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REVIEW

The role of botanical triterpenoids and steroids in bile acid metabolism, transport, and signaling: Pharmacological and toxicological implications

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Rheumatoid arthritis

Abstract Bile acids (BAs) are synthesized by the host liver from cholesterol and are delivered to the intestine, where they undergo further metabolism by gut microbes and circulate between the liver and intestines through various transporters. They serve to emulsify dietary lipids and act as signaling molecules, regulating the host's metabolism and immune homeostasis through specific receptors. Therefore, disruptions in BA metabolism, transport, and signaling are closely associated with cholestasis, metabolic disorders, autoimmune diseases, and others. Botanical triterpenoids and steroids share structural similarities with BAs, and they have been found to modulate BA metabolism, transport, and signaling, potentially exerting pharmacological or toxicological effects. Here, we have updated the research progress on BA, with a particular emphasis on new-found microbial BAs. Additionally, the latest advancements in targeting BA metabolism and signaling for disease

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treatment are highlighted. Subsequently, the roles of botanical triterpenoids in BA metabolism, transport, and signaling are examined, analyzing their potential pharmacological, toxicological, or drug interaction effects through these mechanisms. Finally, a research paradigm is proposed that utilizes the gut microbiota as a link to interpret the role of these important natural products in BA signaling.

1. Introduction

Bile acids (BAs) are amphiphilic molecules derived from cholesterol, constituting the primary components of animals' bile. Synthesized in the liver, they are excreted into the intestine and can subsequently be reabsorbed. The metabolism and transport of these steroid acids mediate many physiological functions, including hepatic elimination of excess cholesterol, excretion of xenobiotics, emulsification of lipids in the intestine tract, and maintenance of intestinal acidity. In recent years, BAs have been found as signaling molecules, playing an important role in the occurrence and regulation of cholestasis, metabolic diseases and immune diseases[1–4].

Triterpenoids and steroids are recognized as vital active components in botanicals. They, along with cholesterol, belong to the isoprenoid family and share similar frameworks in the initial biosynthesis steps[5,6]. Triterpenoids are secondary metabolites of plants that play a role in plant defense, growth and development[7]. Phytosteroids can serve as integral components of plants within plant plasma membrane lipid rafts, or be used in the synthesis of saponins and phytoecdysteroids[8]. Due to the similarities in backbones, triterpenes and steroids may have profound effects on BA metabolism, transport and signaling after entering the human body. In this review, we will combine our recent work, elaborating the mechanistic paradigm of these important natural products on BA metabolism, transport and signaling. This is critical to understanding the mechanisms by which botanicals alleviate diseases, as well as their potential toxicity.

2. Structures and biosynthesis of botanical triterpenoids and steroids

2.1. Triterpenoids

Triterpenoids are triterpenes containing heteroatoms, composed of six isoprene units. Tetracyclic and pentacyclic triterpenoids represent the most prevalent triterpenes found in botanicals. Tetracyclic triterpenoids encompass five primary types: lanostane, dammarane, cucurbitane, cycloartane, and protostane; whereas pentacyclic triterpenoids include oleanane, lupane, ursane, and friedelane varieties (Fig. 1A).

The biosynthesis of triterpenoids primarily includes the creation of the triterpene backbone and the subsequent oxygenation processes[6,9]. The initiation of triterpene backbone synthesis starts with the mevalonate (MVA) pathway, where acetyl-CoA undergoes a series of enzymatic reactions, producing isopentenyl pyrophosphate (IPP). And IPP is subsequently converted into dimethylallyl pyrophosphate (DMAPP). These activated isoprene units then merge to form farnesyl pyrophosphate, which, in turn, combine to generate squalene under the influence of squalene synthase. Subsequently, squalene transforms into 2,3-oxidosqualene and is cyclized by oxidosqualene cyclase (OSC). Various OSCs in plants lead to the creation of diverse triterpene backbones such as β-amyrin, lupeol, and bauerenol, which correspond to oleanane, lupane, and ursane types, respectively (presenting a chair−chair−chair conformation). Conversely, OSCs also generate cucurbitadienol, cycloartenol, and lanosterol, which align with cucurbitane, cycloartane, and lanostane types (exhibiting a chair-boat-chair conformation). Following the triterpene backbone's formation, cytochrome P450 isozymes facilitate scaffold oxidation, introducing hydroxyl, carboxyl, ketone, or epoxy groups, producing different kinds of triterpenoids. Notably, fungi primarily produce lanostane-type triterpenoids compared to the diversity found in plants[5]. Additionally, triterpenoids can be glycosylated by diverse glycosyltransferases, attaching glucose, arabinose, rhamnose, xylose, or glucuronic acid onto carboxyl or hydroxyl groups[5,6,9]. The biosynthetic framework of triterpenoids is shown in Fig. 1B.

2.2. Steroids

Steroids, a diverse class of cyclopentane polyhydrophenanthrene compounds, encompass sterols, sterones, and steroidal saponins in herbal medicines. Phytosterols vary mainly in the alkyl group at C-24, classified as C-24 methyl sterols (*e.g.*, campesterol) and C-24 ethyl sterols (like β-sitosterol and stigmasterol). They can also be categorized based on the degree of unsaturation into sterols and stenols (*e.g.*, stigmastanol). Steroidal saponins branch into spirostane, isospiranostane, furostane, and cholestane types depending on aglycone backbone variations (Fig. 1C).

The biosynthesis of steroids, shared with triterpenes, begins similarly *via* the TMA and the subsequent squalene synthesis pathway. In plants, cycloartenol, a cyclization product of 2,3-oxidosqualene, plays a pivotal role in steroid synthesis. Most cycloartenol molecules enter the C-24 alkyl sterol pathway mediated by sterol methyl transferase 1, whereas there is also a small amount of molecules flux to the cholesterol biosynthesis pathway, although plants only produce little cholesterol. In the C-24 alkyl sterol pathway, cycloartenol undergoes transmethylation, redox, demethylation, and isomerization steps. Sterols and cholesterol then generate furostanol or spirosanol derivatives through hydroxylation, oxyheterocyclic fusion, and glycosylation[10]. Although cholesterol and phytosterols can be synthesized from lanosterol in some plants, direct evidence for steroidal saponin synthesis *via* this pathway is lacking[11]. Notably, fungi produce steroids (like ergosterol) mainly from lanosterol, rather than cycloartenol[5] (Fig. 1B).

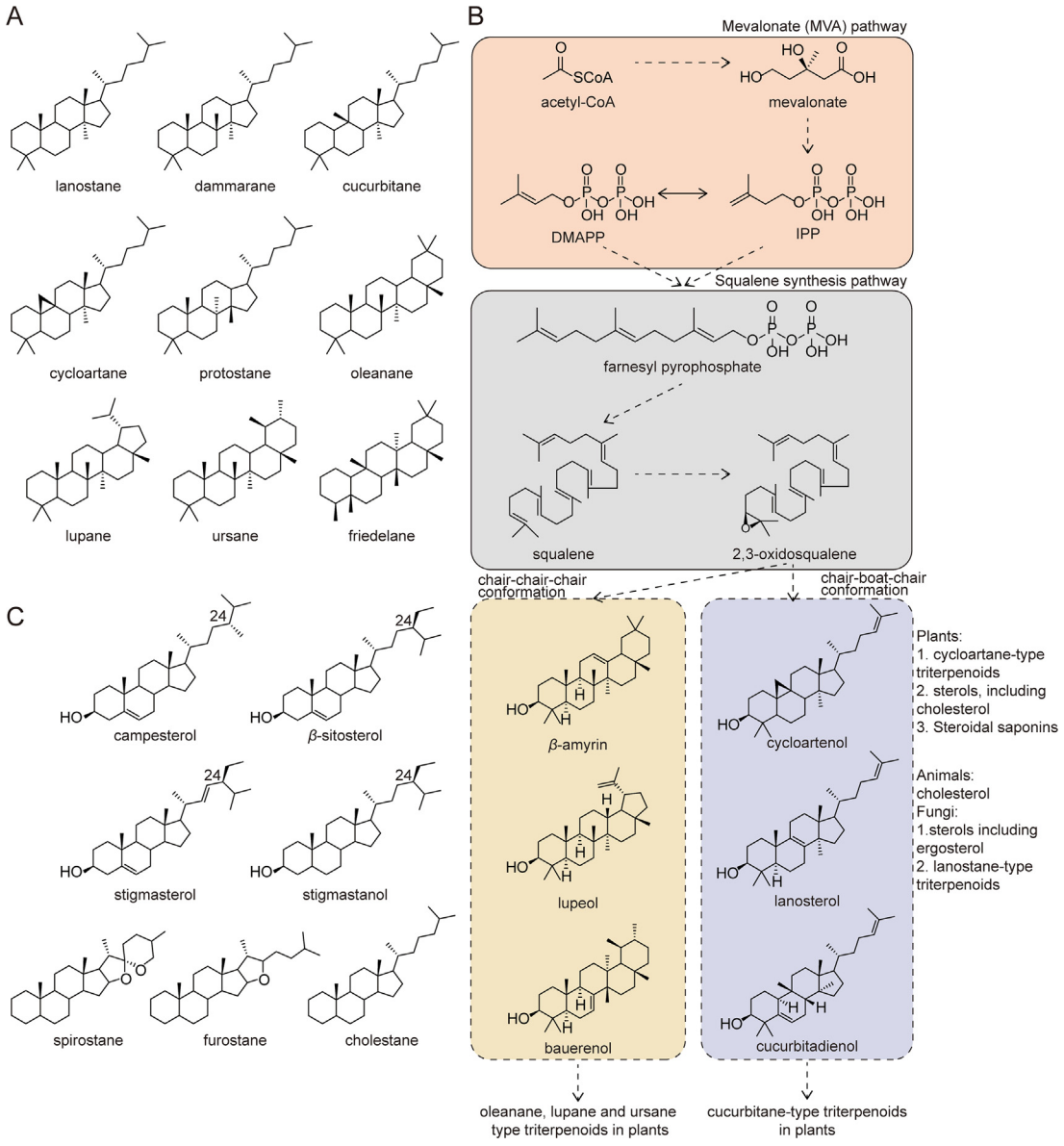

Figure 1 (A) The structures of various botanical triterpenoid types; (B) The biosynthesis framework of triterpenoids and steroids; (C) The structures of common phytosterols and types of steroidal saponin aglycones.

3. Categorization and metabolism of BAs

3.1. Structures and categories of BAs

BAs are steroid acids with a backbone of 24 carbon atoms, metabolized from cholesterol. In humans and rodents, a BA molecule usually features a C-24 carboxyl group, a C-5β hydrogen and a C-3α hydroxyl group, with additional hydroxylation possible at C-7, C-12 or C-6 (Fig. 2A). These groups can undergo oxidation and isomerization, forming *iso*-BAs, *allo*-BAs, *epi*-BAs, *oxo*-BAs, and others[12] (Fig. 2A). Moreover, the C-24 carboxyl group of BAs can be amidated by amino acids, predominantly resulting in the formation of glycine-conjugated BAs (GBAs) in humans, while taurine conjugation is more prevalent in rats and mice[13]. In addition, non-amino acid carboxyl conjugations and hydroxyl modifications, such as sulfation and glucuronidation, also occur[14] (Fig. 2B).

BAs can be categorized as primary BAs and secondary BAs. Primary BAs are mainly synthesized in hepatocytes, while secondary BAs are formed from primary BAs by gut microbes. In humans[13], major primary BAs include cholic acid (CA), chenodeoxycholic acid (CDCA), and their conjugated derivatives, while prominent secondary BAs consist of deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA). In mice[13], primary BAs encompass CA, CDCA, α-muricholic acid (α-MCA), β-muricholic acid (β-MCA), UDCA, and their conjugated derivatives, while the secondary BAs can be DCA, LCA, ω-muricholic acid (ω-MCA), hyocholic acid (HCA),

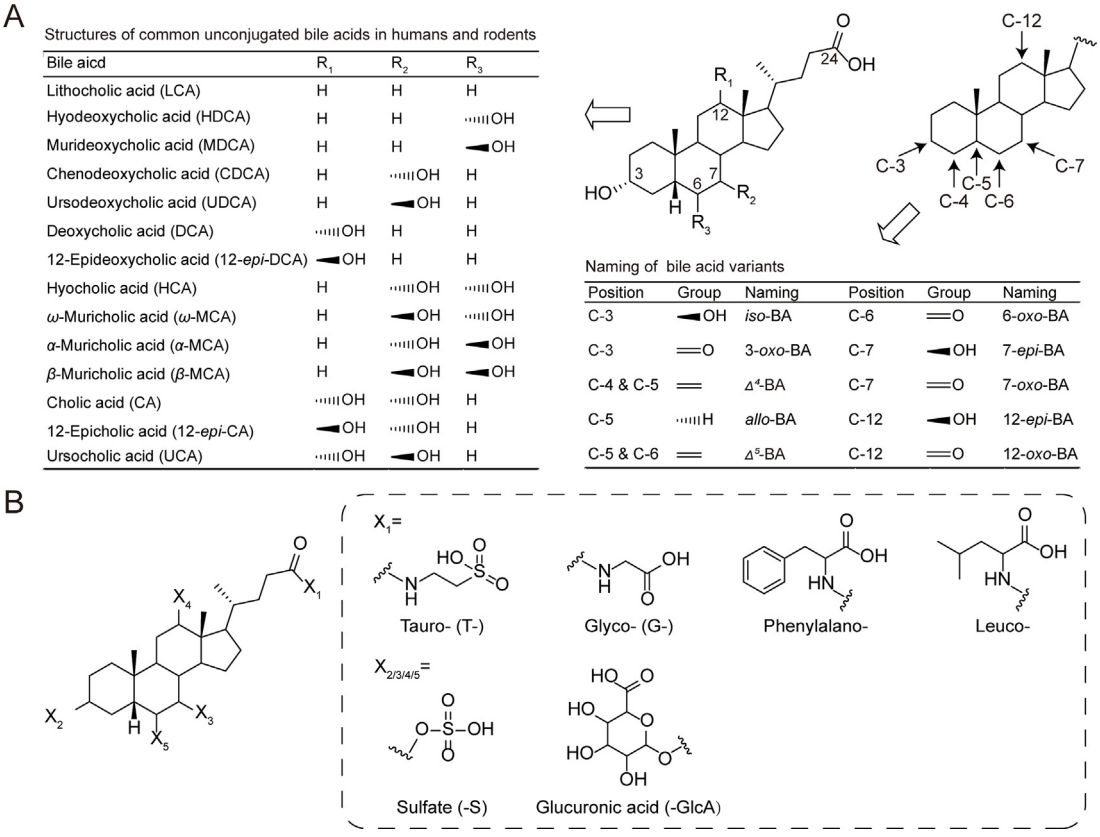

Figure 2 (A) Structures of common unconjugated BAs and naming of BA variants; (B) Conjugation and hydroxyl modifications of BA backbone.

murideoxycholic acid (MDCA), hyodeoxycholic acid (HDCA), among others. Notably, reabsorption of secondary BAs leads to the formation of new conjugates in the liver, thereby expanding the host BA pool[12,15,16].

3.2. *Biosynthesis of primary BAs*

Cholesterol is the major sterol in animals, which can be incorporated into cell membranes, or serves as the precursor for the biosynthesis of primary BAs, steroid hormones, and vitamin D. Endogenous synthesis and dietary intake are two main sources of mammalian cholesterol. The early biosynthetic steps of cholesterol are similar to those of plant triterpenoids and sterols, proceeding through the MVA pathway and the squalene synthesis pathway, although there might be differences in the enzymes involved (Fig. 4B). Subsequently, animals employ lanosterol as an intermediate, which undergoes a 19-step process, involving the removal of two methyl groups and the rearrangement of the alkenyl group, ultimately forming cholesterol.

Primary BA biosynthesis involves both the classical (neutral) pathway and the alternative (acidic) pathway[17]. In the human liver, BAs produced *via* the classical pathway predominate, with those produced *via* alternative pathways accounting for no more than 10%. However, under pathological conditions, the proportion of BAs from alternative pathways may increase[1,18].

The classical pathway begins with the 7α-hydroxylation of cholesterol in the endoplasmic reticulum, catalyzed by the rate-limiting enzyme, 7α-hydroxylase (CYP7A1). This conversion produces 7α-hydroxy-cholesterol (7αHC), which is further transformed into 7α-hydroxy-4-cholesten-3-one (C4) by 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (encoded by *HSD3B7* gene). C4 then undergoes C-12 hydroxylation *via* the action of 12α-hydroxylase (CYP8B1). Subsequently, 5β-reductase (encoded by *AKR1D1* gene) and 3α-hydroxysteroid dehydrogenase (encoded by *AKR1C4* gene) mediate the hydrogenation reaction in the cytosol. The intermediate then undergoes side-chain oxidation by sterol 27-hydroxylase (CYP27A1) in the mitochondria, eventually resulting in the formation of CA following side-chain cleavage (in the peroxisomes). In the classical pathway, some intermediates do not undergo C-12 hydroxylation and ultimately result in CDCA formation. The alternative pathway is initiated by the production of 27-hydroxy-cholesterol (27HC) through the action of CYP27A1. 27HC undergoes further hydroxylation catalyzed by oxysterol 7α-hydroxylase (CYP7B1), as well as side-chain oxidation and double bond rearrangement. This series of reactions ultimately lead to the formation of 7α-hydroxy-3-*oxo*-4-cholestenoate, which then experiences 5β-hydrogenation, 3-*oxo* group reduction and side chain cleavage, resulting in the production of CDCA. Similarly, the alternative pathway can also generate some metabolic flux towards CA. Notably, differences in metabolic enzyme activities contribute to interspecies variations in the composition of the BA pool. Enzymes encoded by the mouse *Cyp2c70* gene possess both 6β hydroxylation and 7α epimerization capabilities, leading to the conversion of CDCA into α-MCA, CDCA into UDCA, UDCA into β-MCA, and α-MCA into β-MCA[19]. However, the human homolog of *Cyp2c70*

gene doesn't perform these functions, causing a higher concentration of 6-OH BAs in the BA pool of mice in comparison to humans[20].

In primary BA biosynthesis, a large portion of bile acid-CoA is not hydrolyzed to free BAs but rather reacts with glycine or taurine under the action of bile acid-CoA:amino acid *N*-acyltransferase (encoded by the *BAAT* gene in humans) located in the cytosol, forming glycine-conjugated BAs (mainly in humans) or taurine-conjugated BAs (mainly in rats and mice), such as GCA, GCDCA, TCA, and TCDCA[21]. Furthermore, hepatic sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs) can modify the hydroxyl groups of BA molecules, resulting in the formation of sulfated or glucuronidated BAs. Sulfation can occur in both free and conjugated BAs, while glucuronidation primarily targets free BAs[14]. These modifications of the hydroxyl groups enhance the water solubility of BAs, reduce their cytotoxicity, and promote their excretion *via* feces and urine[22,23].

3.3. Metabolism of secondary BAs

Primary BAs undergo deconjugation, dehydroxylation, redox and isomerization processes in the intestine, mediated by gut microbes, leading to the formation of secondary BAs. Deconjugation and dehydroxylation reactions increase the hydrophobicity of BAs and reduce their detergency, thus enhancing BA tolerance of gut microbes. The initial step in secondary BA metabolism is the C24 amide hydrolysis of conjugated BAs, which is carried out by bacterial bile salt hydrolase (BSH). Numerous genera of gut microbes are found to express BSH, including *Enterococcus*, *Lactobacillus*, *Bacteroides*, *Staphylococcus*, among others. To gain a better understanding of these enzymes, researchers have undertaken extensive phylogenetic analysis, classifying BSHs from 591 bacterial strains spanning 117 genera into eight distinct phylotypes (BSH-T0−T7). Distinct phylotypes display selectivity for specific substrates. For example, BSH-T5 and T6 demonstrate lower catalytic hydrolysis activity on GCA compared to TCA, while BSH-T3, exclusively found in *Lactobacillus*, exhibits potent activity in hydrolyzing various conjugated BAs[24]. These diverse BSHs not only exhibit varying distributions among different human populations but also showcase differences in prevalence between patients and individuals without specific health conditions[24]. Therefore, intestinal bacterial BSH may be related to the health status or disease progression of the host, which will be further elaborated in the following content.

In the 1980s, the research team led by Phillip B. Hylemon made an initial breakthrough by revealing that the intestinal bacterium *Clostridium scindens* VPI 12708 could convert CA into DCA and CDCA into LCA[25]. Their subsequent discovery of the *bai* gene cluster, a substantial operon within this bacterium, revealed the presence of eight specific genes responsible for facilitating the crucial 7α-dehydroxylation process[26]. Over time, several other strains and gene clusters with the capability for this enzymatic activity were uncovered. Nevertheless, the *bai* operon is a shared feature among all known strains exhibiting this function, and it's worth noting that only recently has this pathway been fully elucidated[27]. Within this cluster of genes, *baiB* and *baiF* are responsible for encoding BA CoA ligase and CoA transferase, respectively, while *baiA2* encodes 3α-hydroxysteroid dehydrogenases (3αHSDH) that catalyze oxidation and reduction reactions at the C-3 position. Furthermore, *baiCD* encodes an Fe−S flavoenzyme, which plays a crucial role in dehydrogenation and hydrogenation reactions between C4 and C5, whereas *baiE* and *baiH* mediate dehydration and hydrogenation reactions between C6 and C7, respectively. The entire reaction process involves 6 genes and a total of 8 steps (Fig. 3). Among the remaining two genes, *baiG* encodes a BA transport protein, enabling the bacterium to uptake BAs; while *baiI* is a homolog of *baiE*, but it's not essential to the 7α-dehydroxylation pathway[26,28]. In addition to 7α-dehydroxylation, gut microbes also demonstrated 7β-dehydroxylation capability, converting UDCA into LCA and β-MCA into MDCA in mice[13,16].

Although the principal product of 7-dehydroxylation is DCA and LCA, there are also many isomeric secondary BAs that can be detected. Michael A. Fischbach's research team identified a 3β-hydroxysteroid dehydrogenase (3βHSDH), encoded by Rumgna_00694 from the intestinal bacterium *Ruminococcus gnavus*. This enzyme influences both the conversion of 3-*oxo*-LCA into *iso*-LCA and the transformation of 3-*oxo*-DCA into *iso*-DCA[29] (Fig. 3). Furthermore, 5α epimers constitute another significant category of secondary BAs. It has been reported that 5α-reductase enzymes, originating from some strains of *Bacteroidales* or *Firmicutes*, are responsible for catalyzing the conversion of 3-*oxo*-Δ^4-LCA into 3-*oxo*-*allo*-LCA. This further leads to the generation of *allo*-LCA and *iso*-*allo*-LCA[30,31] (Fig. 3). In addition, gut microbes derived 7α/β-hydroxysteroid dehydrogenase (7α/βHSDH) and 12α/β-hydroxysteroid dehydrogenase (12α/βHSDH) mediate the oxidation and isomerization reactions of the corresponding hydroxyl groups of CA and CDCA, resulting in production of 7-*epi*-cholic acid (Ursocholic acid, UCA), 12-*epi*-CA and UDCA, among others[16,26]. Considering that humans lack the enzymes required for the synthesis of 7β-OH BA, this pathway is likely the main contributor of both unconjugated and conjugated UDCA concentrations to the human BA pool[16] (Fig. 3). In mice, gut microbes isomerize α-MCA and β-MCA into HCA and ω-MCA, respectively, and convert MDCA into HDCA[13].

It should be noted that the above metabolic processes can be synergistic among bacterial species, and a specific bacteria strain does not need to possess all of the metabolism-related genes. Since most reactions use unconjugated BAs as substrates, the deconjugation process is considered as the "gateway reaction" of secondary BA metabolism[16], and recent research supports this opinion. In the work, BSH inhibitor decreased the production of 3-*oxo*-LCA, *iso*-LCA, LCA and DCA by *Parabacteroides distasonis*, thus reducing the immune regulation effect of this bacterium on the host[2].

3.4. New-found microbial BAs

In recent years, researchers have discovered a range of novel amino acid-conjugated-BAs, including phenylalano-CA, tyroso-CA and leuco-CA in both mice and humans[15] (Fig. 2B). Through a comparison of BA profiles between germ-free (GF) and specific pathogen-free (SPF) mice, these BAs were identified as production of gut microbes[15] and were termed microbially conjugated BAs (MCBAs)[16]. Subsequently, an increasing number of MCBAs have been identified in human samples, such as alano-, glutamimo-, glutamato-, tryptophano-, and argino-BAs[32]. The latest research has indicated a high proportion of MCBAs in human fecal BAs, with their total concentration possibly matching or exceeding that of primary BAs and approximating one-third of the total concentration of secondary BAs[33]. Surprisingly, their production is intimately linked with a specific type of BSH. N-terminal nucleophilic BSH identified from *Clostridium perfringens* exhibits acyltransferase activity and can convert TCA or

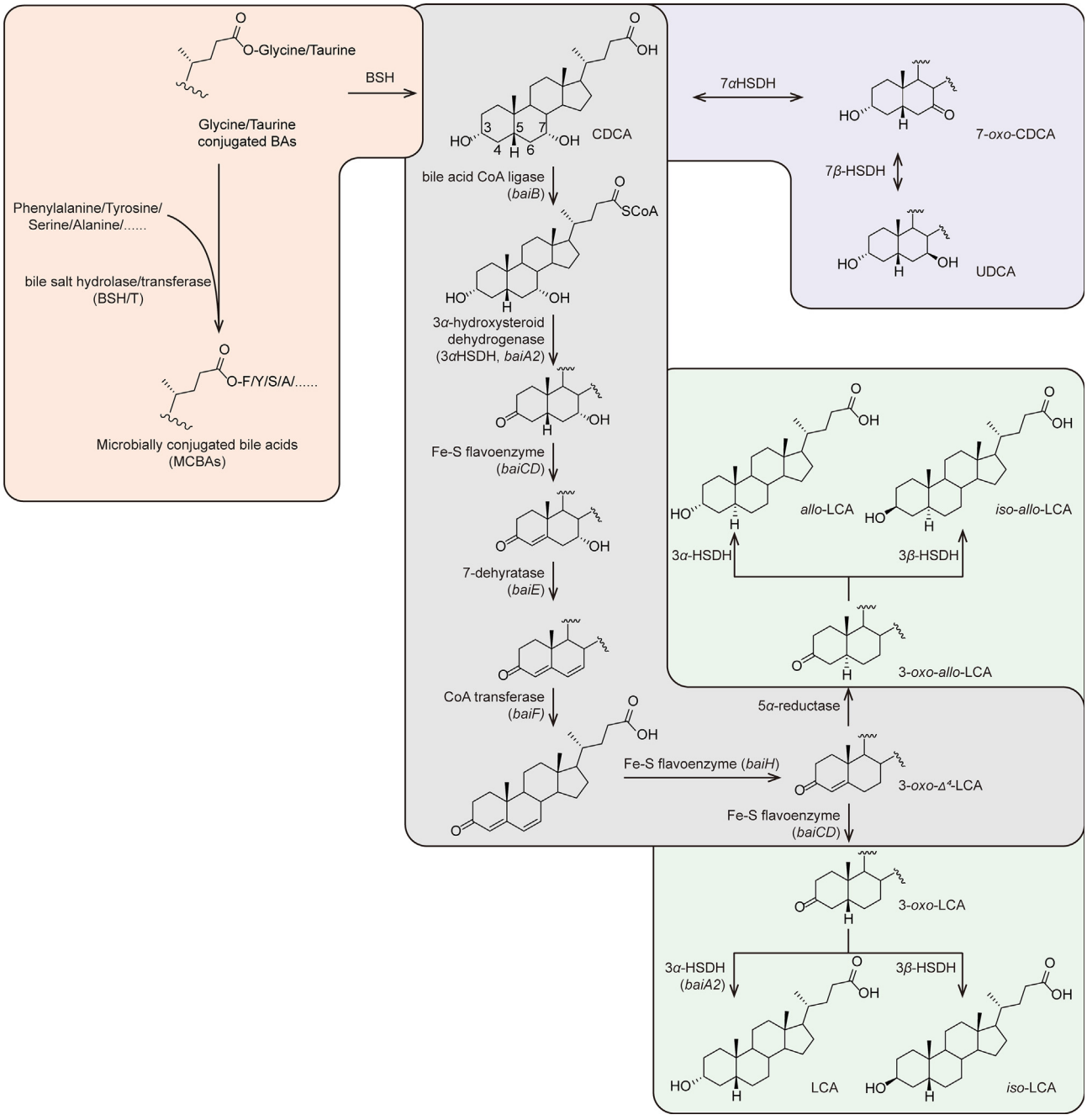

Figure 3 Gut flora-mediated BA metabolism in humans.

GCA into the respective amino acid-conjugated CA in the presence of specific amino acid substrates[33] (Fig. 3). This type of BSH is referred to as BSH/transferase (BSH/T). BSH/T is widely distributed among gut microbiota and has been found in *Bifidobacterium*, *Enterococcus*, *Lactiplantibacillus*, and *Ruminococcus* genera[33–35]. Compared to host-derived TBAs and GBAs, MCBAs conjugated with hydrophobic amino acids, such as PheCA and LeuCA, exhibit stronger antibacterial activity[33]. It indicates that, by producing MCBAs, gut bacteria may regulate the toxicity of BAs and increase their own survival advantage. Besides, MCBAs also interact with host receptors such as farnesoid X receptor (FXR), pregnane X receptor (PXR), aryl hydrocarbon receptor (AHR), and others, and receptor selectivity depends on the type of conjugated amino acid and the type of BA backbone[34,35]. Hence, MCBAs may participate in host physiological and pathological processes. In addition to conjugation at the carboxyl group, gut microbiota can also modify the hydroxyl groups on BA molecules. 3-*O*-Acetyl/propionyl/butyryl CA produced by gut commensal *Christensenella minuta* is a type of FXR antagonist that may influence host metabolism[36]. Additional research is necessary to understand the relevance of these recently discovered BAs within physiological contexts to disease states, along with exploring their metabolic pathways and regulatory mechanisms.

The effects of isomerization and oxidation catalyzed by gut flora elevate the diversity of BA backbone types, while the novel re-conjugation process mentioned above further expands the

potential number of BA species to thousands in both humans and rodents[16]. In this context, the development of new BA detection methods is highly important. Recently, researchers have introduced the concept of reverse metabolomics and applied it to the discovery of novel MCBAs in biological samples. In the reverse metabolomics strategy, researchers can first synthesize reference standards for the interested MCBAs, obtain their mass spectra, and then search for them in the public mass spectra databases to investigate the changes of these potential novel MCBAs occurring in diseases[34]. A non-targeted BA profile analysis software named BAFinder has also been developed using a strategy similar to reverse metabolomics[37]. In developing this analysis workflow, 84 reference standards of BA, either commercially purchased or synthesized in-house, underwent ionization and fragmentation in positive and negative ESI modes. Fragmentation information was collected to construct a computer simulation database, which was used to differentiate BA isomers and identify potential novel BA molecules. Through its application, a total of 112 BAs in human plasma and 244 in urine samples were annotated[37]. This methodology breaks the limits of traditional target analysis methods, which are restricted by the available number of reference standards. The updated BAFinder 2.0 library now covers BAs conjugated with 18 common amino acids to enhance the detection of MCBAs. Using this workflow, researchers can efficiently annotate and identify MCBAs in various biological samples[38]. Additionally, they can conduct *in silico* conjugation of self-defined small molecules with BAs, and further search for validation in sample datasets. Through this approach, four new conjugates were discovered: D-Ala-D-Ala, Lys(*iso*)-Gly, L-2-aminobutyric acid, and ornithine[38].

4. BAs transport mechanisms

After their synthesis in the liver, BAs are released into the intestine through the biliary system, where they emulsify fats and enhance the digestion and absorption of lipophilic nutrients. Around 95% of these BAs can be reabsorbed by the small intestine, primarily through active uptake by enterocytes in the ileum. Subsequently, these reabsorbed BAs are transported back to the liver *via* the portal venous system. The entire process is known as enterohepatic circulation (EHC), occurring roughly 8 to 12 times daily in the human body. A small portion of BAs are passively reabsorbed by cholangiocytes or other segments of the intestine. Some BAs reach the colon, where they are metabolized by gut microbes, absorbed passively, or eliminated in feces. Additionally, a fraction of BAs is transported into the circulation by hepatocytes and eventually eliminated through urine. As a result, approximately 400−800 mg of BAs escape the EHC daily, necessitating their re-synthesis by the liver to maintain the BA pool size. The critical transporters involved in the BA transport process are elaborated upon below.

4.1. *Bile salt export pump (BSEP)*

BSEP, encoded by the *ABCB11* gene, is an ATP-dependent BA transporter. It primarily localizes in the canalicular membrane of hepatocytes and is responsible for secreting BAs from the liver into the bile canaliculus lumen (Fig. 4A). BAs are the primary endogenous substrates of BSEP, and studies report that human BSEP can transport various monovalent BAs like TBAs, GBAs and unconjugated BAs[39,40]. Notably, unlike rodents, human BSEP can also mediate the output of some sulfated BAs[41].

4.2. *Sodium taurocholate cotransporting polypeptide (NTCP)*

NTCP, encoded by the *SLC10A1* gene, is a Na^+-dependent BA transporter located mainly on the basolateral plasma membrane of hepatocytes. Unlike BSEP, NTCP controls the vectorial flow of BAs into hepatocytes (Fig. 4A). NTCP-mediated BA transport is reliant on extracellular Na^+ concentration, taking up two Na^+ ions with one BA molecule. Research indicates that NTCP transports both conjugated BAs and unconjugated BAs, but exhibits a higher affinity for conjugated BAs[39,40].

4.3. *Apical sodium-dependent BA transporter (ASBT)*

Similar to NTCP, ASBT, encoded by the *SLC10A2* gene, is a sodium-dependent BA transporter involved in the uptake of BAs. It exhibits high expression in the apical plasma membrane and microvilli of the distal ileal enterocytes, absorbing BAs from the intestinal lumen and thereby playing a crucial role in the ileal reabsorption of BAs (Fig. 4A). Moreover, a minor expression of ASBT is observed in cholangiocytes and proximal renal convoluted tubule cells, respectively facilitating BAs reabsorption in the bile ducts and renal tubules[42,43]. ASBT demonstrates a narrow endogenous substrate specificity, primarily transporting BAs. Research suggests that both conjugated and unconjugated BAs can be transported by ASBT, with a higher transport efficiency observed for conjugated BAs[43].

4.4. *Ileal BA-binding protein (IBABP)*

Differing from the aforementioned transmembrane transporters, IBABP is a binding protein encoded by the *FABP6* gene. It predominantly resides in the cytoplasm of enterocytes, functioning as a facilitator for the transport of BAs from the apical to the basolateral side (Fig. 4A). IBABP accelerates the flow of BAs within ileal enterocytes, thus diminishing the harmful impact of BAs on cells. As reported, compared to free BAs, taurine/glycine-conjugated BAs exhibit a stronger affinity for IBABP. Additionally, a hydroxyl group at the C-7 position decreases the affinity of BA molecules for IBABP, while a hydroxyl group at the C-12 position has the opposite effect[44].

4.5. *Organic solute transporter alpha/beta (OSTα/β)*

OSTα and OSTβ are transmembrane transporters encoded by *SLC51A* and *SLC51B* genes, respectively, mainly situated on the basolateral membrane of cells. They are widely distributed throughout various tissues, with notably high expression levels in the liver, small intestine, and colon in humans. OSTα and OSTβ are usually combined into heterodimers to perform transport functions. Unlike BSEP, NTCP and ASBT, the functionality of the OSTα−OSTβ dimer does not rely on ATP or transmembrane electrolyte concentration gradients. Instead, it mediates the basolateral efflux of BAs from hepatocytes or enterocytes *via* facilitated diffusion. Therefore, the OSTα−OSTβ dimer plays a crucial role in allowing reabsorbed BAs to enter the hepatic portal vein and facilitating the leak of hepatic BAs into circulation (Fig. 4A). It's noteworthy that OSTα−OSTβ exhibits broad substrate specificity, capable of transporting various compounds such as prostaglandin E2, estrone 3-sulfate, among others, besides BAs. Among BAs, their transport efficiency level is as follows: TBAs > GBAs > unconjugated BAs. Additionally, compared with

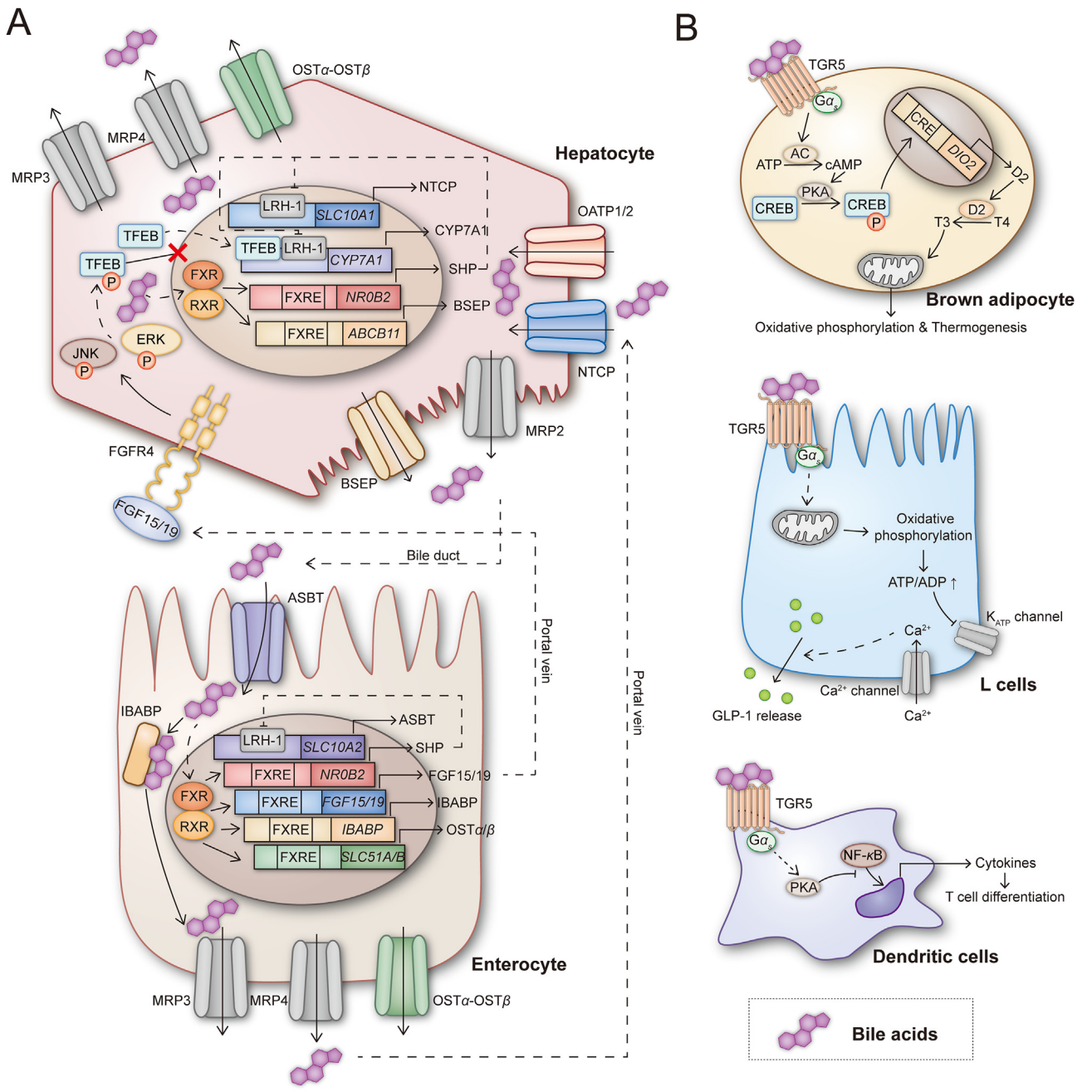

Figure 4 (A) FXR signaling mediated BA metabolism and transport in hepatocytes and enterocytes; (B) TGR5 signaling in representative cells.

ASBT, OSTα−OSTβ transporter also has substrate selectivity for sulfated TBAs[45].

4.6. *Organic anion-transporting polypeptides (OATPs)*

OATPs are a class of Na^+-independent transporters, transporting solutes through an anion (*e.g.*, HCO_3^- and glutathione) exchange process. Human OATPs are classified into six families (OATP1−6). In the liver, OATP1A2, OATP1B1 and OATP1B3, located on the basolateral plasma membrane of hepatocytes and encoded by the *SLCO1A2*, *SLCO1B1* and *SLCO1B3* genes respectively, are responsible for BA transport. The OATPs in rodents differ from humans[46,47]. In rats, OATP1A1/3/4/5/6 encoded by the *Slco1a1/3/4/5/6* genes are orthologs to human OATP1A2, while OATP1B2 encoded by the *Slco1b2* gene is the closest ortholog to human OATP1B1/3, and compared to rats, mice lack OATP1A3[46,47]. Similar to NTCP, OATPs play a role in transporting reabsorbed BAs from the liver sinusoids into hepatocytes (Fig. 4A). However, unlike NTCP, OATPs exhibit a preference for unconjugated BAs over conjugated ones[48,49].

4.7. *Multidrug resistance-associated proteins (MRPs)*

MRPs, a group of ATP-binding cassette transporters known for inducing multidrug resistance, have been implicated in BA transport. Among them, MRP2, MRP3 and MRP4, encoded by *ABCC2*, *ABCC3* and *ABCC4* respectively, play pivotal roles in this process. MRP2 is crucial for transporting sulfated and glucuronidated BAs from hepatocytes into the bile canaliculi[50]. While MRP3 and MRP4, located in the basolateral plasma membrane of hepatocytes and enterocytes, mediate the efflux BAs from the liver to the circulation and the entry of reabsorbed BAs into the portal vein[51] (Fig. 4A).

In summary, the journey of BAs begins with their synthesis in hepatocytes, followed by active secretion into the bile canaliculus lumen facilitated by BSEP and MRP2, located in the canalicular membrane. Stored in the gallbladder, BAs are subsequently released into the duodenum *via* the bile duct. Upon reaching the ileum, most BAs are absorbed by ASBT at the apical side of enterocytes and, transported to the basolateral side by IBABP. Once there, OSTα−OSTβ, MRP3, and MRP4, located in the basolateral plasma membrane, facilitate BAs' efflux from enterocytes into the portal vein. These reabsorbed BAs then journey back from the liver sinusoids into hepatocytes through NTCP and OATPs located on the basolateral plasma membrane, thus completing an entire EHC process.

5. BAs signaling pathways

In addition to aiding in the digestion and absorption of lipophilic nutrients, BAs function as signaling molecules, actively involved in various physiological and pathological processes within the body. These BA-responsive receptors include takeda G protein-coupled receptor 5 (TGR5, encoded by *GPBAR1* gene), FXR (encoded by *NR1H4* gene), PXR (encoded by *NR1I2* gene), liver X receptor (LXR, encoded by *NR1H3* gene), vitamin D receptor (VDR, encoded by *NR1I1* gene), RAR-related orphan receptor gamma (RORγ, encoded by *RORC* gene), among others. Notably, TGR5 and FXR exhibit the most potent selectivity for BAs[52].

5.1. *Takeda G protein-coupled receptor 5 (TGR5)*

TGR5 is a G protein-coupled receptor located on the cytoplasmic membrane. Human TGR5 is encoded by the *GPBAR1* gene and exhibits widespread expression across various organs and tissues, including the intestine, spleen, gallbladder, and adipose tissues. BAs serve as endogenous ligands for TGR5, with secondary BAs displaying higher affinity compared to primary BAs (LCA > DCA > CDCA)[53]. TGR5 activation triggers adenylyl cyclase (AC)−cAMP−PKA signaling, promoting the recruitment of cAMP response element-binding protein (CREB) to the cAMP response element (CRE) of target genes (Fig. 4B). In addition, it stimulates various signaling pathways such as extracellular signal-related kinase 1/2 (ERK1/2), protein kinase B (AKT), and mammalian target of rapamycin complex 1 (mTOC1)[54]. TGR5 plays a crucial role in metabolic and inflammatory responses. In brown adipocytes, TGR5 enhances cAMP-dependent type 2 iodothyronine deiodinase (D2) expression, facilitating the conversion of tetraiodothyronine (T4) into triiodothyronine (T3). While in L cells, TGR5 activation prompts oxidative phosphorylation, elevating the intracellular ATP/ADP ratio. This leads to the closure of the ATP-dependent potassium channel and facilitates calcium influx, ultimately triggering the release of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY)[55]. In addition, TGR5 signaling has also been found to regulate the activation of various types of immune cells. For instance, activation of TGR5/cAMP/PKA signaling in dendritic cells (DCs) reduces nuclear factor kappa B (NF-κB)-dependent cytokine expression, thus inhibiting DCs activation and T cell differentiation[56]. In addition, in the RAW246.7 mouse macrophage cell line, the activation of TGR5 induces CREB binding to the *Il10* gene promoter, promoting the transformation of macrophages from a classical activated state to an alternative activated state[57]. The functionality of TGR5 signaling in brown adipocytes, L cells, and DCs is shown in Fig. 4B.

5.2. *Farnesol X receptor (FXR)*

FXR was initially identified as a nuclear orphan receptor until its de-orphanization with the discovery of BAs as its endogenous ligands. Humans only express FXRα (encoded by the *NR1H4* gene), which generates four isoforms (FXRα1−4) through alternative promoter usage and splicing[58−60]. These isoforms exhibit distinct tissue-specific distribution in humans, with FXRα1/2 prominently expressed in the liver, while FXRα3/4 are more prevalent in the intestine[59,61,62]. The FXR protein consists of ligand-independent transcriptional activation domains (AF1 and AF2), a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain (LBD). FXRα1/2 and FXRα3/4 differ in AF1 length, while FXRα2/4 lack MYTG in the hinge region, unlike FXRα1/3[58,62,63]. All FXRα isoforms can bind to the IR-1 motif on the genome DNA, while FXRα2/4 additionally binds to the ER-2 motif[64]. Differences in the binding properties may account for functional differences among different FXRα isoforms. For instance, FXRα2/4, rather than FXRα1/3, can regulate mitochondrial pyruvate transport in liver organoids[64]; FXRα2, instead of FXRα1, can regulate the expression of genes involved in fatty acid metabolism[65]. Furthermore, different FXRα isoforms display varying sensitivities to FXR ligands; thus, disease-induced changes in BA pool composition may lead to differential activation among these isoforms[62]. Consequently, it becomes evident that for targeting FXR in different diseases like diabetes, fatty liver, and liver cancer, isoform selectivity should be considered[60].

The LBD of human FXR comprises 12 α-helices (H1−12), and ligand binding can alter H12's conformation, modulating the nuclear receptor's activity. FXR agonists disrupt or weaken the LBD-corepressor interaction, allowing H12 to bind to the LBD and recruit coactivators like SRC-1[63]. On the contrary, FXR antagonists stabilize the FXR-LBD-corepressor interaction, maintaining H12 away from the LBD, rendering it inactive[63]. Initial research based on reporter gene assays showed that among endogenous BAs, CDCA exhibits the highest activation magnitude on human/murine FXR[66], followed by DCA and LCA. However, coactivator recruitment assays showed that DCA and LCA did not promote interactions between the FXR-LBD and SRC-1, and could disrupt the CDCA−FXR−SRC1 complex[66]. Subsequent studies have shown that they are potential FXR antagonists in the BA pool[67,68]. In reporter gene assays based on Caco-2 cells, CA, TCA, and GCA initially showed no effect on FXR. However, upon transfection with the human *SLC10A2* gene plasmid, they all exhibited an FXR agonistic effect[69]. Additionally, UDCA[70], Tβ-MCA[71], TUDCA[72] and GUDCA[72] can reduce agonist-induced FXR activation, suggesting that a subset of BAs in the BA pool serves as FXR antagonists. These results suggest that different BAs vary in

their ability to activate or antagonize FXR, and changes in BA composition in the BA pool may influence the activity of human FXR.

FXR forms heterodimers with retinol X receptor (RXR), binding to FXR response elements (FXREs) located on promoters or introns of the target genes, thereby regulating their expression. The FXR/RXR heterodimer mediates two feedback mechanisms in BA biosynthesis regulation[73]. In the first mechanism, the heterodimer binds to FXREs situated on the promoter of the hepatic small heterodimer partner (SHP, encoded by *NR0B2* gene), facilitating its transcription. Subsequently, SHP binds to liver receptor homolog-1 (LRH-1) on the *CYP7A1* gene promoter, forming a heterodimer that suppresses the expression of the *CYP7A1*[74] gene. The second pathway depends on hormonal crosstalk between the intestine and the liver. Specifically, the FXR/RXR heterodimer promotes the expression of human fibroblast growth factor 19 (FGF19, while FGF15 is the orthologous protein in mice) in the ileum[75], and this endocrine hormone then acts on hepatic fibroblast growth factor receptor 4 (FGFR4), augmenting the phosphorylation of ERK and c-Jun N-terminal kinase (JNK)[75,76]. Consequently, this triggers the phosphorylation of transcriptional factor EB (TFEB), reducing its nuclear translocation and thus inhibiting the expression of the *CYP7A1* gene[77]. Notably, the FXR−FGF15/19 signaling pathway appears to have a more potent inhibitory effect on the expression of *CYP7A1*, *CYP27A1* and *CYP7B1* compared to *CYP8B1*[78]. FXR can also regulate the expression of BA transporters. In the ileum, FXR/RXR directly binds to the promoter region of the *IBABP* gene[69], thereby promoting its transcription, and repressing the expression of *SLC10A2* gene by upregulating SHP[79]. In the liver, FXR activation leads to the upregulation of the *ABCB11* gene, enhancing the efflux of BAs from the liver into the bile canaliculus lumen[80]. Additionally, it inhibits the expression of the *SLC10A1* gene through SHP upregulation, consequently reducing the liver's uptake of BAs[81]. The role of FXR signaling in BA metabolism and transport is shown in Fig. 4A.

5.3. *BA signaling in metabolic homeostasis*

BA signaling is crucial not only for the regulation of cholesterol metabolism but also for the homeostasis of glucose and lipid metabolism. TGR5 signaling maintains energy metabolism balance and triglyceride homeostasis by promoting thermogenesis in brown adipose tissue and browning of white adipose tissue[82]. In enteroendocrine cells, TGR5/GLP-1 signaling regulates the body's glucose and lipid homeostasis by modulating appetite and insulin release[55].

In addition to TGR5, FXR also plays a crucial role in maintaining glucose and lipid metabolism homeostasis. In the liver, FXR enhances the expression of peroxisome proliferator-activated receptor alpha (PPARα)[83], potentially promoting fatty acid β-oxidation. Moreover, FXR suppresses hepatic de novo lipogenesis by upregulating the expression of SHP, which in turn downregulates sterol regulatory element-binding protein-1c (SREBP-1c) expression[84,85]. A recent study has shown that FXR can also independently inhibit the expression of key lipogenic genes[86]. In addition to hepatic FXR, intestinal FXR signaling also regulates lipid homeostasis. Intestinal FXR-FGF15/19 signaling can increase the expression of hepatic peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) by inhibiting phosphorylation of CREB, thus reducing the expression of fatty acid oxidation-related proteins, such as carnitine palmitoyltransferase 1 (CPT1)[77,87]. Furthermore, intestinal FXR can upregulate the expression of sphingomyelin phosphodiesterase 3 (SMPD3), increasing circulation ceramide levels, thus affecting hepatic lipid homeostasis and insulin sensitivity[36,88]. The role of FXR in regulating hepatic gluconeogenesis is complex and somewhat contentious. A study indicated that FXR agonists upregulated the expression of phosphoenolpyruvate carboxykinase (PEPCK) in both mice liver and primary human hepatocytes, enhancing gluconeogenesis[89]. Consistently, FXR antagonist HS218 could inhibit the binding of FXR to PGC1α promoter, thereby attenuating hepatic gluconeogenesis[90]. However, another research demonstrates that dietary supplementation with the FXR agonist CA suppressed the expression of PEPCK and other gluconeogenesis-related proteins, thereby reducing fasting blood glucose levels, and this effect was depleted in FXR$^{-/-}$ and SHP$^{-/-}$ mice[91]. Notably, FGF15/19 reduces PGC1α expression and subsequently decreases the expression of gluconeogenesis-related proteins[87]. It suggests that the activation of intestinal FXR might inhibit hepatic gluconeogenesis through the crosstalk between the intestine and the liver. Therefore, from a global perspective, the regulatory role of FXR in hepatic glucose homeostasis is complex and may require further investigation using tissue-specific FXR knockout models.

6. Targeting BA metabolism, transport, and signaling in diseases

Primary biosynthesis and secondary metabolism control the BA pool's composition, while transport processes regulate BAs' spatial distribution, collectively influencing BA signaling across tissues, and participating in physiological and pathological processes. BA metabolism, transport, and signaling are implicated in numerous conditions: cholestasis, metabolic diseases (including diabetes, NAFLD, hyperlipidemia, etc.), autoimmune disorders, inflammatory bowel diseases, colorectal cancer, liver cancer, and others. Modulating endogenous BAs or targeting BA signaling offers potential intervention in these diseases.

6.1. *Intrahepatic cholestasis (IHC)*

IHC arises from impaired BA metabolism and excretion in the liver, commonly observed in conditions like cholestatic liver disease (CLD) or intrahepatic cholestasis of pregnancy (ICP). IHC can inflict damage on hepatocytes, and prolonged cholestasis might result in liver fibrosis or cirrhosis. Enhancing hepatic bile excretion, reducing intrahepatic BA synthesis and curbing ileal BA reabsorption are key approaches to ameliorate IHC. UDCA application stimulates impaired bile excretion, protecting cholangiocytes from BA-induced toxicity[92]. To block ileal BA reabsorption, ASBT inhibitors (*e.g.*, maralixibat) and BA sequestrants (*e.g.*, cholestyramine) can be employed[93,94]. Furthermore, activating FXR proves effective[95]. Hepatic FXR activation diminishes BA biosynthesis by suppressing the *CYP7A1* gene, alongside downregulating *SLC10A1* and upregulating *ABCB11* genes, thus decreasing hepatic BA intake while promoting excretion. Simultaneously, intestinal FXR activation lowers *SLC10A2* gene expression, reducing BA reabsorption. 6α-Ethyl CDCA (INT747), also known as obeticholic acid (OCA), is a semi-synthetic FXR agonist, approved as a second-line treatment for patients with primary biliary cholangitis when UDCA shows inadequate response or intolerance[96].

6.2. *Metabolic diseases*

6.2.1. *TGR5 activation*

The incretin GLP-1 is vital in attenuating metabolic diseases by reducing appetite and enhancing insulin sensitivity. Drugs like semaglutide and liraglutide, analogs of GLP-1, are widely used for managing weight and blood glucose levels. TGR5 activation induces calcium influx, triggering GLP-1 release from enteroendocrine L-cells. Endogenous TGR5 activators, such as HCA, CA-7-sulfate, and semisynthetic agonist INT-777, have been noted for their ability to stimulate TGR5-mediated GLP-1 release, thereby enhancing glucose regulation[55,97,98].

In addition to the ileum, TGR5 is also present in skeletal muscle and adipose tissues. Research indicates that selective TGR5 agonists can mitigate insulin resistance in skeletal muscle through the TGR5/cAMP/PKA signaling pathway[99]. Therefore, modest rises in circulating BA levels might contribute to enhanced insulin sensitivity. TGR5 activation also stimulates white adipose tissue browning and increases adaptive thermogenesis in brown adipose tissue[82]. Previous studies suggest that cold exposure induces hepatic BA synthesis in mice, increasing circulating BA levels, thereby activating TGR5 in adipose tissue[100,101]. This might raise energy consumption and aid in countering obesity. Notably, a recent study revealed that modulating endogenous BA levels could trigger TGR5 activation in the hypothalamus, subsequently stimulating the sympathetic nervous system and resulting in weight and fat loss[102].

6.2.2. *FXR regulation*

Hepatic activation of FXR plays an important role in alleviating nonalcoholic steatohepatitis (NASH) by mitigating lipid accumulation, BA-induced cellular damage, inflammation and fibrosis progression[103,104]. In a study conducted by Clifford et al., FXR activation in mice liver lowered the expression of key lipogenesis genes, *Scd1*, *Dgat2*, and *Lpin1*, through a pathway independent of SHP[86]. Additionally, intestinal FXR activation in mice reduced lipid absorption by lowering CA and TCA concentrations, which are the main emulsifiers of lipids in the small intestine. These combined pathways contribute to preventing NAFLD[86]. Furthermore, a recent study has revealed that FXR agonists Turofexorate and Fexaramine upregulate the expression of GPX4, FSP1, and PPARα in mouse primary hepatocytes and human induced pluripotent stem cells (iPSCs), consequently inhibiting lipid peroxidation and ferroptosis[105]. Given the beneficial effects of inhibiting ferroptosis in anti-NASH, this mechanism may represent a crucial pathway through which hepatic FXR activation ameliorates NASH and warrants further investigation[106]. GW4064 is a synthetic non-steroidal FXR agonist that has shown promise in preclinical studies for alleviating insulin resistance and fatty liver symptoms[107,108]. However, its potential toxicity and poor bioavailability have hindered further clinical application[109]. Another FXR agonist, OCA, was once considered one of the most promising candidates as the first FDA-approved drug for NASH[110,111]. Numerous preclinical studies demonstrate that OCA can ameliorate NASH-related symptoms such as hepatic steatosis, inflammation, liver injury, and fibrosis across various animal models[112–115]. Although OCA has shown promise in alleviating NASH in clinical studies[116–119], its potential side effects, such as pruritus, dyslipidemia, and hepatotoxicity, have resulted in a relatively low benefit-risk ratio[103,120], hampering its path to approval as an anti-NASH drug. Therefore, given the safety concerns related to full FXR activation, strategies involving partial FXR agonism or modulating endogenous BA levels to activate FXR might offer better prospects[63].

Research suggests that targeted inhibition of intestinal FXR proves beneficial for metabolic diseases. Teams led by Jiang and Gonzalez found that intestine-specific FXR knockout increased resistance to high-fat diet-induced obesity and insulin sensitivity impairment in mice[71]. Moreover, administering FXR antagonists like GUDCA, Tβ-MCA, and Gly-MCA alleviated NAFLD and diabetes symptoms[72,88,121,122]. Other findings by Jia's team showed that TUDCA, TCDCA, and HDCA attenuated hyperlipidemia or NAFLD symptoms through intestinal FXR inhibition[123,124], while HCA enhanced glucose homeostasis by dual effects of intestinal FXR inhibition and TGR5 activation[98]. The possible mechanism is that FXR inhibition reduced FGF15/19 signaling, enhancing hepatic cholesterol metabolism and fatty acid oxidation[77,123], while also reducing ceramide levels, thereby alleviating hepatic steatosis and cholesterol levels[88,125]. In addition, since FXR−FGF15/19 signaling mainly regulates the alternative pathway of BA biosynthesis, selectively inhibiting ileal FXR can alter the BA composition in the liver. This alteration increases the proportion of CDCA, subsequently leading to the activation of hepatic FXR[123]. Notably, in enteroendocrine L cells, FXR inhibition increased GLP-1 production by upregulating proglucagon expression and intracellular ATP levels[126]. Notably, in contrast to antagonizing intestinal FXR signaling, studies have found that the intestine-restricted FXR agonist Fexaramine can induce browning of white adipose tissue in HFD-fed mice and alleviate symptoms of obesity, insulin resistance, and hepatic fat accumulation[127]. However, further research has revealed that the beneficial effects of Fexaramine on metabolic homeostasis may not be directly related to the restricted activation of intestinal FXR. Fexaramine increases the abundance of intestinal *Acetatifactor* and *Bacteroides*, thereby elevating the concentration of intestinal LCA, stimulating the release of GLP-1 mediated by TGR5, and thus improving metabolism[128]. The benefits of Fexaramine can be reversed by antibiotic-mediated depletion of the gut flora.

In addition to regulating metabolic diseases related to glucose, lipids, and cholesterol, FXR has also been found to be associated with iron-related metabolic diseases. In adults with hyperferritinemia and children with β-thalassemia, serum FGF19 levels decrease while conjugated BA levels increase, indicating potential inhibition of FXR signaling[129]. Moreover, in *in vivo* and *in vitro* iron overload models, the use of GW4064 or overexpression of *Fxr* can ameliorate iron overload-induced hepatotoxicity. This suggests that there is further potential to explore the role of FXR in regulating metabolic diseases.

6.2.3. *Intestinal BA reabsorption inhibition*

Blocking the reabsorption of BAs in the ileum has been found beneficial to maintaining metabolic homeostasis. For example, using BA sequestrants can increase GLP-1 production by reducing BAs-stimulated FXR activation in enteroendocrine L cells[126]. Another example involves utilizing a high-fiber diet to sequester BAs, such as oat bran, thereby increasing the excretion of BAs and cholesterol in feces, consequently improving metabolism[130]. ASBT inhibition is a similar strategy. As reported, synthetic ASBT inhibitors alleviate NAFLD in mice through reduced ileal FXR-FGF15 signaling[131,132].

6.2.4. Gut microbiota regulation

There are conflicting perspectives on the regulation of secondary BA metabolism in metabolic diseases. Some studies indicate that the application of specific bacteria, *e.g.*, *Lactobacillus johnsonii* and *Bacteroides intestinalis* can enhance the production of secondary BAs (including LCA and DCA) in diet-induced obesity mice. These secondary BAs then stimulate TGR5 in brown adipose tissues, prompting increased energy expenditure and consequent reduction in the host's body weight[133].

Another perspective suggests that targeting BSH-related bacteria to increase the concentration of conjugated BAs in the gut and suppress intestinal FXR signaling could be beneficial in alleviating metabolic diseases. For instance, in a study, theabrownin decreased the abundance of BSH-related bacteria such as *Lactobacillus*, *Streptococcus*, and *Clostridium* in the feces of volunteers[123]. This led to an increase in the concentration of FXR antagonists (*e.g.*, GUDCA), suppressing intestinal FXR signaling and reducing serum FGF19 levels. Consequently, this activated hepatic BA synthesis, resulting in decreased blood lipid levels. Another example is metformin, which reduces the abundance of *Bacteroides fragilis* in the intestines of diabetic patients, thereby increasing intestinal levels of GUDCA, TUDCA, and others[72]. This leads to inhibition of FXR/FGF-19 signaling, thus alleviating symptoms. Additionally, tempol[71], caffeic acid phenethyl ester[134] and vancomycin[135], and others, have also demonstrated similar potential.

6.3. Autoimmune diseases (rheumatoid arthritis)

BAs have been found closely related to various autoimmune diseases, including PBC[136], type 1 diabetes[137], and rheumatoid arthritis[138], likely due to the influence of receptor-mediated BA signaling on immune cells. GDCA and TUDCA, through TGR5-mediated GATA binding protein 3 signaling, stimulate interleukin-22 secretion in type 3 innate lymphoid cells[139]. Interestingly, due to varying receptor affinity, secondary BAs exhibit more potent efficacy in immune regulation compared to primary BAs. For instance, TLCA and LCA, *via* TGR5/PKA signaling, inhibit NLRP3 inflammasome activation in macrophages, demonstrating greater potency than CA and CDCA[140]. In addition, microbial BAs like 3-*oxo*LCA, *isoallo*LCA, *iso*DCA and LCA modulate the differentiation of Th17 cells and Treg cells by interacting with VDR, RORγt, and FXR[141−144].

Recent work has uncovered a link between microbial BA metabolism, immune regulation, and RA symptoms[2]. In this work, *Parabacteroides distasonis* was found to display a negative correlation with Disease Activity Score-28 in rheumatoid arthritis patients. *P. distasonis* converted primary BAs into secondary BAs, including LCA, DCA, *iso*LCA, and 3-*oxo*-LCA, which were found to directly inhibit Th17 differentiation, and promote macrophage M2 polarization by activating TGR5, synergistically alleviating symptoms.

6.4. Intestinal damage, inflammatory bowel diseases (IBD) and colorectal cancer (CRC)

The role of BA signaling in intestinal physiology has received significant attention in past decades, as it has been found to be closely associated with gut barrier function, intestinal inflammation, and tumorigenesis[145]. Secondary BAs are generally considered to damage the intestinal barrier, especially DCA. Studies have shown that DCA reduces the expression of tight junction proteins in the mouse intestine and decreases the number of Paneth cells and goblet cells[146,147]. This destructive effect may be associated with the activation of epidermal growth factor receptor (EGFR) or ERK1/2[146,148], but the specific details remain unclear. Recently, it has been reported that the abundance of *Parabacteroides goldsteinii* in the feces of volunteers taking aspirin decreased; this bacterium produces 7-*oxo*-LCA through 7αHSDH, which has been shown to antagonize intestinal FXR, promoting Wingless/integrated (Wnt) signaling in intestinal stem cells, maintaining their stemness, and ultimately reversing aspirin-induced intestinal damage[149]. Additionally, research has found that the FXR agonist OCA improves tight junctions in the mouse intestine and increases the number of goblet cells[150]; however, considering OCA activates both intestinal and hepatic FXR, it may also activate hepatic FXR, reducing BA synthesis and thus alleviating intestinal damage. Therefore, further research is needed to better understand the role of FXR signaling in intestinal damage.

IBD primarily includes ulcerative colitis (UC), Crohn's disease, and others. Disruption of BA homeostasis has been observed in fecal samples of IBD patients. For instance, a study found that levels of secondary BAs such as LCA and DCA were decreased in the feces of active IBD patients compared to healthy individuals, while the concentration of 3-OH-sulphated BA was elevated[151]. Similarly, concentrations of LCA and DCA in feces from ileal pouches of UC patients were lower compared to control patients[152]. Although secondary BAs are generally considered to be intestinal toxicants[145,153,154], studies have found that they alleviate intestinal inflammation in dextran sulfate sodium (DSS)-induced colitis mice by stimulating TGR5 signaling in immune cells[152]. Additionally, research has shown that TGR5 signaling in intestinal stem cells promotes epithelial cell regeneration, and mice with intestinal stem cell TGR5 deficiency experienced exacerbated colitis symptoms when induced with DSS[155]. Two other secondary BAs, 3-*oxo*LCA and *iso*LCA, have recently been found to be decreased in IBD patients. They can alleviate symptoms by inhibiting RORγT to suppress Th17 cell differentiation, thus relieving symptoms[144]. FXR signaling has also been found beneficial in ameliorating IBD. Immunohistochemical scoring results indicated a significant negative correlation between colonic FXR expression in patients and the severity of UC[156]. DCA has been reported to inhibit intestinal FXR signaling in mice, inducing colonic inflammation, while the intestine-restricted FXR agonist Fexaramine reverses this effect[153]. OCA reduces colitis symptoms induced by DSS or 2,4,6-trinitrobenzenesulfonic acid (TNBS) in wild-type mice, but this effect is lost in FXR knockout mice[150]. FXR signaling may alleviate colitis by blocking the induction of IL17 in innate lymphoid cells (ILCs)[150]. Therefore, the role of BA homeostasis, especially the levels of secondary BAs, in IBD warrants further investigation.

According to immunohistochemical evaluations, the expression of FXR in tumors of CRC patients is significantly lower than that in surrounding tissues, suggesting that this nuclear receptor may be associated with the prognosis of CRC[157,158]. This phenomenon may be attributed to DNA methylation and KRAS signaling[159]. FXR deficiency promotes the occurrence of CRC in mice[160,161]; meanwhile, HFD can elevate the levels of Tβ-MCA and DCA in *Apc*$^{min/+}$ mice, antagonize intestinal FXR, and finally promote CRC[68]. Further research has found that the antagonistic effects of Tβ-MCA and DCA on FXR, together with *Apc* gene (encoding adenomatous polyposis coli protein) mutation, negatively regulate Wnt signaling, inducing malignant transformation of Lgr5^{+} tumor stem cells[68]. Conversely, the administration of

intestine-restricted FXR agonist Fexaramine can reverse this process[68]. In addition to FXR, the involvement of TGR5 in the occurrence and progression of colorectal cancer (CRC) is plausible. Elevated expression of BSH in fragile Bacteroides enhances the levels of LCA and DCA in the colon of *Cdx2Apc*$^{f/w}$ mice[162]. These two BAs, by activating TGR5, stimulate the β-catenin/CCL28 axis, inducing an increase in the levels of immunosuppressive T reg cells within the tumor, ultimately accelerating CRC progression[162]. The above process can be reversed by inhibiting BSH. Furthermore, dysbiosis of the intestinal microbiota in mice exposed to cigarette smoke leads to an increase in TDCA concentration, which activates the MAPK/ERK pathway, thereby promoting CRC[163].

6.5. *Hepatocellular carcinoma (HCC)*

The relationship between HCC and BAs has been revealed in recent years[164]. In clinical cohorts and animal experiments, it has been demonstrated that the BA profile of patients or animals with HCC can undergo alterations[165-169]. BAs are involved in the occurrence of HCC, and different BAs have different effects on HCC[164]. TCA can promote IL4-induced M2-like macrophage polarization, creating an immunosuppressive tumor microenvironment conducive to tumor development in HCC[170]. The mixture of CA, β-MCA, and TCA can enhance the stemness and expression of inflammatory factors in tumor-initiated stem-like cells and primary mouse hepatocytes[169]. Additionally, UDCA, CDCA, LCA, DCA, GCDCA, TCDCA, and GDCA have also been found to play roles in either promoting or inhibiting HCC[164,171]. BA signaling is also related to HCC. Whole-body FXR knockout, rather than hepatocyte-specific FXR deficiency, leads to spontaneous HCC, whereas liver-specific hepatic deficiency serves as an initiator of HCC[172−174]. Using BA sequestrant colesevelam can reduce the size of the total BA pool, thereby alleviating HCC[170,174,175].

7. Role of botanical triterpenoids and steroids in BA metabolism, transport and signaling

7.1. *Regulating BA metabolism*

7.1.1. *Regulating primary BA biosynthesis*

Primary BA biosynthesis directly affects the balance of cholesterol and BA levels in the body. Many botanicals abundant in triterpenoids, such as *Astragalus membranaceus*[176], *Alisma orientale*[177], *Panax ginseng*[178], *Poria cocos*[179], etc., demonstrated the ability to regulate the expression of BA metabolism enzymes in disease animal models[180,181]. Some phytosterols, on the contrary, promote excessive hepatic BA synthesis, thus causing IHC[182,183]. However, little work has focused on the direct regulatory effects of these natural products on the activities of BA synthases, most studies have primarily showcased their ability to modulate enzyme expression *in vitro* and *in vivo*. Remarkably, most of these botanical triterpenoids and steroids serve as FXR ligands to regulate primary BA biosynthesis through FXR/SHP or FXR-FGF15/19 signaling, which will be elaborated in the following sections.

7.1.2. *Modulating secondary BA metabolism*

Triterpenoid and steroidal saponins are known for their characteristic low bioavailability and, when orally administered, distribute more widely throughout the gastrointestinal tract, offering increased opportunities for interactions with gut microbes[184]. Many saponins or sapogenins have been found to regulate intestinal flora, thereby influencing secondary bile acid metabolism (Fig. 5). Astragaloside IV (**1**), the most representative active compound in *Astragalus membranaceus*, has been observed to decrease the proportions of BSH-expressing flora, including *Enterococcus*, *Streptococcus*, *Lactobacillus*, and *Lactococcus*, in HFD-fed mice. This consequently leads to an increase in ileal Tβ-MCA content, inhibiting intestinal FXR/FGF15 signaling, thus alleviating hepatic steatosis[185]. Fecal microbiota transplantation (FMT) experiments have confirmed this action of astragaloside IV (**1**) is gut microbiota-dependent. Soyasaponin A2 (**2**), another triterpenoid saponin, sourced from the *Fabaceae*, reduces *Faecalibaculum* and *Lactobacillus* populations in NASH mice subjected to a methionine and choline-deficient (MCD) diet, thus elevating THDCA levels to ameliorate NASH symptoms[186]. Dioscin (**3**) is a spirostane saponin found in *Dioscoreaceae* botanicals, which demonstrated protective effects against chemical injury-induced Parkinson's disease in mice. Likewise, this saponin reduces the intestinal abundance of *Lactobacillus*, *Enterococcus*, *Streptococcus*, inhibiting deconjugation of TBAs, consequently regulating BA signaling to intervene in the disease[187].

In addition, sapogenins and secondary saponins also exhibit regulatory effects on secondary BA metabolism. For instance, diosgenin (**4**), the sapogenin of dioscin, regulates the abundance of *Clostridia* in mice induced with NASH *via* an MCD diet. This action, in turn, modulates BA-mediated activation of hepatic and intestinal FXR signaling pathways[188]. 2α-OH-Protopanoxadiol (**5**), a triterpenoid sapogenin derived from *Gynostemma yixingense*, has been found to decrease the abundance of *Ruminiclostridium* and *Desulfovibrio* in mice with metabolic syndrome. Likewise, this effect is associated with a reduction in the hydrolysis of conjugated BAs within the intestine, resulting in the inhibition of ileal FXR signaling, ultimately leading to increased GLP-1 release and improved insulin sensitivity[189]. Ginsenoside C-K (**6**), a secondary saponin converted from protopanaxadiol ginsenosides by gut microbes, has been shown to reverse gut microbiota dysbiosis in *db/db* mice, increasing intestinal levels of secondary BAs (like LCA and DCA), consequently attenuating symptoms of diabetes through the TGR5/GLP-1 pathway[190].

However, the mechanism by which saponins regulate these gut microbes involved in BA metabolism remains unclear, though there is a possible link to their anti-biofilm potential. Saponins are generally acknowledged as molecules that serve a defensive function in plants, because of their amphipathic properties, which give them surfactant activity, disrupting the integrity of microbial membrane systems and resulting in cellular collapse[7]. As has been reported, ginsenosides can inhibit the formation of bacterial cell envelope at a certain concentration and, at the minimal bactericidal concentration (MBC), can penetrate biofilms to kill bacteria[191]. Moreover, the antibacterial effectiveness of ginsenosides is linked to their hydrophile−lipophile balance number, where saponins with a single glycosyl group demonstrate greater antibacterial activity compared to those with two or more glycosyl groups. This trend may be attributed to the enhanced affinity of less polar saponins for microbial cell membranes[191]. Notably, several investigations have indicated that saponins exert more pronounced inhibitory effects on Gram-positive bacteria than on Gram-negative bacteria[7,192−195]. This phenomenon could potentially elucidate why Gram-positive enteric bacteria, such as *Enterococcus*, *Streptococcus*, and *Lactobacillus*, are more

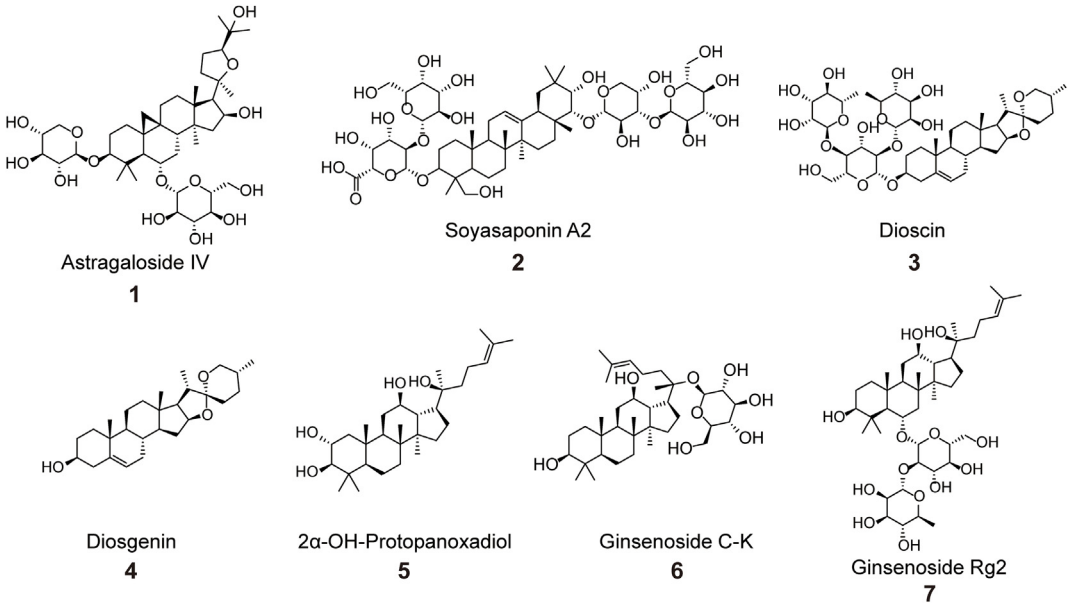

Figure 5 Botanical triterpenoids and steroids that regulate gut microbes-based BA metabolism.

sensitive to saponin intervention in aforementioned studies, although further verifications are required.

Recently, a pattern of saponin-induced up-regulation in BA-metabolizing bacteria has been discovered[2]. In this research, *P. distasonis* alleviated symptoms of RA mice through secondary BAs-mediated immunoregulation. Interestingly, saponins could function as natural modulators of *P. distasonis* through *in vitro* co-incubation experiments. Among the 112 saponins tested, ginsenoside Rg2 (**7**) showed the most potent effect. It significantly promoted the growth of *P. distasonis* both in *in vitro* and *in vivo* settings, although the mechanism remains elusive. One possible explanation is that, within a certain concentration range, saponins may enhance the permeability of bacterial membranes rather than disrupting them, thereby facilitating the influx of nutrients into the bacteria[196]. Hence, the regulatory effect of saponins on the abundance of BA-metabolizing bacteria may be connected to their concentrations.

7.2. *Regulating BA transport*

Many botanical triterpenoids and steroids have been documented for their capacity to regulate the spatial distribution of BAs *in vivo* by modulating BA transporters, consequently inducing or alleviating cholestasis[178,182,197–201]. While some of these natural products regulate the expression of BA transporters *via* FXR signaling, a detailed discussion of which will follow in subsequent sections, others directly influence the function of these transporters (Fig. 6). This section focuses on elucidating the direct action of these compounds on the transporters.

7.2.1. *Acting on ASBT, NTCP and OATPs*

Triterpene acids isolated from *Poria cocos* have been identified as competitive inhibitors of ASBT and NTCP. Cai et al.[202] conducted an investigation by introducing plasmids carrying the human *SLC10A1* and *SLC10A2* genes into *Xenopus laevis* oocytes to assess the impact of triterpene acids on the transmembrane transport efficiency of a fluorescent derivative of BA. The research demonstrated that polyporenic acid C (**8**) and dehydrotumulosic acid (**9**) inhibit NTCP and ASBT, respectively, while poricoic acid A (**10**) and poricoic acid B (**11**) exhibited inhibitory effects on both of these two transporters. Researchers proposed that the inhibition of BA transporters by triterpene acids might contribute to the hypolipidemic effects associated with this fungus.

Compared to ASBT and NTCP, OATPs exhibit wide substrate specificity. Through a high-throughput *in vitro* assay for OATP1Bs inhibition, various steroids like hecogenin (**12**), smilagenin (**13**), and triterpenoids such as euphol acetate (**14**), hederagenin (**15**), madecassic acid (**16**), echinocystic acid (**17**), chrysanthellin (**18**) and isogedunin (**19**) were identified as inhibitors of human OATP1B1[203]. Additional research found betulinic acid (**20**) as a rat OATP1Bs inhibitor[204], and glycyrrhetinic acid (**21**) served as a human OATP1B1 transport inhibitor[205]. Notably, some phytochemicals can be transported by OATPs, while others only possess inhibitory properties without being subject to transport. For example, timosaponin B2 (**22**), a furostane-type saponin from *Anemarrhena asphodeloides*, is taken up by hepatocytes mainly *via* OATP1B[206], whereas ginsenoside Rb1 (**23**), Rc (**24**), and Rd (**25**) are not transported by OATP1Bs but significantly inhibit substrate transport mediated by it[207].

7.2.2. *Acting on MRPs and BSEP*

MRP2 and BSEP mediate the flux of BAs from the liver into the bile duct. According to the research conducted by Jiang et al.[207], 20(*S*)-protopanaxatriol-type ginsenosides, including ginsenoside Rg1 (**26**), Re (**27**) and R1 (**28**), rather than 20(*S*)-protopanaxadiol-type ginsenosides, can be transported by BSEP and MRP2, rapidly eliminated through hepatobiliary excretion.

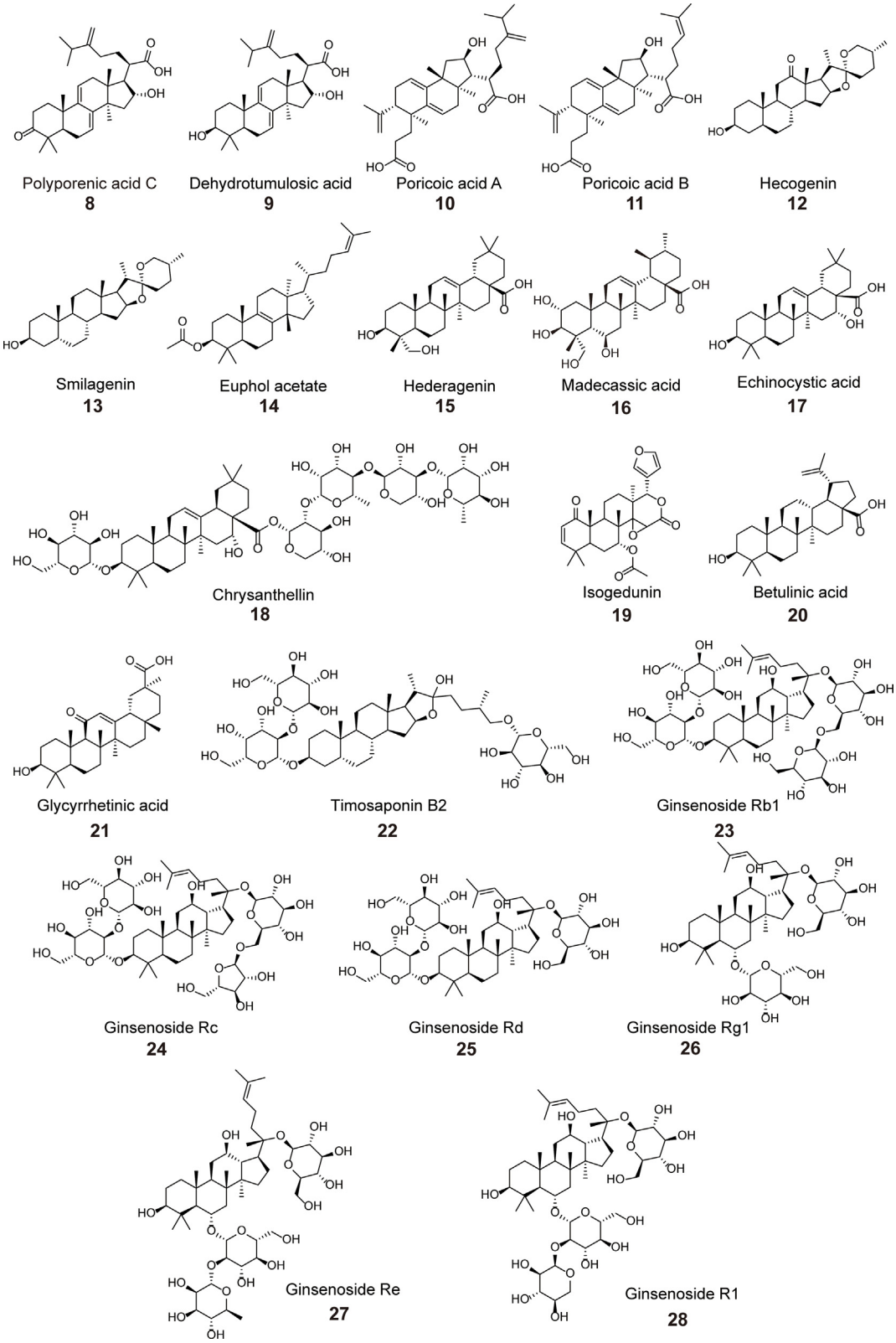

Figure 6 Botanical triterpenoids and steroids that act on BA transporters.

Similarly, timosaponin B2 (**22**) is transported by MRP2, causing its high biliary excretion index[206].

Researching the interactions between phytosteroids/triterpenoids and BA transporters is vital for comprehending their ADME (absorption, distribution, metabolism, and excretion) processes and potential drug−drug interactions. However, there is a lack of studies focused on assessing the impacts of these phytochemicals on BA transport under physiological or pathological conditions. Can

inhibitors targeting BA uptake transporters lessen the influx of BAs, consequently reducing hepatic and ileal FXR activity? Do phytochemicals that are carried by BA transporters affect the regular EHC process? Numerous questions await comprehensive answers.

7.3. *Regulating BA signaling*

7.3.1. *Activating TGR5*

Some pentacyclic triterpene acids have been identified as natural TGR5 agonists based on TGR5-dependent reporter gene assay (Fig. 7). In a study conducted by Genet et al.[208], it was found that, although the maximum agonistic potency was weaker than that of LCA (eff. = 100%), betulinic acid (**20**, EC_{50} = 1.04 μmol/L, eff. = 83%), oleanolic acid (**29**, EC_{50} = 2.25 μmol/L, eff. = 72%), and ursolic acid (**30**, EC_{50} = 1.43 μmol/L, eff. = 65%) exhibited a lower half-maximal effective concentration on TGR5 compared to LCA (EC_{50} = 5.60 μmol/L), as assessed by luciferase reporter assay. Furthermore, through various chemical modifications involving oxidation, esterification, oximation of the hydroxyl group, and the conversion of the carboxyl group into ester, amide, and urea, researchers elucidated the critical role of the hydroxyl group at C-3 and the carboxyl group at C-17 in enabling triterpene acids to activate TGR5. Therefore, compared to betulinic acid (**20**), betulonic acid (**31**) and betulin (**32**) had shown almost no TGR5 agonistic ability. In addition to hydron donors, hydrophobic interactions and steric effects also proved significant. The presence of additional hydroxyls at the α and β positions around C-3 would hinder molecular binding to the receptor, explaining the lack of activity in arjunic acid (**33**) and asiatic acid (**34**).

The TGR5 activation capacity offers insights into the pharmacological mode of action of some triterpene-rich botanicals. Allspice (*Pimenta dioica*) and clove (*Syzygium aromaticum*) contain numerous triterpene acids, such as ursolic acid (**30**), oleanolic acid (**29**), maslinic acid (**35**) and corosolic acid (**36**), activating TGR5 to alleviate metabolic diseases[209]. Also, ursolic acid (**30**) mediates the improvement of insulin sensitivity induced by guayusa (*Ilex guayusa*) and maté (*Ilex paraguariensis*) by stimulating intestinal TGR5 to induce GLP-1 release[210]. In addition, nomilin (**37**) and obacunone (**38**), two naturally occurring lemonoids (highly oxygenated triterpenoids), play a role in mediating the anti-obesity effects of *Citrus* botanicals by activating TGR5. Research indicates that these two compounds exhibit TGR5 agonistic effects in luciferase assays, without affecting FXR activities[211]. In contrast, limonin (**39**), another abundant lemonoid in *Citrus*, did not demonstrate any such activity. Interestingly, as previously mentioned, ginsenoside C-K (**6**) modulate secondary BA metabolism and indirectly trigger TGR5 activation[190]. While in a separate study, it was identified as a direct agonist of TGR5, leading to an increase in intestinal GLP-1 release[212]. This dual effect could represent a significant mechanism through which ginseng facilitates the amelioration of metabolic diseases.

Furthermore, natural triterpenoids have the potential for direct use in disease management. For example, oleanolic acid (**29**) can promote intestinal peristalsis and ameliorate constipation by activating colonic TGR5[213]. Similarly, betulinic acid (**20**) demonstrates its potential to reduce neuroinflammation during hepatic encephalopathy through TGR5 signaling in the frontal cortex[214]. Glycyrrhizic acid (**40**) increases GLP-1 release in L cells through TGR5-mediated increase of intracellular calcium and cAMP levels, thus playing a role in reducing symptoms of diabetes[215].

7.3.2. *Modulating FXR*

FXR was initially identified as a nuclear orphan receptor activated by farnesol, a sesquiterpene alcohol, before the discovery that its endogenous ligands were BAs. Subsequently, through luciferase

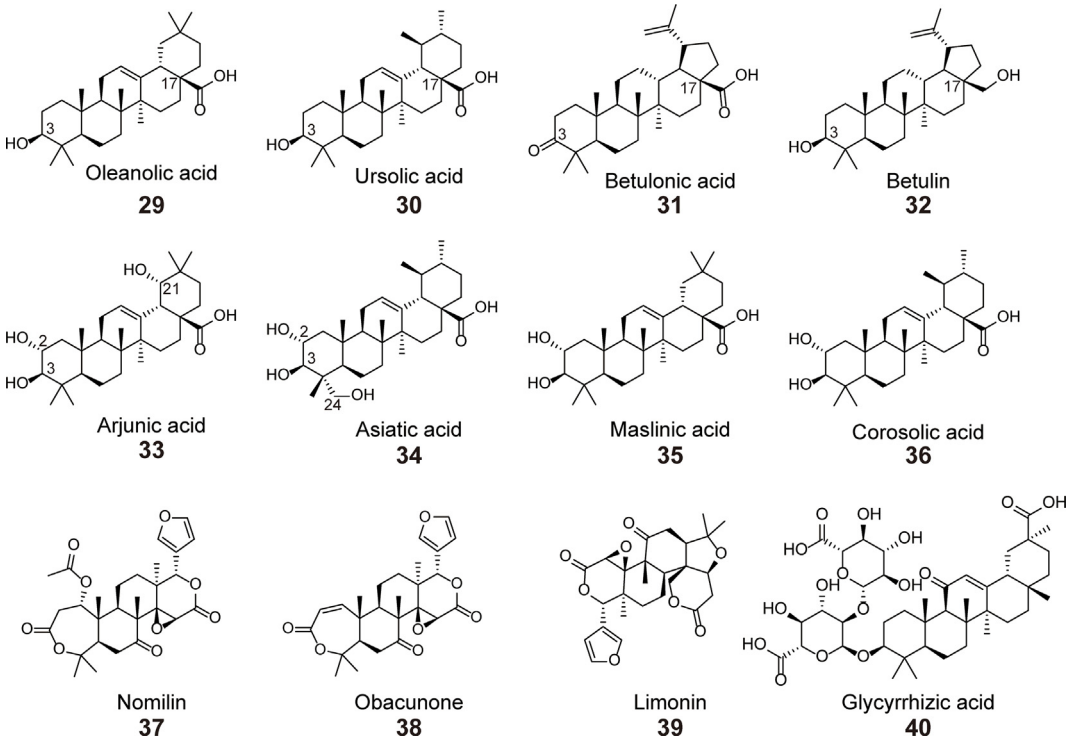

Figure 7 Botanical triterpenoids and steroids in regulation of TGR5 activities.

reporter assay, various nature-source triterpenols and sterols were also recognized as FXR activators (Fig. 8). *Ganoderma lucidum*, a traditional Chinese herbal medicine known for its immune and metabolic regulatory properties, was found to contain five lanostane-type triterpenols and sterols capable of activating FXR in a concentration-dependent manner. Notably, ergosterol peroxide (41, EC_{50} = 0.85 μmol/L), ganodermanontriol (**42**, EC_{50} = 2.5 μmol/L), and ganoderiol F (**43**, EC_{50} = 5.0 μmol/L)

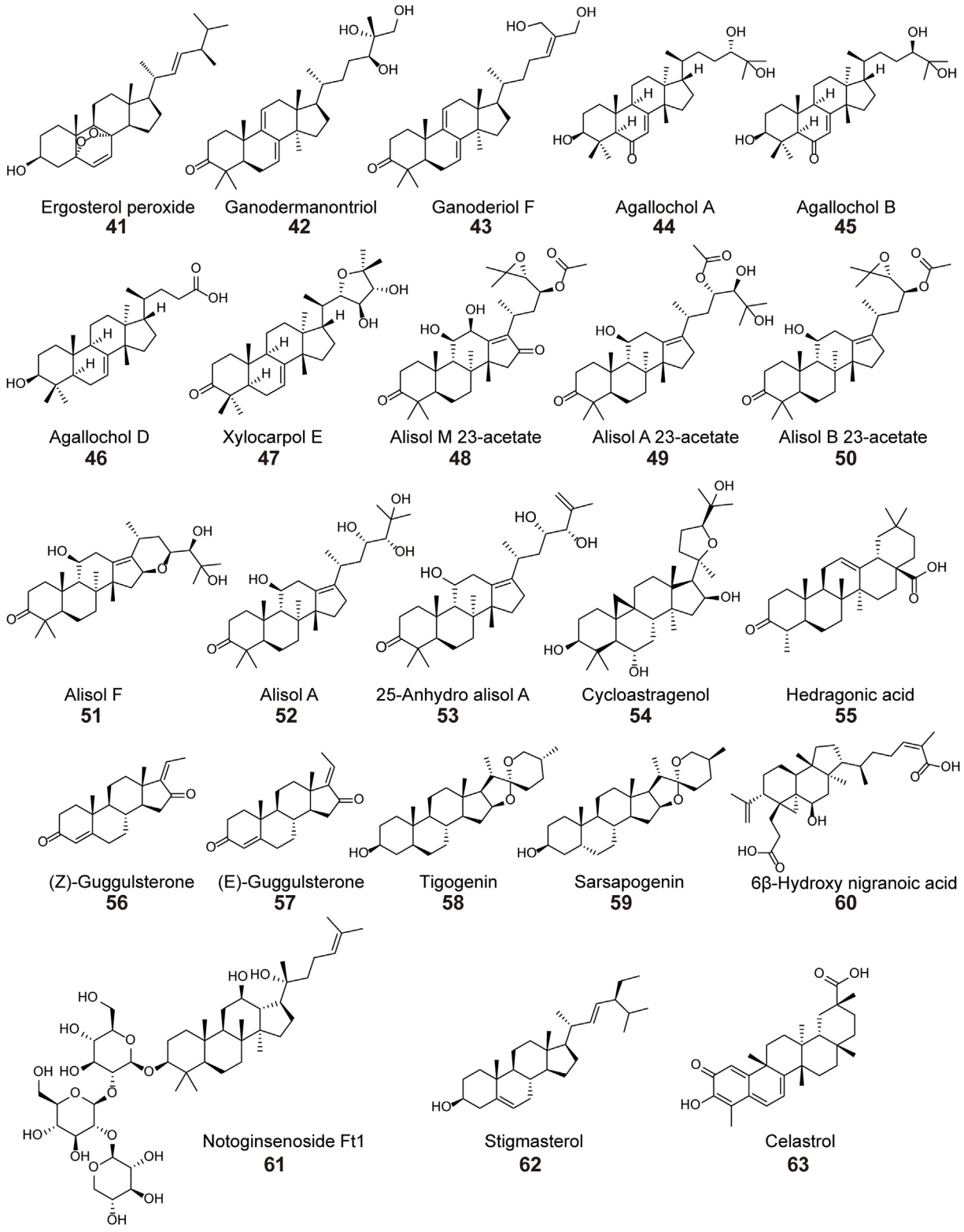

Figure 8 Botanical triterpenoids and steroids in regulation of FXR activities.

exhibited agonist potency surpassing that of CDCA (EC_{50} = 16.8 μmol/L)[216]. Another category of FXR activators is the tirucallane-type triterpenols, as reported by Jiang et al. Compounds such as agallochol A (**44**), B (**45**), D (**46**) and xylocarpol E (**47**), isolated from *Xylocarpus granatum*, *Xylocarpus moluccensis* and *Excoecaria agallocha*, markedly activated FXR at a concentration of 10 μmol/L[217]. In addition, a series of protostane-type triterpenols obtained from Genus *Alisma*, such as alisol M 23-acetate (**48**), alisol A 23-acetate (**49**), alisol B 23-acetate (**50**), alisol F (**51**), alisol A (**52**), 25-anhydro alisol A (**53**), etc., activated hepatic FXR, inhibiting *de novo* lipogenesis to alleviate lipid accumulation, or decreasing *CYP7A1* expression to attenuate cholestasis[177,180,218,219]. Cycloastragenol (**54**), a cycloartane-type triterpenol, serves as the aglycone for most astragalus saponins. According to the findings of Gu et al.[220], it was also an activator of FXR, contributing to improved hepatic fatty acid oxidation and a reduction in BA synthesis in mice with NAFLD. Interestingly, this compound not only exhibits directly in the roots of *Astragalus membranaceus*, but also represents the main gut flora metabolite of astragalus saponins. This may account for the observed regulatory effect of astragalus saponins on hepatic FXR signaling in cholestatic liver fibrosis models[176].

Besides triterpenols and sterols, some triterpene acids and triterpenoid saponins also function as FXR agonist ligands (Fig. 8). Hedragonic acid (**55**), an oleanane-type triterpene acid sourced from *Celastrus orbiculatus*, activates FXR rather than TGR5, which differs from the effect of oleanolic acid (**29**)[208,221]. These differing effects between the two compounds may be attributed to variations in their structures at the C-3 and C-4 positions[221]. Glycyrrhetinic acid (**21**), another oleanane-type triterpene acid, increases FXR transcription-mediated expression of the luciferase reporter gene in the presence of CDCA but is ineffective when CDCA is absence[222]. Since it is the main absorbed form of orally administered glycyrrhizic acid (**40**) after being metabolized by intestinal bacteria, this mechanism provides valuable insights into the specific aspects of licorice's protective effects on cholestatic liver injury.

Some botanical triterpenoids and steroids are identified as FXR antagonists (Fig. 8). (*Z*)-Guggulsterone (**56**) and (*E*)-guggulsterone (**57**), sterones obtained from gugal, a gum resin of *Commiphora mukul*, were among the earliest phytochemicals recognized as FXR antagonist ligands, determined through luciferase reporter assay and FRET-based coactivator binding assay. They triggered BA biosynthesis, resulting in reduced hepatic cholesterol in mice. Notably, this effect could be eliminated by FXR knockout. Interestingly, these two sterones inhibited the activation of FXR caused by agonists, rather than directly affecting FXR activity itself[223]. Dioscin (**3**) has been shown to alleviate hyperuricemia-caused atherosclerosis in mice by blocking hepatic FXR signaling[224]. In a study conducted by Chen and colleagues, tigogenin (**58**), a gut flora metabolite of dioscin (**3**), was identified as an FXR antagonist through a coactivator recruitment assay, mediating the aforementioned effect, while dioscin itself, alongside other metabolites such as diosgenin (**4**) and sarsapogenin (**59**), did not demonstrate any inhibitory effect on FXR[224].

In addition to steroids, triterpene acids, exemplified by oleanolic acid (**29**), represent another category of FXR antagonists. In the study by Genet et al., oleanolic acid (**29**) was identified as an effective TGR5 agonist but didn't have a direct effect on FXR activity, as demonstrated by luciferase reporter assay[208]. However, further studies showed that it could block the interaction between FXR LBD and SRC-3, thereby repressing CDCA-induced expression of FXR target genes[225,226]. Moreover, nortriterpenoids and lanostane-type triterpenoids derived from *Schisandra glaucescens* exhibited an antagonistic effect against FXR. Among them, 6β-hydroxy nigranoic acid (**60**, IC_{50} = 1.50 μmol/L) was the most potent antagonist[227,228]. Recently, Ding et al.[229] reported that notoginsenoside Ft1 (**61**), a dammarane-type triterpenoid saponin found in the traditional Chinese herbal medicine Radix Notoginseng (roots of *Panax notoginseng*), acted as an agonist of TGR5 and an antagonist of FXR. Since saponins exhibit a broader distribution within the digestive tract after oral administration, notoginsenoside Ft1 activated ileal TGR5, stimulating GLP-1 secretion. Simultaneously, it inhibited ileal FXR/FGF15 signaling, leading to elevated serum BA levels, activating TGR5 in adipose tissue and enhancing energy metabolism. This dual action finally alleviates insulin resistance and obesity in HFD mice[229].

It should be noted that the regulatory effects on FXR have not only revealed the pharmacological mechanisms of some botanical products rich in steroids and triterpenoids, but have also contributed to our understanding of their toxicological impacts. Parenteral nutrition lipid solutions derived from soy can sometimes cause liver injury in infants. One possible explanation lies in the FXR-inhibiting properties of stigmasterol (**62**), a phytosterol found in soy lipids. This compound has been shown to suppress CDCA-activated, FXR-dependent reporter gene expression in HepG2 cells. It also reduces the gene expression of hepatic BA transport-related proteins downstream of FXR signaling both *in vitro* and *in vivo*, ultimately leading to cholestasis[182,230] (Fig. 9A). *Tripterygium wilfordii* root is a traditional Chinese herbal medicine known for its potent immunosuppressive functions, commonly used in the treatment of conditions such as rheumatoid arthritis, lupus erythematosus, Behcet's disease, and psoriasis. However, its widespread use is limited due to severe gastrointestinal, liver, and renal toxicity. Celastrol (**63**) and triptolide have been identified as significant contributors to these toxic effects. Celastrol (**63**) is a pentacyclic nortriterpen quinone, recognized as an FXR antagonist through luciferase reporter assays and the determination of FXR-targeted gene expression in small intestinal organoids. By inhibiting intestinal FXR, it leads to increased JNK phosphorylation, thus exacerbating triptolide-induced intestinal bleeding[231].

Some botanical steroids and triterpenoids have been observed to play a role in disease intervention as regulators of FXR expression, rather than acting as a ligand to play an agonistic or antagonistic role. For instance, ginsenoside Rg1 (**26**) has been shown to upregulate FXR expression in rats treated with α-naphthylisothiocyanate, thereby mitigating cholestasis[232]. Similarly, diosgenin (**4**) has been found to increase hepatic FXR expression while reducing ileal FXR levels in rats with hypercholesterolemia[233]. It has also been reported to mitigate fatty liver in rats fed a high-fat diet through hepatic FXR/SHP signaling[234]. However, in the study conducted by Bao and colleagues, it did not show an affinity for FXR[224]. Due to the absence of direct evidence of ligand−receptor interaction, it is possible that these phytochemicals indirectly modulate FXR

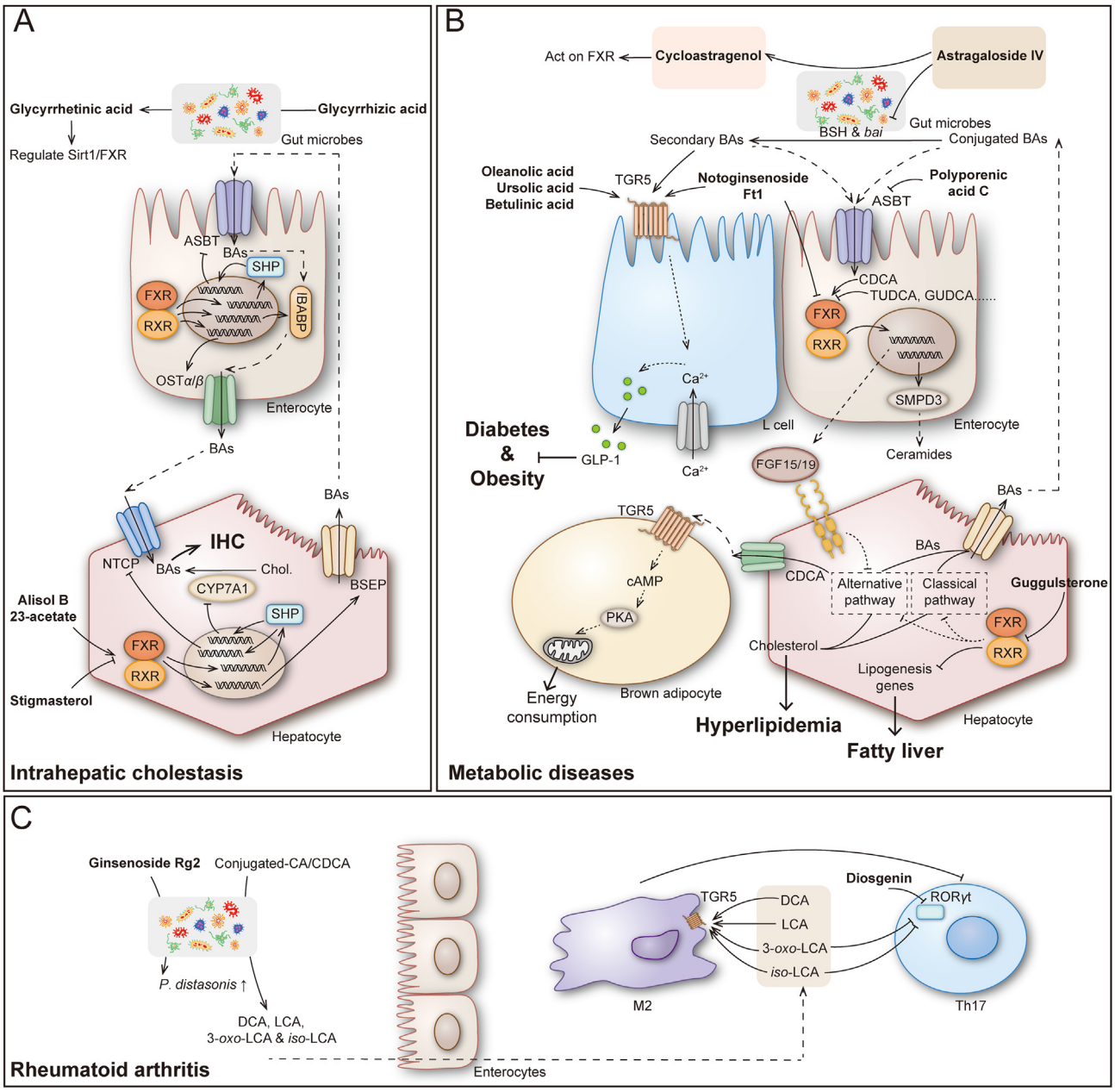

Figure 9 An overview of the roles of triterpenes and steroids from medicinal plants in IHC (A), metabolic diseases (B), and rheumatoid arthritis (C).

signaling through indirect pathways[188,235], necessitating further experimental confirmation.

8. Botanicals rich in triterpenes and steroids treat diseases by regulating BA metabolism, transport, and signaling

8.1. IHC

Licorice is a traditional hepatoprotective herbal medicine, and its triterpenoids have been found beneficial in the treatment of IHC. Glycyrrhetinic acid, as an aglycone, is the main form of triterpenoids absorbed after oral administration of licorice. In models of cholestasis induced by α-naphthylisothiocyanate (ANIT), glycyrrhetinic acid has been observed to alleviate symptoms while increasing the expression of hepatic FXR and its downstream transporters such as BSEP and MRP2[222,236]. Additionally, *in vitro* experiments have demonstrated that the inhibition of Sirtuin 1 can reverse the upregulation of FXR expression induced by glycyrrhetinic acid[222]. Therefore, glycyrrhetinic acid may regulate Sirt1/FXR-mediated BA transport, which could be an important mechanism by which licorice mitigates IHC (Fig. 9A).

The rhizome of *Alisma orientale* is a traditional Chinese medicine used clinically for diseases such as cholestasis, hyperlipidemia, and fatty liver[237]. This herbal remedy is rich in over 80 triterpenoid compounds, most of which are protostane-type triterpenols. In a rat model of cholestasis induced by ANIT, *Alisma orientale* extract has been shown to reduce the expression of

CYP7A1, thereby alleviating symptoms of intrahepatic cholestasis (IHC)[177]. Triterpenols such as alisol B 23-acetate (**50**), alisol F (**51**), alisol A (**52**) and 25-anhydro alisol A (**53**) act as FXR agonist[177,218], inhibiting BA synthesis and uptake in hepatocytes, increasing BA excretion into the bile canaliculus lumen, which may lead to alleviation IHC symptoms. In ANIT-induced IHC mice, alisol B 23-acetate (**50**) has been found to downregulate the expression of CYP7A1, CYP8B1 and NTCP, while upregulating the expression of BSEP, thus alleviating liver injury[238] (Fig. 9A). The effect can be reversed by FXR antagonist.

8.2. *Metabolic diseases*

Gynostemma pentaphyllum herbs and their total saponin (gypenosides) extracts are commonly used clinically for treating diabetes, hyperlipidemia and other metabolic diseases[239,240]. Modern clinical trials have confirmed that *Gynostemma pentaphyllum* extract can alleviate obesity[241,242], insulin resistance[243], and high blood glucose levels[244] in patients. In HFD-fed mice, gypenosides extract can ameliorate metabolic disorder symptoms, upregulating hepatic FXR expression and activating FXR signaling[245]. Similarly, in a mouse model of NASH induced by a Western diet, gypenosides extract upregulated hepatic expression of FXR and SHP proteins, and downregulated the expression of downstream proteins involved in de novo lipogenesis, such as SREBP1-c and FASN[246]. And in *Fxr*$^{-/-}$ mice, the anti-NASH effect of gypenosides was eliminated[246]. However, there is a lack of direct evidence indicating that any specific gypenoside serves as a ligand for FXR, and the mechanisms by which gypenosides regulate FXR protein expression also remain unclear. Further research is needed to investigate the mechanism by which gypenosides extract regulates hepatic FXR signaling in metabolic diseases.

Astragalus membranaceus root is a botanical medicine rich in triterpenoid saponins, clinically used for metabolic diseases[247]. In animal models induced by a high-fat diet, astragaloside IV (**1**) and cycloastragenol (**54**) have both been found to alleviate symptoms associated with fatty liver, although their mechanisms of action differ[185,220]. Astragaloside IV (**1**) primarily regulates BSH-related bacteria, altering the composition of intestinal BAs, thereby inhibiting intestinal FXR−FGF15 signaling indirectly[185]; whereas cycloastragenol (**54**) has been identified as an FXR ligand, activating hepatic FXR signaling[220] (Fig. 9B). Despite higher concentrations of saponins in astragalus extract, cycloastragenol (**54**) is the main metabolite formed *in vivo* following oral administration of astragalus extract. Further research is needed to investigate the comprehensive effects of astragalus extract on FXR signaling in preclinical models.

Reishi mushroom (*Ganoderma lucidum*) is a botanical medicine known for its ability to lower blood lipid and glucose levels[248]. A clinical trial demonstrated that Reishi extract alleviated symptoms of hyperlipidemia and insulin resistance in patients[249]. In HFD-fed mice, its derivative triterpene acids were found to modulate the composition of gut microbiota, potentially regulating secondary BA metabolism[250]. Moreover, ganoderiols and ergosterol derivatives, contained in this mushroom, have been identified as ligands for FXR; however, their agonistic effects have yet to be confirmed in preclinical models[216].

Ginseng is an ancient herbal remedy that has been found beneficial for metabolic diseases in recent clinical research. Ginseng extracts, rich in ginsenosides, can reduce weight and BMI in obese volunteers[251,252], and lower fasting insulin levels in type 2 diabetes patients[253,254], mitigating insulin resistance symptoms. In HFD-induced obese mice, ginseng extract increased serum GLP-1 levels and alleviated obesity-related metabolic symptoms; however, intestinal TGR5 restricted knockout abolished this effect, indicating that ginseng extract may promote GLP-1 release by stimulating intestinal TGR5 signaling[255]. Ginsenoside Ro, an oleanane-type saponin, similar to ginseng extract, promotes GLP-1 secretion in mice with HFD-induced metabolic syndrome, but has no effect in TGR5 knockout mice[256]. While ginsenoside C-K directly activates TGR5/GLP-1 signaling in enteroendocrine cells[212]. Additionally, it has been shown to regulate intestinal BA secondary metabolism in *db*/*db* mice, increasing the abundance of secondary BAs and indirectly activating TGR5 signaling[190].

Besides IHC, the rhizome of *Alisma orientale* is also commonly utilized in the treatment of metabolic diseases[237,240]. In a diet-induced NAFLD model, *Alisma orientale* extract reversed hepatic steatosis and liver function damage in mice, while upregulating the expression of FXR in liver tissue[257]. Moreover, in female *Ldlr*$^{-/-}$ mice subjected to bilateral ovariectomy, alisol B 23-acetate (**50**) promoted the excretion of BAs and cholesterol by activating hepatic FXR/BSEP signaling, resulting in reduced serum cholesterol levels and improved atherosclerotic symptoms[258]. However, while most *Alisma* triterpenes have been identified as FXR ligands, regulating FXR signaling, the mechanism by which they regulate FXR expression in the aforementioned studies remains unclear.

8.3. *Rheumatoid arthritis*

Tripterygium wilfordii, a traditional Chinese medicine, has been developed into Tripterygium Glycosides Tablets, used to treat autoimmune diseases such as rheumatoid arthritis[259]. *Tripterygium hypoglaucum* extract has demonstrated efficacy in ameliorating inflammation and joint swelling symptoms in mice with complete Freund's adjuvant-induced rheumatoid arthritis. Its potential mechanism appears to involve remodeling in the composition of intestinal BAs, consequently activating the "gut−joint" axis mediated by FXR[260]. However, it is worth noting that celastrol (**63**), as a key triterpenoid component in *Tripterygium wilfordii*, has been shown to be an FXR antagonist[231]. Therefore, further research is needed to clarify these contradictions.

The rhizome of *Dioscorea nipponica*, a Chinese herbal remedy used for treating arthritis, is rich in steroidal saponins, primarily dioscin (**3**), and has been found to be notably effective against rheumatoid arthritis[261]. Dioscin (**3**) alleviates symptoms in collagen-induced arthritis (CIA) DBA/1 mice and inhibits the response of Th17 cells[262]. Results from *in vitro* luciferase reporter gene assays indicate that diosgenin (**4**), one of the main metabolites after oral administration of dioscin (**3**), acts as a ligand and antagonist of RORγt, which may mediate its attenuating effect on Th17 responses[263].

The rhizome of *Panax notoginseng* is a traditional Chinese medicine used for its anti-inflammatory and anti-swelling properties, and in recent years, it has been found to hold potential for treating rheumatoid arthritis. In CIA mice, total triterpene saponins from notoginseng inhibit Th17 cell differentiation, thereby mitigating symptoms[264]. Notably, various saponins from notoginseng, especially ginsenoside Rg2, can increase the abundance of *Parabacteroides distasonis* in the intestine[2]. This bacterium can enhance the abundance of intestinal secondary BAs, suppressing RORγT, thus indirectly inhibiting Th17 cell differentiation[2] (Fig. 9C).

Tripterygium Glycosides Tablets have been clinically used to treat rheumatoid arthritis, with celastrol as a key component.

Additionally, some herbal combinations have similar clinical effects. Granules composed of *Dioscorea bulbifera* and *Acanthopanax senticosus*, rich in dioscin and acanthopanax glycoside A, reduce inflammation markers in rheumatoid arthritis patients[265]. Liuwei Dihuang pills, composed of six botanicals including *Alisma orientale* and *Dioscorea opposita*, rich in triterpenols and dioscin, lower blood glucose in type 2 diabetes patients treated with metformin[266].

8.4. Other diseases

Lysimachia capillipes is a traditional herbal remedy from southeastern China, commonly used for its anti-inflammatory and analgesic properties. Capilliposide A, a triterpenoid saponin isolated from this herb, alleviates colitis symptoms in mice treated with DSS and increases the concentration of DCA in feces[267]. It's suggested that this triterpenoid saponin has the potential to enhance secondary BA levels, regulate Th17 cell differentiation, and thereby mitigate IBD. However, further experimental validation is required.

Waltonitone, a ursane-type triterpenoid isolated from *Gentiana waltonii*, upregulates FXR expression in HCC xenograft mice and inhibits tumor growth[268]. Knocking down FXR eliminates the inhibitory effect of this triterpenoid saponin on cell proliferation. In an orthotopic HCC rat model induced by diethylnitrosamine, celastrol (**63**) reduced the abundance of *Bacteroides fragilis* in feces and increased the levels of liver FXR antagonists such as TUDCA, UDCA, and GUDCA. Interestingly, further research revealed that GUDCA inhibited the proliferation of cancer cells both *in vitro* and *in vivo*, and its mechanism might be associated with mTOR phosphorylation[269]. In HepG2 cells, GUDCA inhibited mTOR phosphorylation, but this effect was lost after interfering with FXR expression. This indicates that celastrol may intervene in the development of HCC through the "*Bacteroides fragilis*-GUDCA-FXR-mTOR axis". However, considering that celastrol itself has also been identified as an FXR modulator, more details of its actions should be explored.

9. Concluding perspectives

9.1. Profound impact of microbial BAs on host health status

Gut commensal microbes intricately influence and adapt alongside the host, fostering a co-evolutionary relationship, which is exemplified by the "host−microbiota" co-metabolism of BAs. Gut bacteria have evolved specific actions—such as deconjugation, dehydroxylation, and isomerization—to tolerate BAs within the gut lumen. The resultant secondary BAs enrich the species of the BA pool, and also cause differences in the effect among the BA receptors. Some BAs notably affect FXR signaling, playing a crucial regulatory role in the host's glucose, lipid, cholesterol metabolic balances, and EHC processes. Conversely, some others exhibit heightened affinity for receptors like TGR5 and RORγt, governing the host's incretins secretion and immune homeostasis. Therefore, the delicate equilibrium of different BAs within the BA pool is vital in maintaining optimal host health. Understanding and harnessing this balance opens promising avenues for targeted drug development aimed at treating conditions such as cholestasis, metabolic disorders, and autoimmune diseases. Moreover, with the advancements in detection methods, MCBAs have been discovered, significantly broadening the anticipated spectrum of BA species within the BA pool to include potentially thousands of variants[16]. Prospective clinical cohort studies are poised to elucidate the correlation between these novel BAs and the host's health status. This endeavor could potentially lead to the discovery of a new "BA-diseases" action mode.

9.2. Pharmacological mechanisms and clinical prospects of botanicals rich in triterpenoids and steroids: Insights from BA metabolism, transport, and signaling

Herbal medicines rich in triterpenoids and steroids have been used for a long time to treat diseases, such as IHC, metabolic diseases, rheumatoid arthritis, IBD and cancer[239,248,259,261,270,271]. However, the detailed pharmacological mechanisms of these botanicals have long remained poorly understood; consequently, their clinical application still tends to be largely empirical. Now, BA metabolism, transport, and signaling offer insights into elucidating their mechanisms of action. Natural triterpenoids, phytosterols, and animal cholesterol share similar frameworks in their initial biosynthesis, while BAs represent metabolites derived from cholesterol. The structural similarities potentially confer upon these natural products the capacity to influence BA metabolism, transport, and signaling[272−274]. This elucidates potential mechanisms through which herbal remedies rich in these natural compounds ameliorate diseases. In summary, TGR5 agonists, FXR antagonists, and ASBT inhibitors found in botanical triterpenoids and steroids hold potential benefits for metabolic symptoms such as obesity, insulin resistance, hepatic steatosis, and dyslipidemia. FXR agonists may have therapeutic value for IHC, NASH, and CRC. Additionally, some compounds may also alleviate metabolic diseases, rheumatoid arthritis, and IBD by modulating the BA metabolism-related gut flora.

However, significant challenges persist in current research. Firstly, although many compounds, such as *Alisma* triterpenols, *Poria* triterpene acids, *Citrus* lemonoids, ergosterol and others, have been identified as ligands of BA receptors or transporters *in vitro*, their potential for treating diseases through regulating BA signaling or transport still needs to be validated in preclinical models. Secondly, while some compounds or herbal extracts have demonstrated modulation of BA metabolism and signaling in animal models, the precise regulatory mechanisms remain elusive. For example, ginsenoside Rg1, diosgenin and gypenosides extract have been found to modulate the expression of FXR in animal models of disease, rather than acting as FXR ligands to play agonistic or antagonistic roles. However, the mechanisms by which they regulate FXR expression remain unclear. Similarly, astragaloside IV (**1**), dioscin (**3**), and 2α-OH-protopanaxadiol (**5**) have been observed in animal models to indirectly improve diseases by modulating gut microbiota and altering BA composition. However, it remains uncertain whether they can exert the same regulatory effects on these bacteria in human settings. This also raises the final challenge the need for clinical data to support these findings. Hence, there is a call for more comprehensive and rigorous preclinical and clinical research to elucidate how these compounds or botanical extracts relieve diseases by modulating BA metabolism, transport, and signaling, thus providing scientific evidence for their clinical use.

9.3. The toxicity of botanicals rich in triterpenoids and steroids: Insights from BA metabolism, transport, and signaling

Although natural compounds generally exhibit weaker activity compared to synthetic agonists/antagonists, their potential toxicity and side effects should not be overlooked. The FXR antagonistic

effects of celastrol (**63**) and stigmasterol (**62**), for example, may respectively explain the intestinal toxicity of *Tripterygium wilfordii* in clinical use[231] and the hepatic toxicity observed in infants receiving parenteral nutrition[182,230,275]. Using FXR agonists may mitigate their toxicity[275]. Oleanolic acid (**29**) also acts as an FXR antagonist[225,226]. In LCA-induced obstructive cholestasis mice, it can reduce the expression of BSEP by antagonizing hepatic FXR, thus decreasing the flow of BAs excreted through the bile canaliculus lumen and alleviating the symptoms[226]. However, excessive or repeated administration of oleanolic acid (**29**) in normal animals may cause IHC and liver injury due to impaired bile excretion[201,276,277], highlighting a cautionary note for the safe usage of natural BA receptor modulators. Moreover, timosaponin A3, a steroidal saponin isolated from the rhizome of *Anemarrhena asphodeloides*, has been found to downregulate the expression of BA metabolism and transport-related proteins such as NTCP, MRP2, BSEP, and CYP7A1 in the liver of normal rats[278]. This leads to bile stasis and liver injury, although the details of this effect are not yet clear. Therefore, attention should be paid to the BA-related toxicity that may be caused by botanical triterpenoids and steroids.

9.4. *Developing novel BA receptor modulators based on botanical triterpenoids*

Considering the activity of natural triterpenoids as ligands for FXR or TGR5, developing more potent and selective semi-synthetic agonists/antagonists based on their scaffold is a highly promising drug development strategy[279–281]. Researchers modified the structure of betulinic acid and discovered that introducing an allyl or 2-methylallyl group at the C-3 position, forming the "*R*" configuration, could decrease the EC_{50} for activating TGR5 from 1.04 to 0.12 and 0.42 μmol/L, respectively. Additionally, the maximum agonist potency was increased from 83% of LCA to 122% and 117%, respectively[208]. Further modification of the 2-methylallyl group to an epoxide could decrease the EC_{50} to 47 nmol/L[282]. Currently, modifications targeting the C-3 hydroxyl, C-28 hydroxyl, and C-19 isopropenyl groups of betulinic acid have resulted in a series of semi-synthetic TGR5 agonists, some of which have been shown to promote the release of GLP-1 in enteroendocrine cells, demonstrating potential therapeutic effects for metabolic disorders[283,284]. It is worth noting that in a recent study, a betulinic acid derivative acylated with glycine at the C-28 position was found to be an FXR antagonist. It can antagonize FXR activation induced by GW4064, with an IC_{50} of only 2.1 μmol/L, and has shown potential in animal models to inhibit intestinal FXR signaling and mitigate NASH symptoms[285]. In addition to betulinic acid derivatives, semi-synthetic oleanolic acid derivatives are also developed as FXR ligands. Wang et al. esterified the C-3 hydroxyl group of oleanolic acid, and among the four derivatives synthesized, two exhibited stronger antagonistic activity than the prototype molecule[286]. Another research group focused on modifying the C-12 alkenyl group of oleanolic acid, synthesizing 12β-*O*-γ-glutamyl oleanolic acid and 12β-oxygenated oleanolic acid alkyl esters[287,288]. These derivatives have been confirmed as selective FXR antagonists, showing the potential to alleviate NASH and hyperglycemia in mice.

9.5. *The potential drug interactions of plant triterpenoids and steroids mediated by BA transporters*

A considerable body of research suggests that BA transporters play a pivotal role in the transport of drugs. For instance, OATP1A2 is known to transport atorvastatin, celiprolol, imatinib, among others[47]; OATP1B1 transports troglitazone, valsartan, estrone-3-sulphate, etc.[47]; MRP2 transports itraconazole, etoposide, methotrexate, etc.[289]; NTCP transports rosuvastatin, pitavastatin and fluvastatin[290]. It's worth noting that many botanical triterpenoids and steroids, such as ginsenosides[207], timosaponins[206], licorice triterpenoids[291], and *Poria* triterpene acids[202], have also been identified as substrates or inhibitors of these transporters. Considering that some patients concurrently use herbal remedies as complementary therapy while taking medication, potential drug interactions may lead to reduced efficacy or increased toxicity due to blocked drug excretion. Moreover, as herbal remedies are typically used in combination, interactions between compounds mediated by BA transporters cannot be overlooked. However, there remains a shortage of adequate research to address these concerns comprehensively.

9.6. *Deciphering the mechanistic paradigm of botanical triterpenoids and steroids in BA signaling: Gut microbiota as the connecting link*

Botanical triterpenoids and steroids, particularly their glycosides, typically engage with gut microbiota subsequent to oral administration[184]. This mode of interaction holds the potential for significant effects on BA signaling pathways. For instance, compounds like dioscin (**3**) and astragaloside IV (**1**) demonstrate the capacity to diminish populations of bile salt hydrolase (BSH)-related bacteria, consequently impeding intestinal FXR signaling. Subsequently, these compounds undergo bacterial metabolism within the gut, transforming into aglycones such as tigogenin (**58**) and cycloastragenol (**54**), both recognized as direct modulators of FXR. In another instance, glycyrrhizic acid (**40**) itself acts as a TGR5 agonist, while its gut flora metabolite, glycyrrhetinic acid (**21**), has been identified as an FXR agonist. Hence, it becomes evident that the intestinal flora serves as a pivotal intermediary enabling these natural products to regulate BA signaling pathways.

Here, we propose two paradigms for studying how botanical triterpenoids, steroids, and their glycosides intervene in diseases through regulation of BA signaling pathways by the gut microbiota. The first paradigm investigates the regulatory effects of phytochemicals on disease-related microbial BAs (Fig. 10A). Firstly, investigating their regulation on microbial BAs. Initially, designing cohorts and collecting clinical biosamples; utilizing BAFinder/BAFinder 2.0 for non-targeted BA profiling to screen out BAs most closely associated with the disease. Subsequently, employing metagenomic approaches to explore disease-associated gut microbiota closely linked with disease and selected BA levels in fecal samples. After isolating these microbiota, investigating their capability for metabolizing selected BAs both *in vitro* and *in vivo*. Then, investigating the efficacy and mechanisms of selected BAs and bacterial interventions in treating diseases. Finally, utilizing a botanical triterpenoids/steroids molecular library for high-throughput *in vitro* screening to identify potential natural compounds capable of modulating these microbial BAs. The second paradigm investigates the direct regulatory effects of saponins and their secondary metabolites on BA receptors (Fig. 10B). Identifying[292,293] and isolating triterpenoid/steroidal saponins from empirically clinically used herbal medicines, and investigating the metabolic effects of gut bacteria on them. Then screening potential ligands of BA receptors from selected saponins or secondary metabolites *in vitro*. Finally, conducting *in vivo* preclinical studies and clinical trials to validate the benefits of these herbal components.

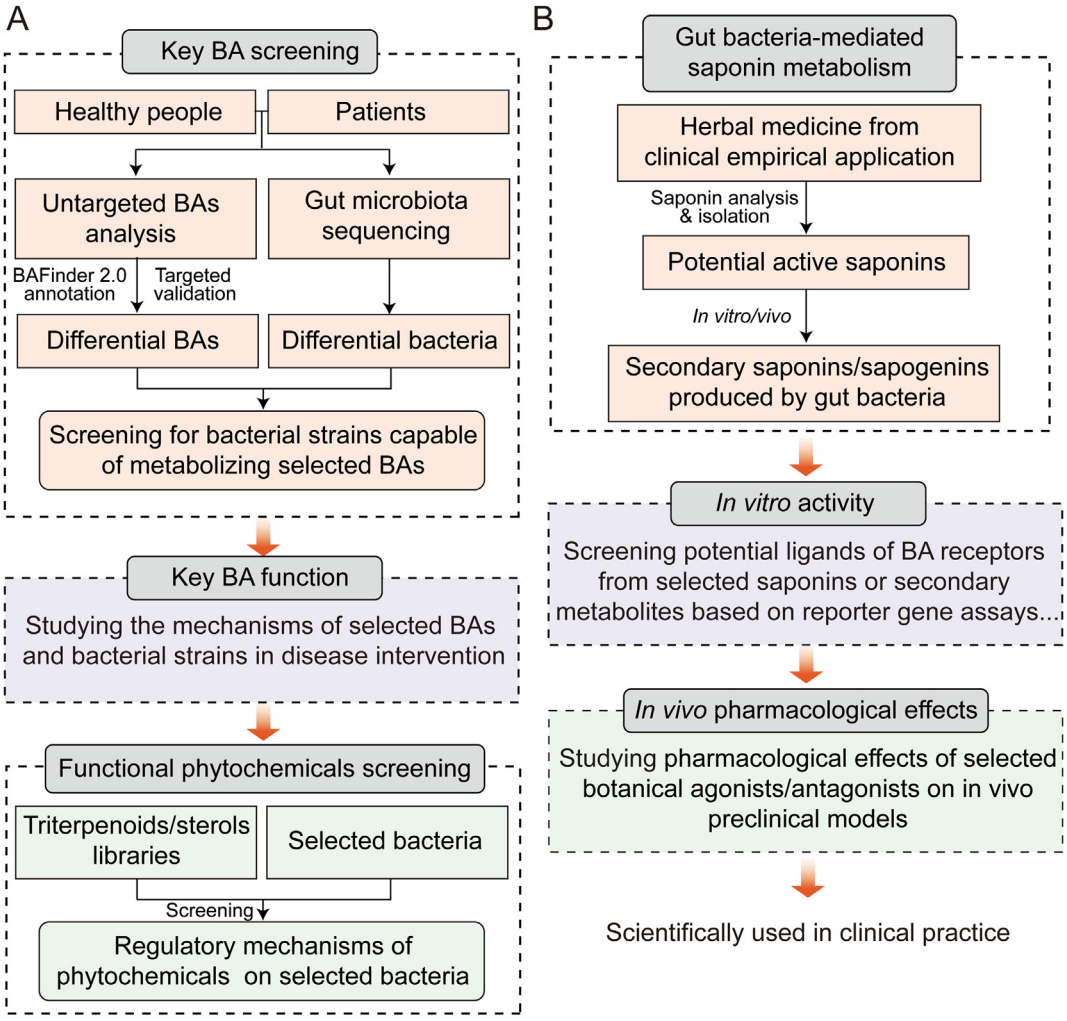

Figure 10 The research paradigm of botanical triterpenoids and steroids in BA signaling. (A) The regulatory effects on disease-related on microbial BAs; (B) The direct regulatory effects on BA receptors.

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Conflicts of interest

We declare no conflict of interest.

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