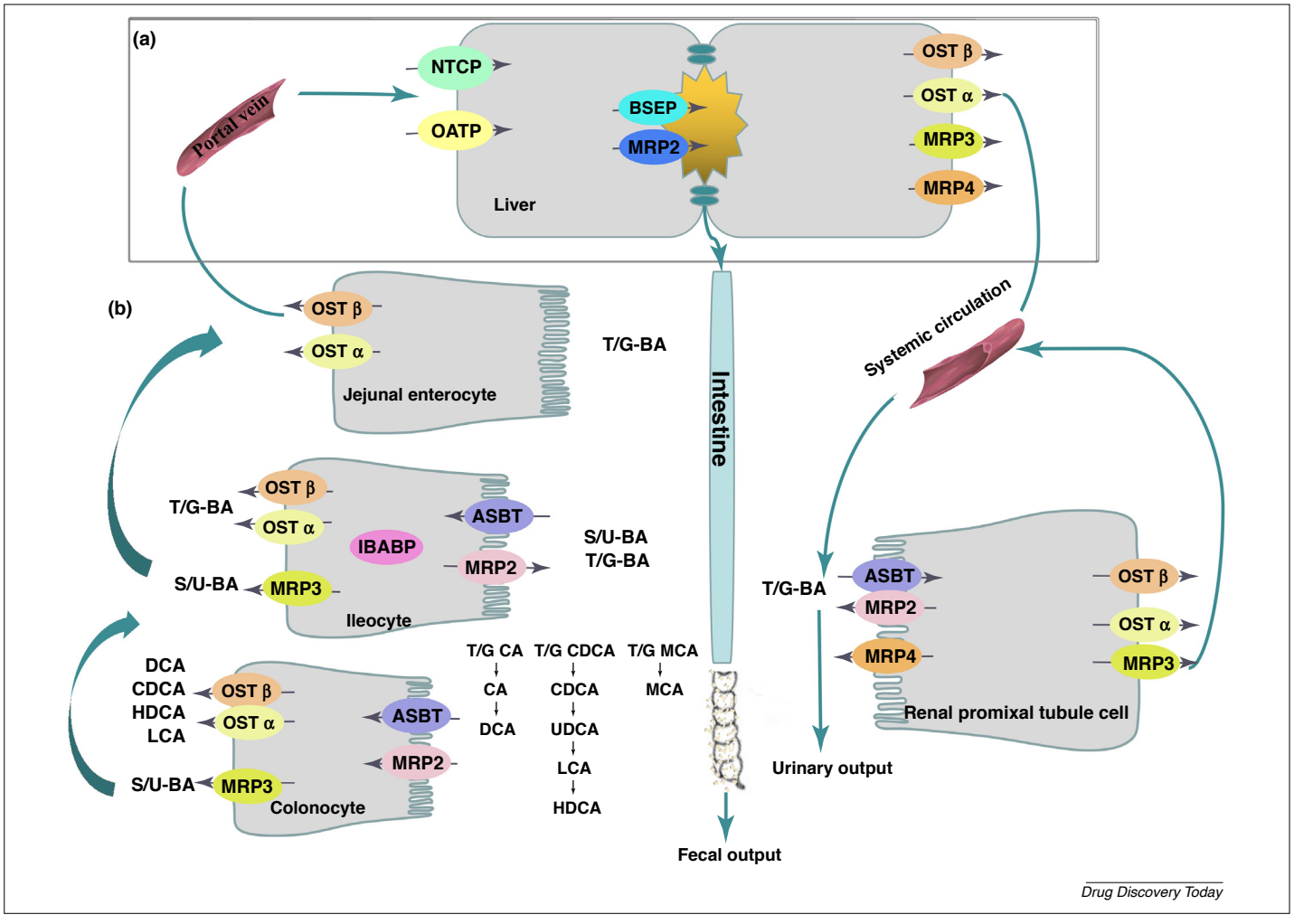

FIGURE 2

Bile acid (BA) transport. (a) After the biosynthesis of BAs, they are excreted in bile canaliculi through the canalicular bile-salt export pump (BSEP) and multidrug resistance-associated protein 2 (MRP2, ABCC2) into the bile. At the basolateral membrane of hepatocytes, the transport of BAs is mainly mediated by members of the MRP family and OST-α/β. (b) Under physiological conditions, bile is released into the intestinal lumen after being stored in the gall bladder. With the assistance of intestinal epithelial cell ASBT, BAs are reabsorbed by the terminal ileum and effluxed by OST-α/β and MRP3, whereas intracellular transport is promoted by the ileal bile-acid-binding protein (IBABP). Similar reuptake of BAs also occurs in cholangiocytes, colonocytes, and proximal convoluted renal tubules, which results in the limited loss of BA via faeces and urine. Once recycled to the liver, BAs are mainly taken up by NTCP and OATPs.

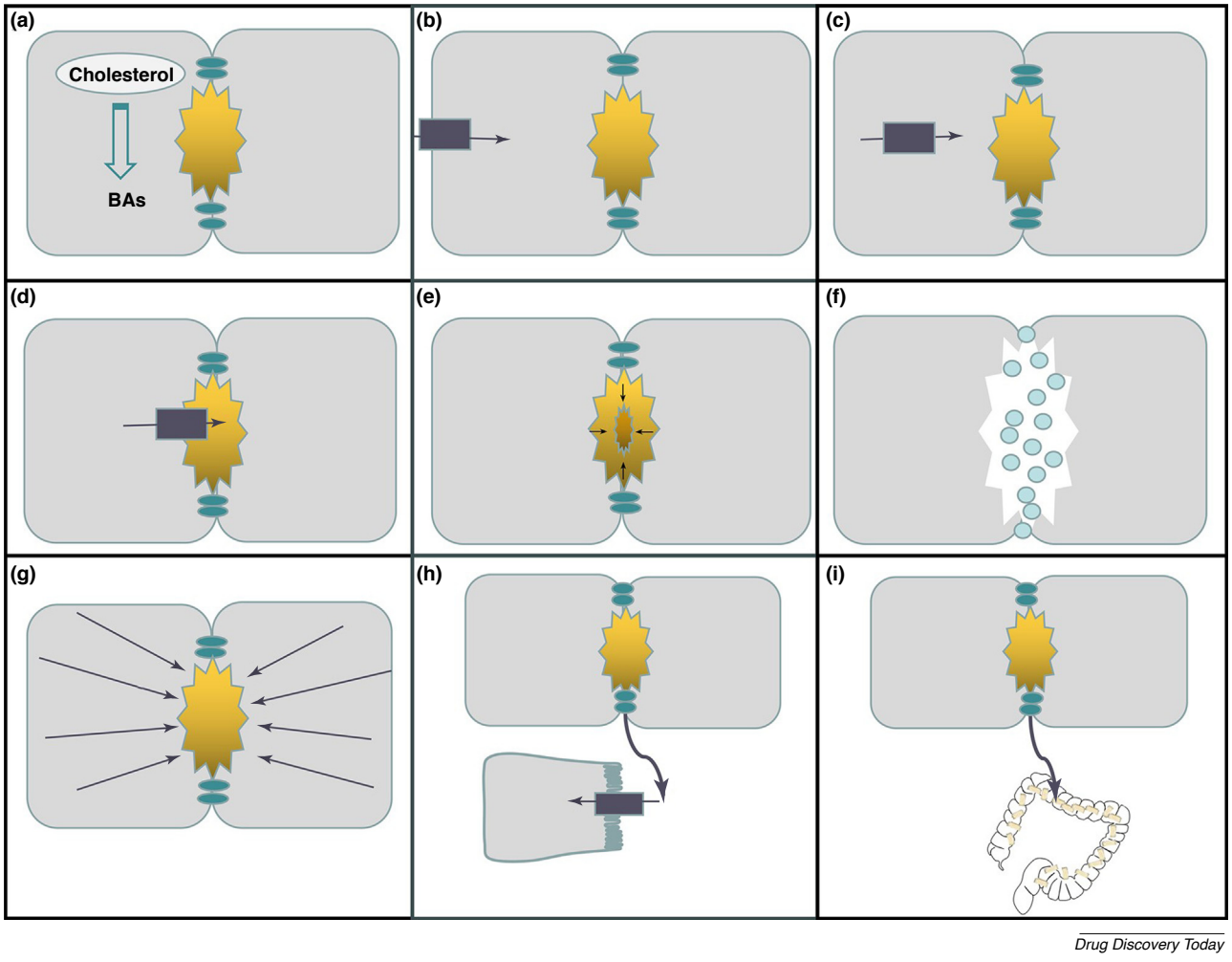

FIGURE 3

Earlier studies of the mechanisms of cholestasis. (a) Metabolism disorders of bile acid (BA); (b) inhibition of BA uptake; (c) decrease in cytoskeleton-dependent endocytosis; (d) bile secretion dysfunction (transporter inhibition, transporter detached from bile canalicular membrane); (e) reduced contractility of the bile duct and decreased ability of bile duct closure; (f) leakage of tight junctions (TJs); (g) accumulation of toxic substances in the vicinity of the bile duct; (h) intestinal absorption disfunction; (i) intestinal microbial homeostasis.

efflux transporters, including MRP3 and 4, are involved in BA-dependent hepatocyte toxicity [35,36]. BA can be transported out of hepatocytes by means of BA transporter systems at basolateral membranes. Therefore, basolateral efflux transporters, including MRP3, MRP4, and OSTα/OSTβ, and especially MRP3, have an essential role in protecting the liver from cholestatic injury. Consistent with the enforcing cellular export, the ingress of BAs into hepatocytes (mainly via NTCP and OATPs) and enterocytes (mainly via ASBT) is reduced. Meanwhile, BAs accumulated in hepatocytes also downregulate the expression of CYP7A1, which further reduces the production of BA. All of these changes are primarily a response to adaptation to the disruption of BA homeostasis and an attempt to slow the progression of cholestasis.

Inborn errors of BA synthesis can grow into life-threatening progressive cholestatic liver disease. Accompanied by the elevated activity of serum aminotransferases and the level of conjugated bilirubin, liver diseases, such as acholic stools, jaundice, hepatomegaly, and deficiency of fat-soluble vitamins, usually appear during early childhood, whereas gamma glutamyl transpeptidase activity remains normal [37]. Congenital defects in BA synthesis include the deficiency of several enzymes, including 3β-dehydrogenase, sterol 27-hydroxylase, oxysterol 7α-hydroxylase, BA-CoA, and BA-CoA ligase, as well as mutations in the *AKR1D1* (*SRD5B1*) gene [38]. Moreover, mutations of NTCP can elevate the serum conjugated BA concentration in patients with cholestasis [39]. Many cholestatic injury drugs have been shown to inhibit BSEP, and most patients with mutations in *MDR3* or *BSEP* were reported to have a higher risk of drug-induced cholestatic liver injury [40].

BA synthesis involves many signalling molecules and several interconnected complex pathways. The mechanisms of BA synthesis and its feedback regulation reported thus far are presented in Fig. 4 [41–56]; these collectively target the maintenance of BA homeostasis.

Gut microbiome–BA axis

Many active BA metabolites, including secondary BAs, are transformed by the intestinal microbiota. DNA damage and cell death can be induced by hydrophobic secondary BAs in abnormally high

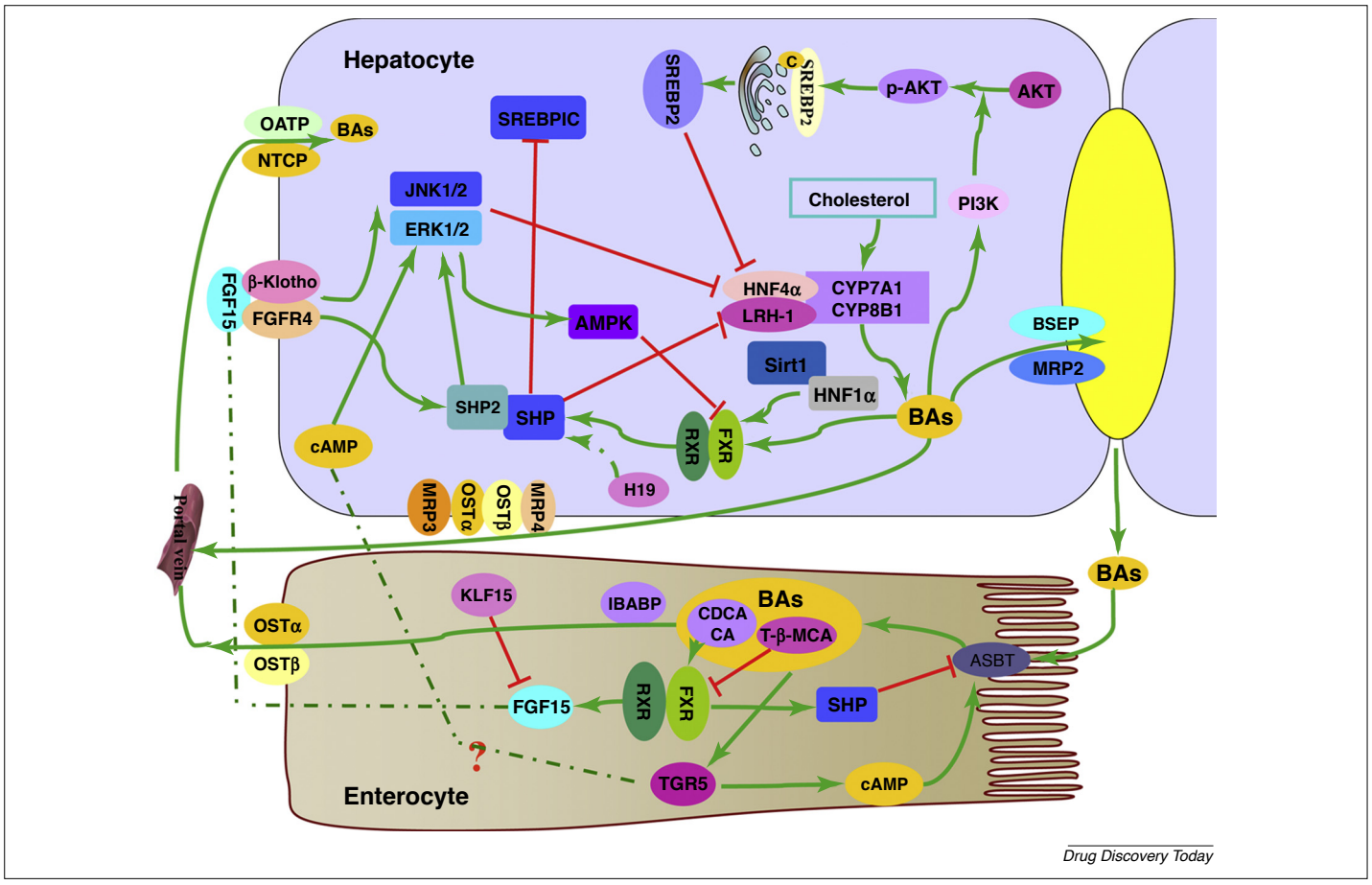

FIGURE 4

Mechanisms of bile acid (BA) feedback inhibition of bile acid synthesis. BA-activated farnesoid X receptor (FXR) signalling in the liver and intestine, and TGR5 in the intestine inhibits synthetases (CYP7A1 and CYP8B1), further reducing hepatic BA synthesis. BA homeostasis is regulated by the FXR-FGF15/19-FGFR4-SHP axis. (a) In enterocytes, after being secreted into the circulation, FGF15/19 is transported to the liver and binds to its cognate receptor FGFR4 and co-receptor β-Klotho on the hepatocytes, activating a signalling pathway that downregulates the expression of CYP7A1. The circadian production of BA is regulated by the KLF15-FGF15 signalling axis. (b) In hepatocytes, SHP represses the action of BA receptors (mainly HNF4α and LRH), and its expression is suppressed by H19 (in extracellular vesicles). Nonreceptor tyrosine phosphatase SHP2 is also a crucial player. Relaying from FGFR4, receptors for FGF15/19, ablating SHP2 in hepatocytes suppresses the signal and attenuates BA activation of FXR signalling. Steroid response element binding protein-2 (SREBP-2) regulates cholesterol synthesis, and the increased expression of SREBP-2 through the PI3K/AKt pathway can decrease nuclear HNF-4α expression, which decreases CYP7A1 transcription. cPKC/p38 MAPK and PI3K/ERK1/2 signalling pathways also participate in cholestasis, mainly through the internalisation and sustained intracellular retention of canalicular transporters. Activation of the cAMP-ERK-LKB1-AMPKα1 and Sirt1-HNF1α signalling pathways is closely related to the dysregulation of the expression of FXR and BA transporters. AMPK phosphorylates and regulates FXR transcriptional activity and is involved in cholestatic liver injury.

concentrations because of their cytotoxicity [57]. Secondary BAs modulate the membrane and inflammatory effects on the colonic mucosa [58]. DCA can induce inflammation by activating COX-2, which is blocked by UDCA in AOM tumours [59]. As shown in Fig. 5 [18,60–63], BAs are also important regulatory factors for the gut microecology environment because of their antimicrobial and amphipathic properties [64]. With the highest taxonomic levels, BAs regulate the profiles of the gut microflora, which results in disease by affecting metabolism. The raised production of hydrophobic secondary BAs is a result of an increase in *Firmicutes*, especially the genus *Clostridium* cluster XIVa, which is caused by increasing levels of primary BA [65,66]. Through activated innate immune genes in the small intestine, the gut microbial composition can be modulated by BAs both directly and indirectly [67]. Certain BAs are agonists or antagonists on G-protein-coupled receptors and FXR, which are collectively known as BA-activated receptors (BARs) [68]. The BA pool can be shaped by perturbations of the microbiota, which can also trigger the activity regulation of BARs [69].

In addition to BAs, the gut microbial composition is also influenced by nutrition, antibiotics, and disease. Approximately 30% of patients with XXXX also have antibiotic-associated diarrhoea, and 70% have antibiotic-associated colitis, with most cases being pseudomembranous colitis. In turn, the growth of *Clostridium difficile* can be promoted or inhibited by alterations in the ratios of different BAs, which result from changes in the gut microbiota composition [70–72]. Diet has a large influence on the gut microbiota profile and function. The impaired intestinal mucosal barrier integrity is observed in mice fed a high-fat diet. At the same time, the BA profile is subsequently modified with an increased level of DCA and a decreased proportion of UDCA. As an important representative of a class of enzyme, the gut sterolbiome-encoded

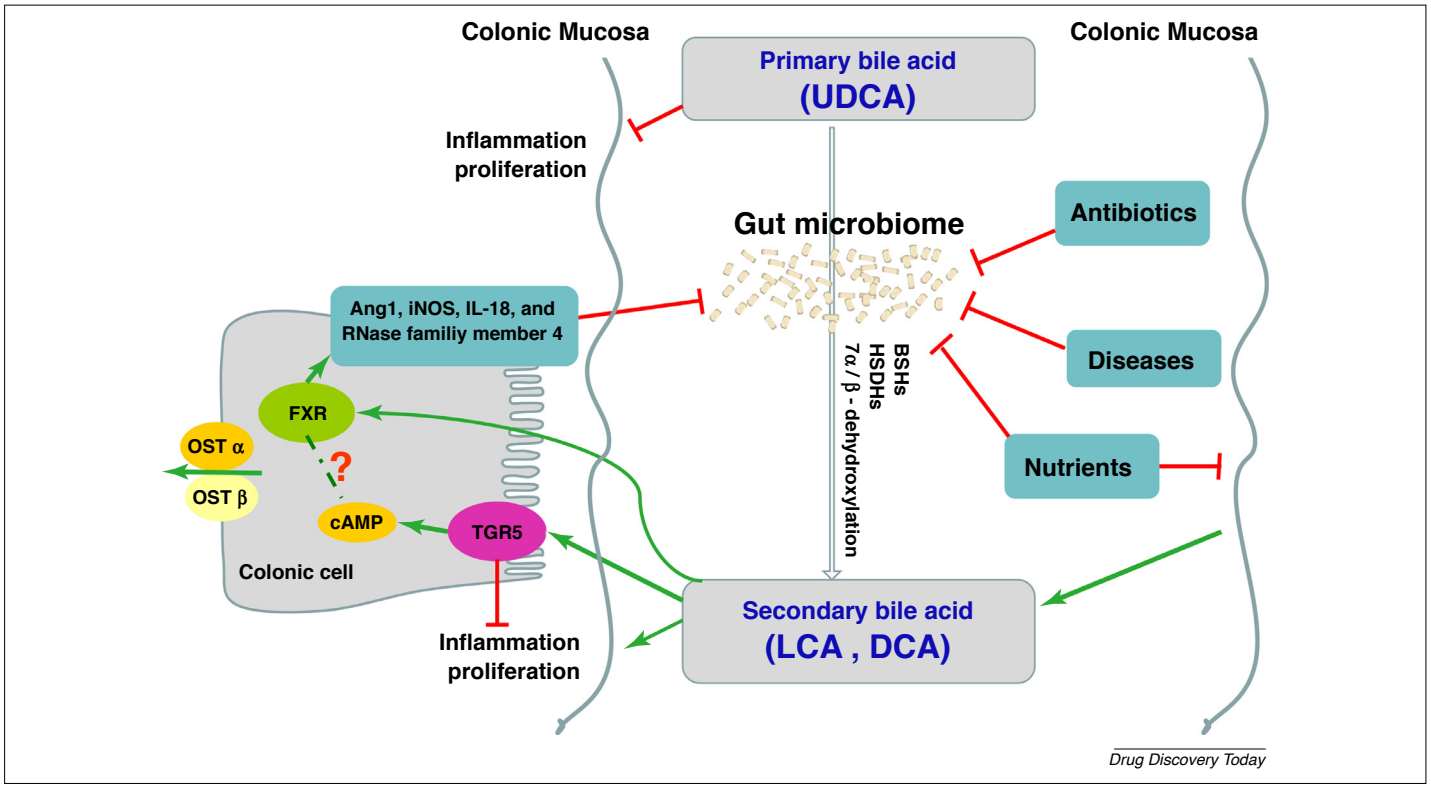

FIGURE 5

Signalling pathways involved in the regulation of the intestinal microbiota–bile acid (BA) axis. The gut microbial composition is influenced by nutrition, antibiotics, and diseases. As a result of microbial transformations, secondary BA metabolites act as signalling molecules and regulate intestinal homeostasis through the TGR5 and farnesoid X receptor (FXR) receptors by inhibiting inflammation, maintaining cell integrity, and so on; activation of the BA receptor FXR in intestinal epithelial cells protects bacterial overgrowth and translocation in the distal small intestine and results in disruption to the gut epithelial barrier through the regulation of genes, including those encoding angiogenin 1 (Ang1), iNOS, IL-18, and RNase family member 4. In this way, BAs inhibit bacterial proliferation directly and indirectly, with recent observations of the effect of FXR activation by secondary BAs showing that the sterolbiome could regulate the size of the BA pool by removing the potent FXR antagonist tauro-β-muricholic acid. The BA-activated membrane G-protein-coupled receptor TGR5 minimises the production of proinflammatory cytokines (IL-1α, IL-2β, IL-6, and TNFα) that are stimulated by lipopolysaccharides in macrophages and Kupffer cells, through the inhibition of NF-κB, and are principally activated by secondary BAs, including DCA and LCA, in which the BA affinities for TGR5 follow the LCA > DCA > CDCA > CA direction.

genes give the microbiome the ability to serve as an endocrine organ with extensive influence on the host. Many factors, such as diet, antibiotics, and probiotics, can perturb the sterolbiome and further influence the composition of the BA pool, which would result in the modulation of physiological effects [73]. An abnormally upregulated concentration of hydrophobic BAs is observed in many enterohepatic diseases, including cholestasis; in addition, a proinflammatory shift in the gut microbial profile is also seen. Aggregately, BAs fundamentally shape the gut microbiome, and vice versa. Thus, the association between bacterially mediated BA dysmetabolism and cholestatic liver injury requires further exploration.

Significant increases in *Escherichia coli* and a marked decrease in the *Clostridium leptum* subgroup and *Bifidobacterium* sp. were reported in patients with diarrhoea-predominant irritable bowel syndrome (IBS). Involved in BA transformation, the *C. leptum* subgroup contains many bacteria (in particular *Ruminococcus* and *Clostridia* spp.), the decrease of which could induce a lower transformation activity of the microbiota and, subsequently upregulate levels of primary BAs and downregulate levels of secondary BAs [74]. The overgrowth of gut bacteria and an increase in BAs in faeces can be observed in most patients with cirrhosis, accompanied by different microbial communities and an increased burden of gut bacteria [75,76].

The microbiota also have a role in the development of nonalcoholic fatty liver disease (NAFLD) [77,78]. The progression of NAFLD and alcoholic liver disease can be accelerated by serious infections induced by bacterial translocation [61,79,80]. The lower expressed FXR and the increased serum levels of triglycerides are observed in patients with NAFLD [81]. Bacterially mediated BA dysmetabolism in FXR signalling pathways impacts the piglet model of short bowel syndrome-associated liver disease (SBS-ALD) [82].

The intestinal microbiota can also be altered by drugs, especially antimicrobial agents. Intestinal homeostasis can be disrupted by treatment with antibiotics, which also has a profound impact on the intestinal metabolome [83]. The ratio of different gut microbial flora can be altered by taking various types of antibiotic, especially fluoroquinolones, clindamycin, second- and third-generation cephalosporins, and macrolides [84]. With the elevation of hepatic BA synthesis, the BA pool size and hepatic BA concentration can be increased by treatment with antibacterial drugs [85]. At the same time, liver synthesis and faecal excretion of BAs are also sharply reduced after treatment with antibiotics, which also disrupt the

gut microflora. The expression of the ileum bile salt transporter ASBT is increased in the absence of microbiota, and a recent study suggested that disruption of BA homeostasis induced by treatment with antibiotics can be prevented by appropriate inactivation and/or blockage of either ASBT or the transcription factor GATA4 [86].

Integrity of the TJ structure

The blood–biliary barrier is required for bile secretion and is not accompanied by leakage into the blood circulation. The integrity of hepatocyte TJs is a guarantee of the maintenance of the structure and function of the canaliculi, and its loss could lead to structural changes of the canaliculi, which further induce bile duct injuries and result in bile components flowing backward to the liver interstitial tissue. The leakage of bile components could induce damage to hepatocytes, vascular endothelial cells, and so on. In the many pathological changes in biliary diseases, the loss of the barrier function of TJs and bile-induced cell injury can also be observed [87]. Damage factors, such as toxins, inflammatory cytokines, and pathogens, can damage the integrity of the TJs, whereas some protectors, such as glutamine, probiotics, and growth factors, can help maintain TJ integrity [88–95]. The most significant evidence demonstrates that damage to TJs and biliary dysfunction are both involved in the pathogenesis of cholestatic diseases, such as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) [96,97].

The altered integrity of hepatocyte TJs can be observed in several types of cholestasis [98–102]. In experimental models of cholestasis and obstructive jaundice in humans, disturbance of the TJ structure and permeability was revealed by physiological and anatomical studies [103,104]. Downregulated TJ functions can also be observed in cholestatic hepatocytes, resulting in the deteriorated barrier function of TJs [105–107].

The integrity of the related proteins of TJs, such as ZO-1 and occludin, was altered in rifampicin-induced cholestasis in mice and the ligation of bile ducts in rats [102,108]. Alterations in the TJ structure were also observed in cells exposed to toxic BAs [109,110]. Intrahepatic cholestasis is frequently accompanied by inflammatory bowel disease, and hepatocyte TJs and canalicular multispecific organic anion transporters were upregulated in rats with trinitrobenzene sulfonic acid-induced colitis [99]. Kan *et al.* showed that the cholestatic oestrogen metabolite oestradiol-17β-glucuronide increased the permeability of TJs in isolated perfused rat liver [111]. Increased permeability of TJs can also be observed in oestradiol-17β-D-glucuronide-induced cholestasis, as well as a significant redistribution of ZO-1, occludin, and MRP2 in addition to increased permeability of the TJ [99]. Additionally, α-naphthylisothiocyanate (ANIT) progressively increased the permeability of the junctional barrier before a reduction in the bile flow [112]. The failure of the liver barrier can also be observed in obstructive cholestasis, which can be induced by infection and surgery. Oral administration of *Lactobacillus plantarum* to rats with obstructive jaundice resulted in the increased expression of TJ-associated proteins and mRNA levels, enhanced TJ continuity, and decreased hepatocyte apoptosis [113].

Recent studies showed that many signalling pathways are involved in the functional integrity of TJs (Fig. 6). Protein kinases and phosphatases [including protein kinase C (PKC), protein kinase A, c-Src, PP2A, and PP1] interact with TJ proteins, which suggests that TJs are regulated by intracellular signal transduction that involves the phosphorylation and localisation of proteins relevant to TJs. Moreover, other signalling protein molecules, such as calcium, AMP-activated protein kinase (AMPK), myosin light chain kinase (MLCK), NF-κB, phosphatidylinositol 3-kinase (PI3K), Rho kinase (ROCK), and Rac, are all involved in the regulation of TJs and other cellular functions. Phosphorylation of specific Tyr residues in occludin blocks its interaction with ZO-1 and disassembles TJs. Phosphorylation and cellular localisation of occludin can be regulated by PKC, and all these phenomena demonstrate that PKC is a regulator of occludin function [114–116]. PKC signalling enhances the barrier function via the transcriptional upregulation of TJ proteins [117,118]. Phospholipase Cγ (PLCγ)-mediated activation of PKCε, PKCβ, and intracellular calcium is involved in the protection of TJs, which is mediated by epidermal growth factor (EGF) [119]. MLCK has served as a major regulatory factor of TJ permeability, which mainly results from its induction of contraction of the perijunctional actomyosin ring through myosin regulatory light chain phosphorylation. In physiological situations, MLCK regulates the TJ barrier mainly via a ZO-1-dependent process, and its inhibition could provide a strategy to prevent gastrointestinal diseases by restoring the TJ barrier function [120]. Two IκB kinases, IKK1/IKKα and IKK2/IKKβ, cooperate with each other to maintain the bile duct integrity and to protect the liver from cytokine toxicity and bile duct diseases. IKK1 participates in maintaining the integrity of the epidermal barrier by the transcriptional regulation of TJ proteins [121]. As a serine/threonine protein kinase, AMPK has an important role in the regulation of epithelial TJ assembly and disassembly [122,123]. The actin cytoskeleton, which is involved in TJ formation, could be affected by the MLC, which can be regulated by AMPK [124]. The phosphorylation of MLC is regulated by Rho-kinase and MLCK [125]. TJs are also regulated by Rho guanosine triphosphatase and, as a downstream effector of Rho, ROCK regulates TJs by its effects on the F-actin cytoskeleton. At the same time, ROCK also has an important role in the assembly of the apical junctional proteins and the organisation of the F-actin cytoskeleton [126]. The small GTPase RhoA promotes junction formation via myosin II signalling [127,128].

The assembly of the TJ is also related to another small GTPase, Rab13 [129]. In addition, Epac downstream of Rap1 also participates in the regulation of TJ formation [130].

Collectively, the proposed hepatocyte polarity–intestinal microbial–BA homeostasis–cholestasis relationship represents a significant step forward in understanding cholestatic liver disease (Fig. 7). The interactions among hepatocyte polarity, intestinal microbiota, BA homeostasis, and cholestasis are not unidirectional but are highly intertwined, and disruption in this crosstalk is responsible for the initiation and progression of cholestatic liver diseases.

BAs as potential biomarkers of liver disease

The role of BAs as biomarkers for liver injury has been proposed for decades (Table 2). In particular, the Bas unique to rodents, α-muricholic acid (α-MCA), β-MCA, ω-MCA, tauro-α-MCA (T-α-MCA), and tauro-β-MCA (T-β-MCA), are useful biomarkers for predicting

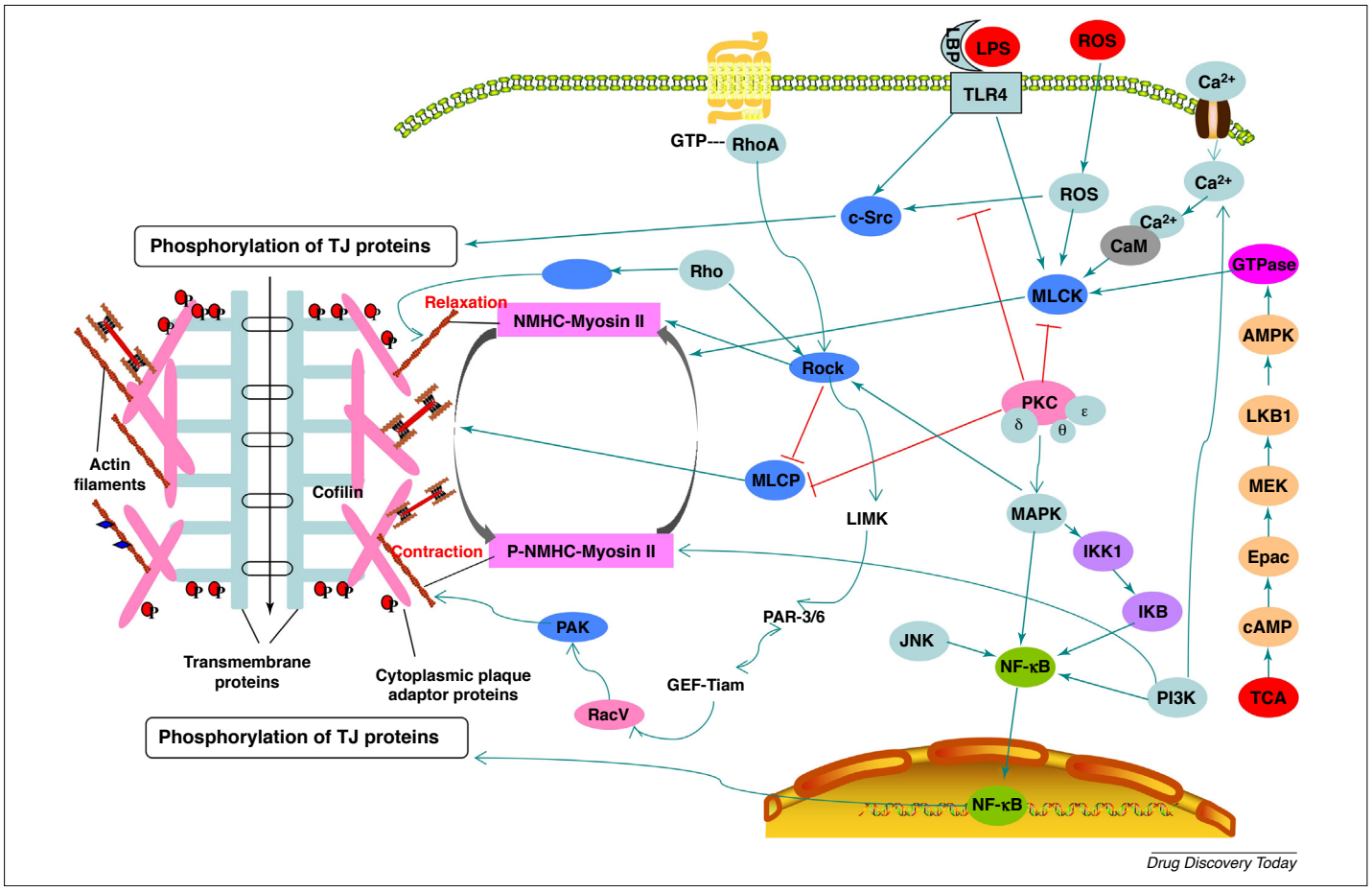

FIGURE 6

Signalling pathways involved in the regulation of tight junctions (TJs). Rho-specific effectors, such as ROCKs, can be activated by the stimulation of Rho. Activated ROCKs not only phosphorylate and inactivate MLCP, but also phosphorylate and activate myosin light chain II, all of which lead to the activation of actinomyosin contractility and increased paracellular permeability. The first step for the activation of the RhoA/ROCK pathway involves G-protein-coupled vasopressor receptors and contractile agonists that further activate the small monomeric GTPase RhoA, which in turn activates ROCK and leads to MLCP inhibition. Myosin II contraction is also stimulated through the phosphorylation of MLC by Ca^{2+}/calmodulin (CaM)-dependent MLCK. LIM kinase (LIMK) is another RhoA/ROCK signalling effector, the phosphorylation of which leads to barrier enhancement. The regulation of the actin cytoskeleton, cell polarity, and paracellular permeability is linked to Rac. Alternatively, ROCK can directly phosphorylate TJ proteins. c-Src and MLCK can be activated by TLR4 activation and reactive oxygen species (ROS). All of these mechanisms synergistically destroy the TJs and result in barrier dysfunction. Protein kinase C (PKC) activation increases the TJ barrier function along with upregulation of the TJ proteins and inactivation of MLCK, MLCP, and c-Src. NF-κB signalling includes MAPK, JNK, PI3K, and IKK1, which are all essential in the liver. GTPase activates MLCK via a cAMP-Epac-MEK-LKB1-AMPK pathway to regulate TJ permeability.

potential cholestatic liver injury risks in preclinical drug development [126]. Another study showed that there is a link between the levels of individual BAs and specific forms of liver injury [131]. GDCA in plasma could serve as a biomarker in patients with acetaminophen-induced acute liver failure [132]. In a model of CCl_4- and ANIT-induced cholestasis, BAs, especially certain conjugated forms, such as TCA, GCA, GCDCA, and GDCA, can serve as sensitive and potential indicators of drug-induced liver injury (DILI) [133]. A study identified GCA, TCA, and GCDCA as pharmacodynamic indicators of ANIT-induced cholestasis after *Paeonia lactiflora* Pall. (PLP) treatment in rats [134]. The roots of rhubarb (*Rheum palmatum*) have been used to abate jaundice. After screening the sensitive and potential pharmacodynamics indicators of rhubarb in the treatment of ANIT-induced cholestasis, GCA, GCDCA, THDCA, and TCA were selected [135]. The perturbation of BAs was observed in *Polygonum multiflorum* (HSW)-induced liver injury, especially changes in GDCA in bile, as well as HDCA in serum [136].

Patients with severe intrahepatic cholestasis of pregnancy (ICP) have higher serum levels of TCDCA, TUDCA, GCA, TCA, and GCDCA [137]. In patients with ICP from Argentina, LCA was proposed as an alternative biomarker and a more sensitive index than total serum BA in the assessment for the effectiveness of UDCA treatment [138]. For hepatobiliary diseases, the changed BA profiles suggested their utility as biomarkers in the clinic [139,140]. Another study showed that the dynamic alteration of serum BAs can be a valuable indicator of exacerbated liver function, which highlights their potential as biomarkers for the grading of liver cirrhosis and the monitoring of its progression [141]. The upregulated levels of conjugated BAs in cirrhosis or chronic hepatitis could be valuable indicators of liver dysfunction for hepatocellular carcinoma (HCC) in patients with liver cirrhosis

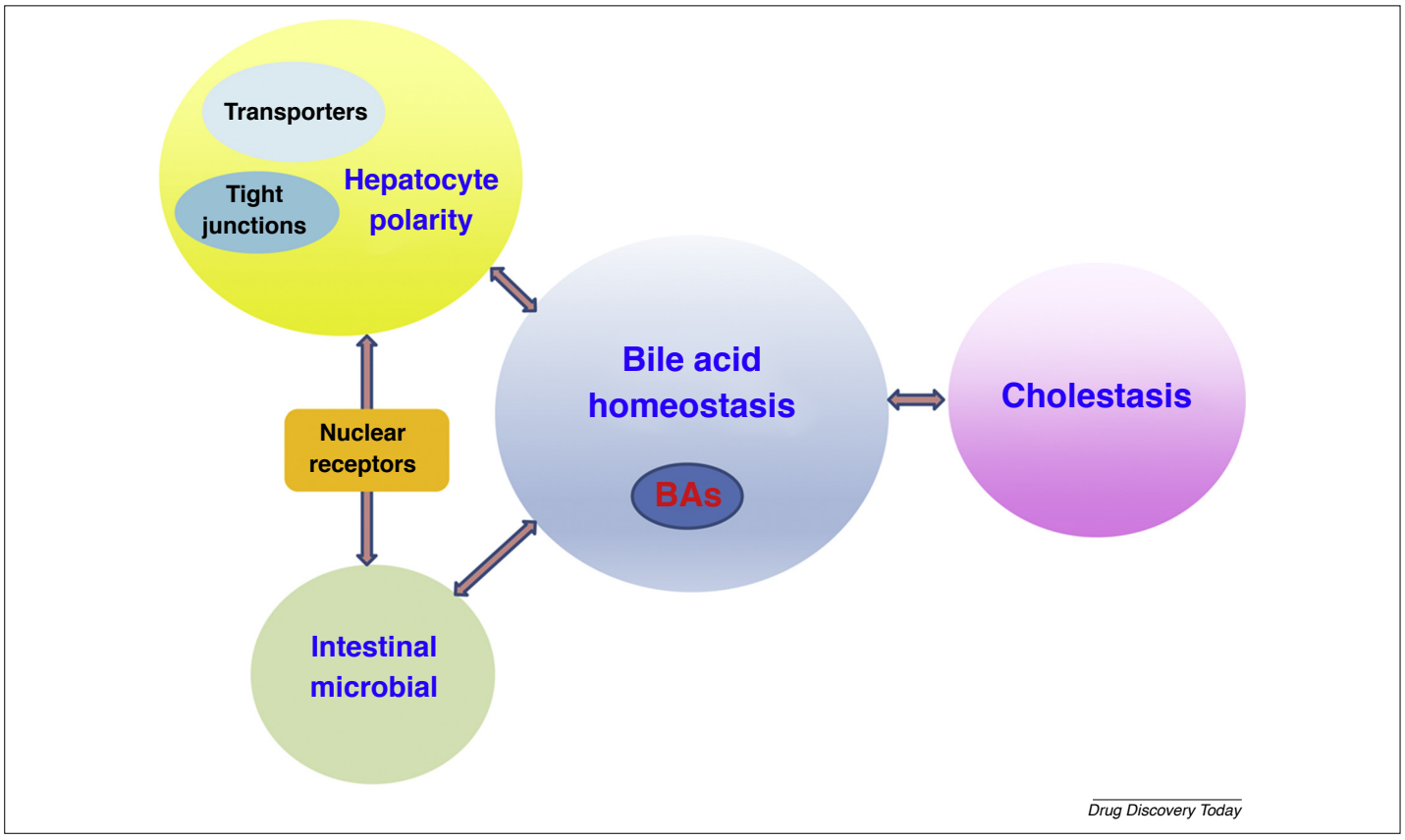

FIGURE 7

Crosstalk among hepatocyte polarity–intestinal microbiota–bile acid (BA) homeostasis–cholestasis. The relationships between hepatocyte polarity, intestinal microbes, BA homeostasis and cholestasis are interrelated, and they interact with one another through different mechanisms. This interaction offers exciting new perspectives for the pathogenesis and treatment of cholestatic liver diseases.

[101]. Other research showed that serum GDCA, GCA, TCA, and CDCA can be used for the early diagnosis of HCC as well as for monitoring the progression of cirrhosis to HCC [142,143].

Latest advances in the treatment of hepatic cholestasis

Cholestasis is an impairment of bile formation and flow at the level of the hepatocytes, as well as cholangiocytes. As shown in Fig. 8, reducing the accumulation of BAs in the hepatic circulation and decreasing the BA pool size are the general goals in treating cholestasis. The latest therapeutic drugs for cholestasis are detailed in Table 4.

TABLE 2

BAs as biomarkers in liver injury

Disease and/or drug	BAs as biomarkers
Liver injures	
Liver disease □□	LCA, DCA, CDCA, CA
Liver cirrhosis	TCA, GCA, TCDCA, GCDCA
HCC	GCA, TCDCA, GDCA
ICP	TCA, GCA, TCDCA, TUDCA, GCDCA
UDCA + TCP	LCA
DILI	
APAP	GDCA
CCl4, ANIT	TCA, GCA, GCDCA, GDCA
PLP + ANIT	TCA, GCA, GCDCA
Rhubarb + ANIT	TCA, GCA, THDCA, GCDCA
HSW	GDCA, HDCA

UDCA and NorUDCA

The first-generation therapy for cholestasis was UDCA. 24-norursodeoxycholic acid (NorUDCA) is a UDCA derivate that shows a relative resistance to amidation with taurine or glycine because it lacks a methylene group. Compared with UDCA, NorUDCA presents additional anti-inflammatory and antifibrotic effects [144–148]. Both of these compounds result in anticholestasis by promoting bile secretion, which targets impaired bile flow.

Another line of anticholestatic strategies has been developed to target the enterohepatic circulation, to mainly reduce the BA pool; these candidate compounds are currently being explored in Phase 2 and Phase 3 clinical trials.

FXR agonists

As a crucial integrator of BA homeostasis, the activation of FXR decreases cellular BA levels. Synthetic and semisynthetic FXR agonists have been successfully examined in animal models of cholestasis because of their relatively higher affinity and potency at activating FXR. FXR agonists are divided into two groups: steroidal and nonsteroidal. Obeticholic acid (OCA) restored the downregulated bile flow and helped recovery from symptoms of cholestatic liver injury in several animal models of preclinical cholestasis [149,150]. Alternatively, as a high-affinity and semisynthetic FXR agonist, INT-747 protected the liver from necrosis and cirrhosis in various rodent models of liver damage via its potent anticholestatic and antifibrotic properties [151]. A recent

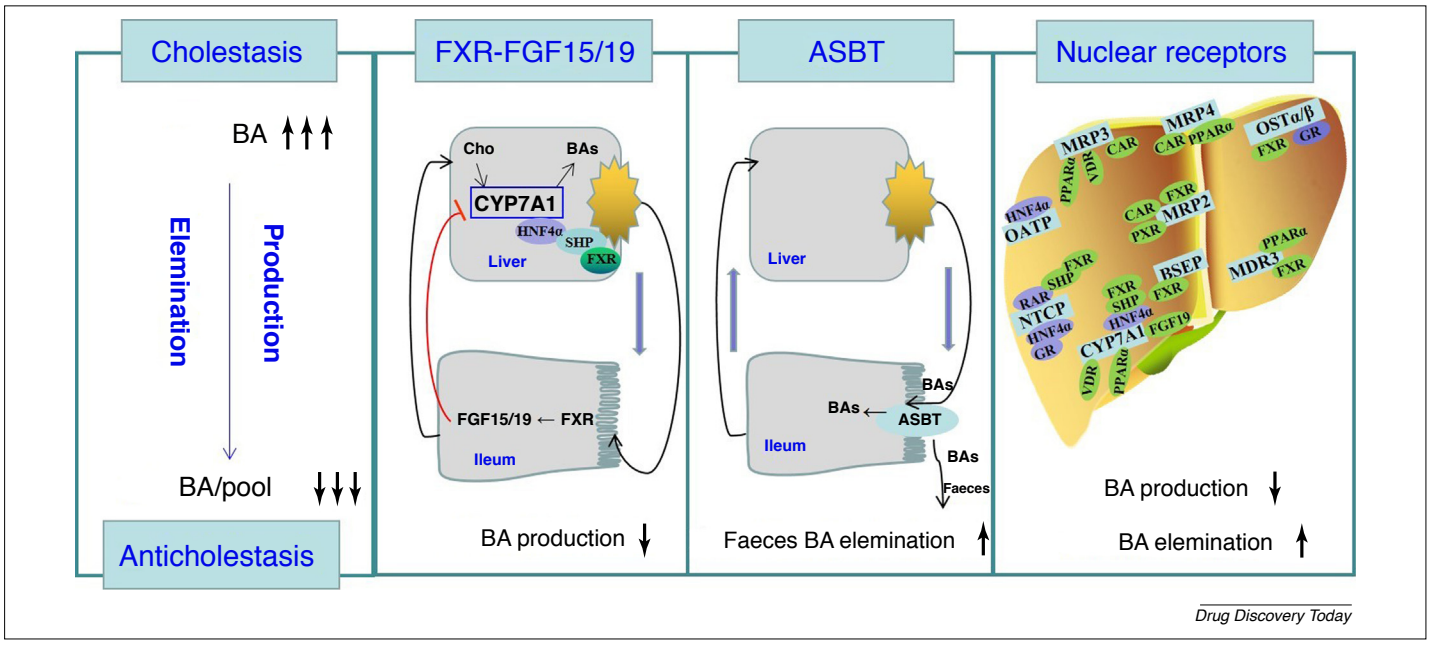

FIGURE 8

Principle anticholestatic mechanism. Fibroblast growth factor15 (FGF15) analogues, apical sodium-dependent bile acid transporter (ASBT) inhibitors, farnesoid X receptor (FXR) agonists, and other nuclear receptors.

study found that tropifexor (LJN452), a novel and highly potent non-BA FXR agonist, was tolerable and effective, and it has advanced into Phase 2 clinical trials in patients with PBC (ClinicalTrials.gov identifier: NCT02855164) [152]. LMB763 is another non-BA FXR agonist currently in Phase 2 clinical trials (ClinicalTrials.gov identifier: NCT02913105). As an orally administered potent FXR agonist, GS-9674 mainly works on the intestinal epithelium and results in a decrease in BA synthesis, largely because of the release of FGF-15/19. A multicentre Phase 2 clinical study is underway on the safety and tolerability of GS-9674 in patients with an inadequate response to UDCA (ClinicalTrials.gov identifier: NCT02943447) [153]. Another Phase 2 clinical trial of the safety, tolerability, and efficacy of GS-9674 is recruiting patients with PBC (ClinicalTrials.gov identifier: NCT02943460). As a potent agonist of FXR, EDP-305 suppresses hepatic stellate cell activation and hepatic fibrosis in a variety of animals models of nonalcoholic steatohepatitis, fibrosis, and cirrhosis [154]. A Phase 1 clinical study in healthy volunteers and in presumed NAFLD has been completed (ClinicalTrials.gov identifier: NCT03207425, NCT02918929, NCT03187496, and NCT03213145), and the subjects of follow-up research will be extended to patients with NASH and PBC (ClinicalTrials.gov identifier: NCT03394924 and NCT03421431).

PPAR agonists

PPAR-δ participates in BA homoeostasis [155]. Seladelpar (MBX-8025) is a selective PPAR-δ agonist, and Phase 2/3 clinical studies are currently recruiting patients with PBC to investigate its safety and efficacy (ClinicalTrials.gov identifier: NCT02955602, NCT02609048, and NCT03301506). PPARα is enriched in the liver. Having shown clinical improvements in small clinical trials with patients with PBC, PPARα ligands are the most promising pharmacological substances among the NR ligands, and researchers are awaiting permission for the confirmation of large multicentre trials [156]. Elafibranor, another orally administered PPAR-α/δ agonist, can decrease the synthesis of BAs, and can also increase the uptake and detoxification of BAs. At the same time, it decreases ALP and GGT levels [157,158]. Both i*n vivo* and *in vitro* studies have shown that fenofibrate has anti-inflammatory, anticholestatic and antifibrotic functions by activating PPAR-α [159–163]. A multicentre Phase I/II clinical study is underway on the safety and efficacy of the fenofibrate-UDCA combination in patients with an inadequate response to UDCA (ClinicalTrials.gov identifier: NCT02965911).

FGF15/19 mimetics and agonists

As an endogenous hormone primarily produced by ileum cells, fibroblast growth factor (FGF) 15 (FGF19 in humans) efficiently suppresses BA synthesis in liver, and controls liver size. Thus, the modulation of FGF19 by M70 could provide an effective means of treating cholestatic liver injury. FXR-dependent induction of FGF19 could have a therapeutic advantage over FXR agonists in cholestasis, and the selective activation of intestine FXR could be a potential treatment target for cholestasis [164]. The results of a recent Phase I clinical trial on healthy volunteers showed that serum C4 and BA precursor levels reduced by 95% with FGF19 mimetic administration, which indicated firm suppression of endogenous BA synthesis without any toxicity or adverse effects [165]. Based on the positive Phase 1 evaluations, FGF19 mimetics have been approved in patients with PBC who showed an inadequate response to UDCA. Data from Phase 2 trials showed that FGF19 mimetics resulted in a profound decrease in C4 along with significantly decreased total BA levels [166]. As a recombinant protein of FGF19, NGM-282 (Clinical Trials.gov identifiers: NCT02026401 and NCT02135536) can effectively simulate the

actions of FGF19 on BA synthesis via binding of the FGFR4-β-klotho co-receptor. A Phase 2 clinical trial of NGM-282 is underway in patients with PBC showing an inadequate response to UDCA [167].

Hepatic BA transporter substrates

The improved expression level of BSEP can repair bile salt excretion, repairing hepatic damage and followed by enrichment of BAs in hepatocytes. Maralixibat (SHP625) is an experimental drug for progressive familial cholestasis type 2 (PFIC) that works by recovering the activity of BSEP in hepatocytes. A Phase 3 clinical study of maralixibat is currently underway [168]. The inhibition of NTCP also has hepatoprotective effects by decreasing the BA load in hepatocytes. Myrcludex B, a selective NTCP inhibitor, reduced BA levels in hepatocytes and increased the biliary phospholipid:BA ratio [169,170] in Phase 2 clinical trials in patients with PBC. Phase 1/2 clinical studies testing the safety and efficacy of myrcludex B have also been completed in patients with hepatitis (Clinical Trials.gov identifiers: NCT02637999 and NCT02881008).

Intestinal BA transporter inhibitors

Located in the luminal surface of ileum enterocytes, ASBT (SLC10A2) prevents the loss of BAs in faeces by the reabsorption of 95% of BAs from the intestine to maintain their enterohepatic circulation [171]. Lopixibat (LUM001) is a novel ASBT inhibitor that, a Phase 2 clinical trial in 61 patients with PBC, showed a significant reduction in pruritus [172]. GSK2330672 is a highly potent ASBT inhibitor that was shown to be beneficial in pruritus associated with PBC [173]. A Phase 1 clinical study has been completed on the dose escalation of GSK2330672 in healthy Japanese male volunteers (Clinical Trials.gov identifiers: NCT02801981). Another Phase 2 clinical study has also been completed on the safety, tolerability, pharmacokinetics, and pharmacodynamics evaluation of repeat doses of GSK2330672 in patients with PBC (Clinical Trials.gov identifiers: NCT01899703). A4250, an ideal BA transporter (IBAT) inhibitor, had substantial effects on BA synthesis along with noticeable variations in their plasma and faecal levels [174]. In a Phase I clinical trial with human volunteers, total serum BAs were nearly halved by A4250, which also increased faecal BA excretion. A clinical study of A4250 is being designed for its utilisation against cholestatic liver diseases, such as PBC [175]. BAT117213, another selective human IBAT inhibitor, was investigated in a Phase 2 clinical trial for patients with PBC and pruritus; the data showed that BAT117213 decreased the serum conjugated BAs and resulted in a more than 50% decrease in the total BA concentration in patients with cholestasis [176,177]. In addition to lopixibat, A4250, and BAT117213, A3309 is also a small-molecular IBAT inhibitor, and the results of Phase 2 clinical trials in patients with chronic idiopathic constipation and functional constipation showed that A3309 induced the loss of BAs [178,179].

Other nuclear receptors

NRs are important regulatory factors of BA homeostasis (including their synthesis, metabolism, and transport) (Table 3). Given the harm caused by accumulated BAs in the liver under cholestatic

TABLE 3

Nuclear receptors as drug targets in cholestasis

NR	Ligand	Therapeutic target	Target gene	General and therapeutic effects	Research progress
FXR	Natural: CDCA > LCA = DCA > CA; synthetic: GW4064, INT-747, PX-102	BA synthesis BA detoxification BA transport	Indirect repressive effects via SHP CYP3A4,SULT2A1, UGT2B4, UGT2B7 MRP2,OATP,OSTα/β, SHP, BSEP, IBABP, MDR3 indirect repressive effects via SHP	Repression of BA synthesis indirectly via SHP Induction of BA detoxification Induction of canalicular and alternative basolateral BA excretion; increase in bile flow; increase in biliary phospholipid content; repression of BA uptake	Experimental and clinical effects
PPARα	Natural: fatty acids, fibrates; synthetic: WY-14643, bezafibrate, fenofibrate, ciprofibrate	BA synthesis BA detoxification BA transport	CYP7A1 SULT2A1, UGT2B4, UGT1A3 MDR2,ASBT	Repression of BA synthesis Induction of BA detoxification Protection of bile duct epithelium via increased phospholipid secretion and increased BA reabsorption	Experimental and clinical effects
PXR	Natural: LCA > DCA > CA; synthetic: rifampicin, statins, corticosteroids	BA synthesis BA detoxification BA transport	CYP7A1 CYP3A4, SULT2A1, UGT1A1, UGT1A3 MRP2, MRP3, OATP, MDR1	Indirect repression via interaction with HNF4α Induction of BA in Phase □ and Phase □□ trials Induction of orthograde canalicular and alternative basolateral BA excretion	Experimental and clinical effects
GR	Natural: cortisol; synthetic: budesonide	BA transport	ASBT, NTCP, BSEP, MRP2, OSTα/β	Contribution of effects on transporters	Experimental and clinical effects
VDR	Natural: 1.25-diOH-vitamin D, 3-oxo-CA = LCA > DCA > CA; synthetic: vitamin D	BA detoxification BA transport	CYP3A4,SULT2A1 MRP3, ASBT	Induction of BA detoxification systems Induction of alternative basolateral BA excretion; induction of biliary reabsorption; intestinal BA reabsorption	Experimental results

TABLE 4

Therapeutic drugs for the management of cholestasis

Aim	Drug	Research stage
Reduce choleresis	UDCA	Currently in use
Reduce BA pool size	FXR agonists	
	PX20606	Experimental results
	Fexaramine	Experimental results
	INT-767	Phase 2 clinical trial
	Tropifexor	Phase 2 clinical trial
	GS-9674	Phase 2 clinical trial
	PX-102	Phase 1 clinical trial
	LMB763	Phase 2 clinical trial
	WAY-362450	Phase 1 clinical trial
	EDP-305	Phase 2 clinical trial
	PPAR agonists	
	Seladelpar	Phase 3 clinical trial
	Elafibranor	Phase 2 clinical trial
	Fenofibrate	Phase 1/2 clinical trial)
	FGF15/19 agonists	
	FGF19 mimetics	Phase 2 clinical trial
	NGM-282	Phase 2 clinical trial
	BA transporters	
	Hepatic BA transporters mimetics	
	Maralixibat	Phase 3 clinical trial
	Myrcludex	Phase 1/2 clinical trial
	Intestinal BA transporter inhibitors	
	Lopixibat	Phase 2 clinical trial
	A4250	Phase 1 clinical trial
	BAT117213	Phase 2 clinical trial
	GSK2330672	Phase 1/2 clinical trial
Enhance hepatic epithelial TJs	S1PRs	
	S1PR1	
	SEW2871	Experimental results
	GSK2018682	Phase 1 clinical trial
	ACT-128800	Phase 1 clinical trial
	ACT-334441	Phase 1 clinical trial
	BAF312	Phase 1 clinical trial
	S1PR2	
	JTE013	Experimental results
	FTY720	Phase 1 clinical trial

conditions, activation of NRs by BAs results in a decrease the hepatocellular BA levels. Therefore, NRs can provide novel therapeutic targets for cholestatic liver diseases. In addition to ASBT, the membrane-bound BA-specific receptor TGR5 is another promising membrane receptor target. Expressed in cholangiocytes, intestinal epithelial cells, and immune cells, TGR5 shows choleretic, anti-inflammatory, and antiapoptotic functions. Thus, TGR5 could have a role in the pathogenesis of biliary diseases, such as PSC [180–182]. LCA is the most potent endogenous TGR5 activator [183]. Decreased total BA pool size, increased expression of CYP7A1, and hydrophobic BA composition are observed in *TGR5*$^{-/-}$ mice. An *in vitro* study showed that progesterone metabolites can activate TGR5 and that delineation of a progesterone sulfate-TGR5 pruritus axis identified a therapeutic target for itch management in ICP [184–187].

Treatment to enhance hepatic epithelial TJs

The S1P signalling pathway is related to the alleviation of cholestatic liver injury by enhancing the barrier function; thus, S1PRs could be a potential target for the improvement of drug-induced hepatic cholestasis. SEW2871 is a selective S1PR1 agonist and it was reported that ANIT-induced cholestatic liver injury can be improved by SEW2871 treatment in mice with induced ANIT [11]. In addition, SEW2871, GSK2018682, ACT-128800, ACT-334441, and BAF312 are all S1PR1 agonists. Based on these compounds, clinical studies are underway on patients with multiple sclerosis. However, there have not yet been any clinical studies with liver disease. Additional research showed that the expression level of S1PR2 was upregulated in the liver of BDL mice, and S1PR2 deficiency significantly alleviated BDL-induced liver injury by reducing liver fibrosis in *S1PR2*$^{-/-}$ mice [188]. JTE013, a selective S1PR2 antagonist, reduced the total serum and liver BA concentration in BDL mice. These results suggest that S1PR2 plays a prominent part in obstructive cholestasis. FTY720 is also a S1PR2 antagonist; although clinical studies on FTY720 have focused on multiple sclerosis, studies on its effects against liver disease are lacking.

Other treatment strategies related to BA homeostasis

In addition to these latest treatments for hepatic cholestasis, another focus in BA research is the identification and characterisation of relevant gut microbiome members. Some microflora encode novel genes that could be involved in the metabolism of these gene products, and the latter could ultimately regulate and improve the pathophysiology of the disease. Earlier studies also demonstrated that targeting the capacity of microbiota to produce DCA and inhibiting the microbial conversion of UDCA to LCA could be a potential novel therapeutic approach [87]. Similarly, recent studies demonstrated that commensal microbiota and their metabolites have the potential to provide protection against biliary bile injury in PSC, which could support further the identification of relevant biomarkers [189]. In addition, thorough evaluation of these agonists and antagonists, including FXR and PPARα in combination with UDCA, could be another strategy for further exploration.

Concluding remarks

Conclusive early studies have placed emphasis on hepatocyte polarity-mediated BA homeostasis and its signalling mechanisms to understand cholestatic liver diseases. However, the detailed changes in the signalling pathways involved in regulating the synthetases, transporters, TJs, and intestinal microbiota remain unclear. Moreover, BA signalling pathways are complex and cross-linked, and their composition in humans differs from that in rodents. This difference needs to be further defined. Furthermore, extensive studies are needed to determine the role of BAs in signal transduction and their clinical applicability as early potential biomarkers for the diagnosis and prophylaxis of cholestatic diseases.

In treatment approaches, the first-generation therapy for cholestasis is UDCA. Superior to the novel BA-based therapeutic strategy, NorUDCA has the most advanced clinical evaluation in patients with PBC. OCA also significantly improved cholestasis liver injury, especially in patients with an inadequate response to UDCA. In addition, other anticholestatic therapeutic strategies are also under investigation, including a series of BA homeostasis regulators, such as BA transporter inhibitors (i.e.,

lopixibat, maralixibat, A4250, BAT117213, and GSK2330672), FXR agonists (i.e., tropifexor, GS-9674, LMB763, and EDP-305), PPAR agonists (i.e., MBX-8025 and elafibranor), and FGF19 mimetics and agonists (NGM-282). The thorough evaluation of these agonists and antagonists, including FXR and PPARα in combination with UDCA, could be another avenue for further exploration. With the in-depth studies of BA homeostasis, the gut microflora and hepatocyte TJs are attracting increasing attention in cholestatic liver diseases. Thus, more novel therapeutic targets and better tolerated therapies for cholestatic liver diseases, along with the advent of new molecules, might be available in the near future.

Uncited reference

[24].

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