Review:

Bile-ology: from bench to bedside[*]

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Abstract: Bile acids (BAs) are originally known as detergents essential for the digestion and absorption of lipids. In recent years, extensive research has unveiled new functions of BAs as gut hormones that modulate physiological and pathological processes, including glucose and lipid metabolism, energy expenditure, inflammation, tumorigenesis, cardiovascular disease, and even the central nervous system in addition to cholesterol homeostasis, enterohepatic protection and liver regeneration. BAs are closely linked with gut microbiota which might explain some of their crucial roles in organs. The signaling actions of BAs can also be mediated through specific nuclear receptors and membrane-bound G protein-coupled receptors. Several pharmacological agents or bariatric surgeries have demonstrated efficacious therapeutic effects on metabolic diseases through targeting BA signaling. In this mini-review, we summarize recent advances in bile-ology, focusing on its translational studies.

Key words: Bile acid; Gut microbiota; Farnesoid X receptor; G protein-coupled bile acid receptor; Metabolic disease

1 Introduction

Bile acids (BAs) are amphipathic molecules synthesized from cholesterol in the liver and efficiently retained within an enterohepatic circulation through a complex biosynthetic pathway, biliary secretion, bacterial modification, ileum reabsorption, and liver reflux (Slijepcevic and van de Graaf, 2017). Because of their unique detergent properties, BAs have long been known to facilitate the absorption, transport, and metabolism of dietary fats and lipid-soluble nutrients. Excitingly, extensive research in the last decade has demonstrated that in addition to the role in cholesterol homeostasis, fat absorption and digestion, BAs play important roles as signaling molecules in regulating physiological and pathological processes including glucose and lipid metabolism, energy expenditure, inflammation, cancer, heart failure, Alzheimer's disease (AD), and aging. In this mini-review, we summarize recent advances in the understanding of BA signaling, gut microbiota, and the related regulatory functions in metabolic diseases (Fig. 1), and discuss the development of new BA-based therapeutics for the treatment of metabolic diseases by targeting nuclear BA receptor farnesoid X receptor (FXR) and membrane Takeda G protein-coupled BA receptor (GPBAR or TGR5).

2 BAs, gut microbiota, and related metabolic diseases

BAs' synthesis and their enterohepatic circulation have been elaborately reviewed previously (Li
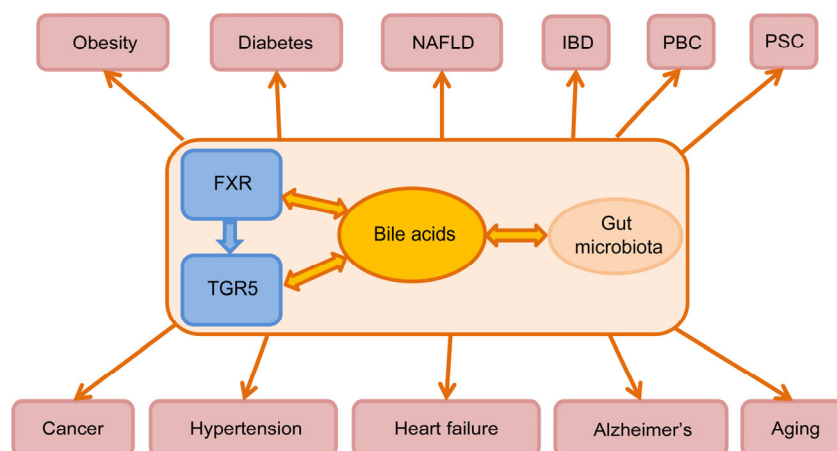
Fig. 1 Bile acid signaling and related diseases
Farnesoid X receptor (FXR) regulates the expression of Takeda G protein-coupled bile acid (BA) receptor (TGR5). Both as BA receptors, FXR and TGR5 are closely involved in BA signaling and the balance of gut microbiota, resulting in the regulation of BA-related diseases. NAFLD, non-alcoholic fatty liver disease; IBD, inflammatory bowel disease; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis

and Chiang, 2014; Mertens et al., 2017). BAs and gut microbiota are closely linked. Gut microbiota is involved in the biotransformation of BAs through deconjugation, dehydroxylation, and reconjugation of BAs (Ridlon et al., 2006). The gut-to-liver axis of microbiota plays a crucial role in the regulation of BA metabolism, BA pool size, and enterohepatic circulation of BAs (Chiang and Ferrell, 2018). Reciprocally, BAs shape the gut microbiome to regulate host metabolism. BAs have antimicrobial activity by damaging the bacterial cell membrane and thus inhibiting bacteria outgrowth (Inagaki et al., 2006; Kurdi et al., 2006). Importantly, BAs link the gut microbiota to both hepatic and intestinal metabolism, and this tripartite relationship has been implicated in different metabolic diseases, which we will highlight below.

2.1 Obesity

Obesity has become one of the most prevalent public health concerns worldwide. Being overweight is related to the incidence of several comorbidities, including type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) (Golay and Ybarra, 2005; Esser et al., 2014), cardiovascular disease (Sommer and Twig, 2018), stroke and cancer (Xu and Mishra, 2018). Elevated serum BA levels have been observed a long time ago in overweight subjects as compared to those with an ideal weight (Halmy et al., 1986). Later, many studies have found increased total BA levels in

humans with obesity (Prinz et al., 2015; Chávez-Talavera et al., 2017). This evidence indicates that BAs may be closely related to the pathogenesis of obesity.

Bariatric surgery represents a viable clinical intervention to efficaciously remit obesity and its associated complications. Interestingly, it has been reported that bariatric surgeries, including Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG), increase circulating BA concentrations (Nakatani et al., 2009; Gerhard et al., 2013). Serum BA levels were higher in obese patients who received RYGB when compared to controls (no RYGB), and elevated levels of BAs were sustained for at least 2 years post-surgery (Nakatani et al., 2009; Patti et al., 2009). Further investigation demonstrated that the mechanism is due to the regulation of specific BAs (Wang et al., 2017; Browning et al., 2019). This might also be explained by the evidence that plasma BAs were negatively correlated with cognitive restraint of eating (Prinz et al., 2015). Obesity is mainly due to a long-term excess energy intake above energy expenditure. Studies using isolated rodent tissues indicated that a BA mixture (cholic acid (CA), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA) in both un-conjugated and glycine- and taurine-conjugated forms) increased secretion of glucose-dependent insulinotropic peptide, glucagon-like peptide-1 (GLP-1), polypeptide YY and neurotensin through activating TGR5, leading to metabolic improvement (Kuhre et al.,

2018). BAs are also identified as regulators of energy expenditure. In brown adipose tissue (BAT), BAs activate TGR5, resulting in increased concentrations of cyclic adenosine 3',5'-monophosphate (cAMP); cAMP subsequently activates type-2 deiodinase (D2) which induces active triiodothyronine (T3), and this cascade results in enhanced energy expenditure in both murine and human BAT by inducing the expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and uncoupling protein 1 (UCP1) (Watanabe et al., 2006; Broeders et al., 2015).

Accumulating evidence indicates that the gut microbiota plays a significant role in the development of obesity and obesity-associated disorders (Shen et al., 2013) by impacting the composition and relative abundance of BA species (Ridaura et al., 2013). Turnbaugh et al. (2006) found that the obese microbiome has an increased capacity to harvest energy from the diet. In this process, the relative abundance of the two dominant bacterial divisions, Bacteroidetes and Firmicutes, contributes to energy homeostasis. Recently, Worthmann et al. (2017) found that BAs are also involved in energy homeostasis regulated by gut microbiota stimulated by cold. They found that cold-induced conversion of cholesterol to BAs in mice shapes the gut microbiome and promotes adaptive thermogenesis. In this process, the crucial enzyme for the alternative BA synthetic pathway, sterol 27-hydroxylase (CYP27A1), is selectively activated in the liver, and thus modulates gut-microbiome composition (Kuipers and Groen, 2017). This research provides a BAT–liver–intestine axis in connecting the BA homeostasis with obesity. Taken together, BAs play dual roles by regulating both energy intake and energy expenditure in balancing body weight.

Interestingly, the obesity-treating strategies by targeting BAs also display some conflicting results. Diet-induced obesity is ameliorated by BA-binding resin (Kobayashi et al., 2007). An additional series of studies demonstrated that BA sequestrants improve metabolic diseases (Beysen et al., 2012; Hansen et al., 2017). The profile of BAs is fluctuated delicately according to many factors, including circumstance temperature (Worthmann et al., 2017), nutrient intake (Matysik et al., 2011; Schmid et al., 2016), drug treatment (Camilleri and Gores, 2015), surgery (Tian et al., 2017), etc. Thus, these reports implicate the sophisticated mechanism of obesity regulated by BA

signaling, and pinpoint the importance of analyzing the composition of BAs in different conditions in terms of therapeutic strategies.

2.2 T2D

Changes in circulating BA profile have been implicated in the pathogenesis of insulin resistance and T2D including gestational diabetes mellitus (Haeusler et al., 2013; Maghsoodi et al., 2019). T2D is associated with an increase in the hydrophobicity of the circulating BA pool in humans (Haeusler et al., 2013). Consistent with this, hydrophilic BA subtypes, such as tauroursodeoxycholic acid (TUDCA), have been shown to protect against inflammation and improve insulin sensitivity in rodent models and patients with T2D (Mahmoud and Elshazly, 2014; Shima et al., 2018). The mechanism of metformin also confirms this observation (Sun et al., 2018). Sun et al. (2018) found that metformin acts through lowering levels of *Bacteroides fragilis* in the gut, resulting in a decrease in the enzyme bile salt hydrolase and a subsequent increase of hydrophilic glycoursodeoxycholic acid (GUDCA), which might inhibit intestinal FXR and increase the level of liver BAs, leading to improvement of metabolic dysfunction including hyperglycemia.

T2D is also proposed to be associated with the ratio of 12α-hydroxylated BAs (CA and DCA) to non-12α-hydroxylated BAs (CDCA and lithocholic acid (LCA)). It has been reported that ratios of 12α-hydroxylated/non-12α-hydroxylated BAs were associated with key features of insulin resistance, including higher insulin, proinsulin, glucose, glucagon and triglyceride levels, and lower high-density lipoprotein associated cholesterol by comparing a cohort of 200 healthy subjects and 35 T2D patients (Haeusler et al., 2013). A similar result was also reported that in patients with verified insulin resistance, enhanced BA synthesis and increased ratio of 12α-hydroxylated (CA and DCA) to non-12α-hydroxylated (CDCA, LCA, and ursodeoxycholic acid (UDCA)) BAs were observed (Kaur et al., 2015). Consistently, sterol 12α-hydroxylase knockout ($Cyp8b1^{-/-}$) mice with low levels of 12α-hydroxylated BAs showed increased GLP-1 and improved glucose tolerance (Kaur et al., 2015). Non-12α-hydroxylated BAs were also reported to be negatively correlated with fasting glucose in women with previous gestational diabetes mellitus with homeostatic model assessment of insulin

resistance (HOMA-IR) >2.8 (Maghsoodi et al., 2019). These results suggest 12α-hydroxylated BAs as negative regulators of insulin action. However, in a randomized trial of lifestyle-induced weight loss in 74 non-smoking men with metabolic syndrome, data show that individuals with improved metabolic control by lifestyle modifications have lower serum levels of BAs and GLP-1, and changes in serum BA composition towards an increased 12α-hydroxylated/non-12α-hydroxylated ratio (Biemann et al., 2016). Although this might due to the difference in the specific health condition of individuals, nevertheless the conflicting results suggest that metabolism is regulated by a dynamically changing profile of BAs.

2.3 NAFLD

The prevalence of NAFLD that is comprised of non-alcoholic fatty liver (NAFL) and its exacerbated condition non-alcoholic steatohepatitis (NASH) is increasing worldwide, reflecting the current epidemics of obesity, insulin resistance and T2D, and metabolic syndrome (Mullish et al., 2018). The relationship between BAs and NAFLD has been extensively investigated previously (Carr and Reid, 2015; Chávez-Talavera et al., 2017). BAs were found to be significantly increased in NASH patients over that when they were in simple steatosis (Bechmann et al., 2013; Jiao et al., 2018). The ratio of CDCA was lower while DCA was significantly increased in NASH patients, and there was a comparable CA and UDCA ratio, suggesting that the elevated 12α-hydroxylated (CA and DCA)/non-12α-hydroxylated (CDCA and UDCA) ratio may also highly correlate with the progress of NAFLD in addition to T2D. Surprisingly, the fibroblast growth factor 19 (FGF19)-FXR pathway was suppressed in NASH patients, indicating that the 12α-hydroxylated/non-12α-hydroxylated ratio of BAs, but not the BA pool size, could be the major regulatory element for NASH (Jiao et al., 2018).

Recently, Puri et al. (2018) found that the presence and severity of NASH are associated with specific changes in circulating BAs. Increase of key BAs was associated with higher grades of steatosis (taurocholic acid (TCA)), lobular (glycocholic acid (GCA)) and portal inflammation (taurolithocholic acid (TLCA)), and hepatocyte ballooning (TCA). Conjugated CA and TCA directly correlated, while the secondary to primary BA ratio inversely corre-

lated to the NAFLD activity score. A higher ratio of total secondary to primary BA decreased and higher conjugated cholate increased the likelihood of significant fibrosis. These observations further provide evidence to support the idea that specific BAs affect specific aspects of NASH.

Gut microbes produce enzymes that convert primary BAs into secondary BAs in the intestines. Consistently, specific species of gut microbe control a diverse effect on NAFLD. For instance, *Parabacteroides distasonis* is one of the 18 core members in the gut microbiota of humans (Falony et al., 2016). Treatment with live *P. distasonis* dramatically altered the BA profile with elevated LCA and UDCA that reduced hyperlipidemia by activating the FXR pathway and repairing gut barrier integrity (Wang et al., 2019). Actinobacteria and Firmicutes are the gut phyla capable of degrading all conjugated BAs, with Bacteroidetes limited to tauro-conjugation activities, exclusively (Jones et al., 2014). It has also been shown that the gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD (Soderborg et al., 2018). Another example is that *Bilophila wadsworthia* aggravates high-fat diet (HFD)-induced metabolic dysfunctions in mice. *B. wadsworthia* acts on both host and microbiota by worsening HFD-induced intestinal inflammation, inhibiting pathways involved in metabolic homeostasis, favoring increased lipopolysaccharides production and translocation, and decreasing butyrate production by the microbiota (Natividad et al., 2018). In the HFD setting, increased production of taurine-conjugated BAs had been proposed to underlie the expansion of *B. wadsworthia* (Devkota et al., 2012). Many studies have investigated the shift of the gut microbiome in NAFLD (Chen et al., 2019). These results further support the idea that different genera of bacteria exert different functions in gut, suggesting that microbiome restoration could be an alternative approach for the treatment of NAFLD.

2.4 Inflammatory bowel disease (IBD)

BAs significantly affect gastrointestinal motor, sensory and secretory functions, intestinal barrier permeability and regulation of the inflammatory response (Panek-Jeziorna and Mulak, 2017). In the healthy gut, these primary BAs aid in the digestion of lipids and are deconjugated by microbes to secondary

BAs, while the primary BAs including CA and CDCA were enriched in IBD (Franzosa et al., 2019). The relative overabundance of primary BAs in the guts of IBD patients is consistent with the disruption of BA transformation activities in the IBD microbiome (Duboc et al., 2013). High-level fecal DCA may act as an endogenous danger signal to activate nod-like receptor protein 3 (NLRP3) inflammasome and contribute to HFD-related colonic inflammation (Zhao et al., 2016). It has also been reported that LCA specifically inhibits NLRP3 inflammasome activation via activating TGR5–cAMP–protein kinase A (PKA) axis (Guo CS et al., 2016). The anti-inflammatory function of TGR5 in protection against IBD is also mediated by inhibition of nuclear factor κB (NF-κB)-dependent proinflammatory cytokine production (Yoneno et al., 2013). Although there has been an increasing number of studies on the role of BAs in IBD (Hegyi et al., 2018; Tiratterra et al., 2018), no cure has been found for treating IBD. Further understanding of the pathogenic mechanisms underlying IBD is still urgently needed.

2.5 Other diseases

BAs, especially secondary BAs, have been well known as strong carcinogens or promoters of colon cancers (Nguyen et al., 2018). The BA receptors FXR and TGR5 not only play key roles in regulating BA homeostasis but also are essential in suppressing BAs' carcinogenic effects (Modica et al., 2008; Wang et al., 2013). This is supported by the facts that FXR-null mice have increased BA pool and spontaneous hepatocarcinogenesis (Kim et al., 2007; Yang et al., 2007; Wang et al., 2008), and deficiency of TGR5 enhances chemically induced liver carcinogenesis (Chen et al., 2013). Additionally, Lee et al. (2019) recently reported that BAs activate yes-associated protein (YAP) through the nuclear vitamin D receptor, resulting in tumor metastasis to lymph nodes. On the other hand, BA metabolism is also involved in antitumor immunosurveillance. Ma et al. (2018) found that gut microbiome-mediated primary-to-secondary BA conversion provides BA messengers to control a chemokine-dependent accumulation of hepatic natural killer T (NKT) cells, leading to selective antitumor in the liver. Therefore, the hypothesis of treating cancer by targeting BAs still needs further detailed investigation. In addition to the above described metabolic diseases, BAs also participate in the development and

progression of cardiovascular diseases, including heart failure. This has been extensively reviewed recently (Tang et al., 2019). BA receptors FXR and TGR5 are expressed in endothelial cells, and a variety of FXR ligands show efficacious antihypertensive effects (He et al., 2006; Verbeke et al., 2014; Schwabl et al., 2017), implying the involvement of BA signaling in hypertension. Recent work suggests that microbial disturbances linked to BA profiles are implicated in neurodegenerative disorders. Because of their involvement in immune, neuroendocrine, and neural pathways, gut microbiota has been shown to regulate microglial maturation and function, and may contribute to AD (Nho et al., 2019). Interestingly, BAs might also be closely related to aging because diet enriched in CA enhances health span and lifespan of progeria mice (Bárcena et al., 2018). All of these results demonstrate the importance of maintaining the homeostasis of BAs as well as gut microbiota. The BA/gut microbiome axis might be a promising therapeutic target for the treatment of these diseases (Fig. 1).

3 Therapeutic strategies by targeting BA signaling

3.1 Drug discovery by targeting BA receptors

BAs are metabolic regulators that act as signaling molecules through receptor-dependent and -independent pathways. The most prominent signaling molecules mediating BA signaling are the nuclear receptor FXR and the membrane receptor TGR5. Both are implicated in the regulation of lipid, glucose, energy metabolism, and inflammation. Dysregulation of these pathways might contribute to the development of the diseases summarized above.

3.1.1 Drug discovery by targeting FXR

FXR is expressed in various tissues but its roles in BA signaling have mostly been studied in the liver and the intestine. FXR regulates a network of genes in hepatic BA synthesis, biliary BA secretion, intestinal BA absorption, and hepatic BA uptake, and thus plays a key role in the regulation of BA homeostasis and related gut microbiota composition that has been extensively reviewed (Li and Chiang, 2014). Extensive research has been undertaken to elucidate the molecular mechanism of BA signaling-related diseases

regulated by FXR, including nuclear translocation (Liu et al., 2015; Thompson et al., 2018), post translational modifications (Kemper et al., 2009; Byun et al., 2018; Blokker et al., 2019), and the transcription activity of FXR (Li and Chiang, 2014). FXR is a promising drug target for metabolic diseases.

As a transcriptional factor, the activity of FXR is mediated by ligand binding and regulates BA signaling-related diseases (Jin and Li, 2010; Ding et al., 2015). Several BAs regulate FXR activity as ligands, for example, CDCA, DCA, LCA, and CA may activate FXR, while UDCA, tauro-α-muricholic acid (T-α-MCA) and T-β-MCA may antagonize FXR. Dozens of FXR ligands, including agonists, antagonists, and selective modulators, have been identified to bind FXR and regulate metabolism by modulating BA homeostasis. The FXR agonists include steroid compounds such as BAs and their derivatives, 17β-(4-hydroxybenzoyl) androsta-3,5-diene-3-carboxylic acid (MFA-1), and non-steroid ligands including isoxazole GW4064 and derivatives (Feng et al., 2009), benzopyran fexaramine and derivatives (Downes et al., 2003), azapyrene WAY-362450 and derivatives (Flatt et al., 2009), benzimidazolyl amide compounds (Richter et al., 2011), etc. Additionally, some drugs on the market are identified as novel FXR modulators and have been repurposed to show promising potential in treating BA-mediated diseases by targeting FXR, such as the anti-parasitic drug ivermectin and its analogues (Jin et al., 2013, 2015), and anti-hypertension drugs 1,4-dihydropyridines (DHPs) (Wei et al., 2018). There are also many natural products identified as FXR ligands. Many of them have been in clinical trial for treating FXR-mediated diseases such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), NASH, duodenal polyps, etc. (Jia et al., 2018). Among them, obeticholic acid (OCA, a derivative of CDCA) and UDCA have been approved by the US Food and Drug Administration (FDA) in treating PBC. OCA is currently the only FDA-designated breakthrough therapy in development for NASH with compensated cirrhosis. The FXR ligands in clinical trials are summarized in Table 1. The development of these candidates strongly supports the feasibility and value of FXR as a target for metabolic diseases.

3.1.2 Drug discovery by targeting TGR5

TGR5 is widely expressed in the intestine, gall bladder, adipose tissue, skeletal muscle, pancreas, and the hepatic sinusoidal endothelial cells and Kupffer cells but is not expressed in hepatocytes (Maruyama et al., 2002; Keitel and Häussinger, 2011). Among all BAs, TLCA is the most potent TGR5 agonist, followed by taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), and TCA (Li and Chiang, 2014). Both as BA receptors, interestingly, the transcription of TGR5 is directly regulated by FXR through binding to the promoter of *Tgr5* (Pathak et al., 2017). The molecular signaling pathways regulated by TGR5 have been extensively reviewed (Guo C et al., 2016). As a metabolic regulator, TGR5 is involved in energy homeostasis, BA homeostasis, as well as glucose metabolism and has been extensively investigated in the current decade (Duboc et al., 2014; Velazquez-Villegas et al., 2018; Donkers et al., 2019; Ferrell et al., 2019; Schmid et al., 2019). Thus, in recent years, TGR5 has been the target of drug discovery efforts in the hope of identifying effective treatments for metabolic diseases including T2D, obesity, atherosclerosis, fatty liver disease, and cancer (Hodge and Nunez, 2016; Tian et al., 2017).

Recently, Finn et al. (2019) identified an orally administered TGR5 agonist RXD8940 that can induce incretin (GLP-1, GLP-2, and peptide YY) secretion which improves liver steatosis and insulin sensitivity in NASH and NAFLD. Berberine exerts powerful renoprotective effects on diabetic nephropathy by activating TGR5 and inhibiting sphingosine 1-phosphate receptor 2 (S1P2)/mitogen-activated protein kinase (MAPK) signaling (Yang et al., 2016). A selective TGR5 agonist SB756050 in clinical trial phase I showed good tolerance and safety, but without therapeutic effects on T2D (Hodge et al., 2013). There are also several dual TGR5/FXR agonists in trial (Table 1), but tolerance and side effects are still critical obstacles for their development. Aside from BAs, a class of plant natural triterpenes, including betulinic acid, oleanolic acid, and ursolic acid, were identified as selective TGR5 agonists with physiologic functions (Sato et al., 2007; Genet et al., 2010; Lo et al., 2017; Chianese et al., 2019). These natural oleanane-type triterpenes have been non-BA drug templates for target TGR5 (Castellano et al., 2013) but not for FXR even though FXR and GPBAR1 have similar binding pockets for BAs. Lu et al. (2018) found that a structurally similar triterpene, hedragonic acid, selectively activates FXR. These results suggest the oleanane-type triterpene may be a novel structure template

Table 1 Clinical trial of compounds for metabolic diseases by targeting FXR and TGR5

Drug candidate	Disease	Type of study	Status	Clinical phase	Reference
FXR agonist					
OCA	Liver cirrhosis, biliary	Double-blind, randomized, placebo-controlled study	Recruiting	Phase IV	US National Library of Medicine. ClinicalTrials.gov. 2019. https://clinicaltrials.gov/ct2/show/NCT02308111
	NASH	Double-blind, randomized, long-term, placebo-controlled study	Recruiting	Phase III	US National Library of Medicine. ClinicalTrials.gov. 2019. https://clinicaltrials.gov/ct2/show/NCT02548351
	NAFLD with raised ALT	Open label	Recruiting		US National Library of Medicine. ClinicalTrials.gov. 2019. https://clinicaltrials.gov/ct2/show/NCT03836937
	PSC	Randomized, double-blind, placebo-controlled study	Completed	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2018. https://clinicaltrials.gov/ct2/show/NCT02177136
	PBC	Open label	Completed	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2018. https://clinicaltrials.gov/ct2/show/NCT01865812
	Obesity	Randomized study	Recruiting	Phase I	US National Library of Medicine. ClinicalTrials.gov. 2018. https://clinicaltrials.gov/ct2/show/NCT02532335
	Alcoholic hepatitis	Double-blind, placebo-controlled study	Completed	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2018. https://clinicaltrials.gov/ct2/show/NCT02039219
Cilofexor (GS-9674)	NASH	Randomized, double-blind, placebo-controlled study	Active, not recruiting	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2018. https://clinicaltrials.gov/ct2/show/NCT03449446; Trauner et al., 2019
Tropifexor (LJN452)	NASH	Randomized, double-blind, placebo-controlled study	Recruiting	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2019. https://clinicaltrials.gov/ct2/show/NCT02855164
	PBC	Randomized, double-blind, placebo-controlled study	Completed	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2018. https://clinicaltrials.gov/ct2/show/NCT02516605
Px-104	NAFLD	Open label	Completed	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2016. https://clinicaltrials.gov/ct2/show/NCT01999101
LMB763	NASH	Randomized, patient and investigator blinded, placebo-controlled study	Active, not recruiting	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2019. https://clinicaltrials.gov/ct2/show/NCT02913105; Laffitte et al. 2017
	Diabetic nephropathy	Randomized, patient and investigator blinded, placebo-controlled study	Recruiting	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2019. https://clinicaltrials.gov/ct2/show/NCT03804879
TGR5 agonist					
SB756050	T2D	Single-blinded randomized, placebo-controlled, staggered-parallel, escalating-dose study	Completed	Phase I	US National Library of Medicine. ClinicalTrials.gov. 2017. https://clinicaltrials.gov/ct2/show/NCT00733577; Hodge et al. 2013
Ursolic acid	Metabolic syndrome X	Randomized, double-blind, placebo-controlled study	Completed	Phase II	US National Library of Medicine. ClinicalTrials.gov. 2015. https://clinicaltrials.gov/ct2/show/NCT02337933
Dual FXR/TGR5 agonist					
INT-767	PSC			Phase I	Intercept Pharmaceuticals Initiates Phase 1 Study of INT-767, a Dual FXR and TGR5 Agonist. 2015. http://ir.interceptpharma.com/news-releases/news-release-details/intercept-pharmaceuticals-initiates-phase-1-study-int-767-dual
UDCA	T2D and chronic liver diseases	Open label	Recruiting	Phase IV	US National Library of Medicine. ClinicalTrials.gov. 2011. https://clinicaltrials.gov/ct2/show/NCT01337440
CDCA	Severe obesity	Open label	Recruiting	Phase IV	US National Library of Medicine. ClinicalTrials.gov. 2016. https://clinicaltrials.gov/ct2/show/NCT02876484

FXR: farnesoid X receptor; OCA: obeticholic acid; TGR5: G protein-coupled BA receptor; UDCA: ursodeoxycholic acid; CDCA: chenodeoxycholic acid; NASH: non-alcoholic steatohepatitis; NAFLD: non-alcoholic fatty liver disease; ALT: alanine transaminase; PSC: primary sclerosing cholangitis; PBC: primary biliary cirrhosis; T2D: type 2 diabetes

for drug design of a selective modulator for FXR or TGR5, or dual FXR/TGR5 agonist with pharmaceutical value.

3.2 Bariatric surgeries

In addition to pharmacological agents, there are other therapies which target BA signaling. Bariatric surgery is the most effective strategy, to date, for the treatment of obesity and its comorbidities. The outcomes and mechanisms for bariatric surgery are under extensive investigation (Tian et al., 2017; Sandoval, 2019). The BA receptors FXR and TGR5 are shown as two potential targets for bariatric surgery VSG (Ryan et al., 2014; Ding et al., 2016; McGavigan et al., 2017). The mRNA expression of FXR, small heterodimer partner (SHP), and peroxisome proliferator-activated receptor α (PPARα) in the liver was significantly higher in the laparoscopic sleeve gastrectomy (SG) group than in the Sham-operated group (Watanabe et al., 2018), further supporting the idea that the therapeutic effect of SG on NAFLD may also be by targeting the FXR signaling pathway. Duodenal-jejunal bypass surgery has a direct effect on NASH improvement. The increase of plasma BA level followed by the stimulation of FXR signaling may contribute to this phenomenon (Tsuchiya et al., 2018).

CYP8B1 is a BA synthetic enzyme expressed primarily in hepatocytes (Wang et al., 2005, 2007). CYP8B1 is required for the synthesis of 12α-hydroxylated BAs and thereby determines the ratio of 12α-hydroxylated to non-12α-hydroxylated BAs (Li and Chiang, 2014). The BA profile hydrophobicity plays a crucial role in intestinal fat absorption, and *Cyp8b1* ablation prevents Western diet-induced weight gain and hepatic steatosis because of impaired fat absorption (Bertaggia et al., 2017). Genetic ablation of *Cyp8b1* decreases BA profile hydrophobicity and protects against several metabolic diseases, including obesity and T2D, in mice (Zaborska and Cummings, 2018). Of note, the percentage of 12α-hydroxylated BAs was reduced after SG, associated with overexpression of V-Maf avian musculoaponeurotic fibrosarcoma oncogene homolog G (MAFG) and lower mRNA and protein levels of CYP8B1 (Wang et al., 2017). Interestingly, MAFG is an FXR target gene that transcriptionally represses CYP8B1 expression (de Aguiar Vallim et al., 2015); these results suggest that in addition to FXR and TGR5, the downstream

CYP8B1 may present an alternative target for bariatric surgery.

4 Future perspectives

Although there are some conflicting reports about the BA level in metabolic diseases, as mentioned in this review, there is increasing evidence that the BA composition plays a more important role than the total BA level in regulating gut microbiome and metabolism. One important example is the BA hydrophobicity modulated by the ratio of 12α-hydroxylated to non-12α-hydroxylated BAs that is switched by the activity of CYP8B1. However, FXR suppresses expression of both CYP7A1 and CYP8B1, implying a possible mechanism for the side effects of FXR agonists. Selective regulation of CYP8B1 expression may lead to the development of BA-based therapeutics with fewer adverse side effects. TGR5 has been shown to reduce hepatic CYP8B1 protein expression with no effect on hepatic CYP7A1 expression (McGavigan et al., 2017), whereas drug development by targeting TGR5 shows other side effects beyond BA signaling. Thus, specific genetic modulation or inhibition by directly targeting CYP8B1 may be a promising therapeutic modality for metabolic diseases.

There is also a conundrum on whether activating or inhibiting FXR will be beneficial for maintaining the homeostasis of metabolism. Numerous data demonstrate that FXR agonists exert efficacious therapeutic effects on metabolic diseases. This can be supported by the drug development processes mentioned in Table 1. However, there is increasing evidence that FXR antagonists ameliorate the disease condition by inhibiting the activity of FXR, especially in the intestine. HFD-fed intestine-specific FXR-null mice show lower diet-induced obesity; tempol improves obesity via inhibition of intestinal FXR by T-β-MCA (Li et al., 2013). It is well known that throughout the gastroenteric tract the most crucial role of FXR is to maintain the BA homeostasis, associated with the homeostasis of gut microbiota, resulting in the final metabolic homeostasis. The regulation of FXR activity by the BAs in the intestine may mainly be the intermediate steps involved in this homeostasis maintenance. With the slow process of drug development targeting BA receptors including FXR and

TGR5, side effects are the most difficult obstacles, suggesting over activating of the BA receptors might cause side effects because of the interruption of this homeostasis, and fine tuning by modulators might be more feasible. Furthermore, directly modulating the downstream enzymes included in BA synthesis might be an alternative strategy for BA-related diseases.

In a similar way, it will be necessary to identify which gut microbes are beneficial and feasible. Careful selection of commensal bacteria for probiotics may lead to an effective therapy for metabolic diseases. Therefore, with an increasing understanding of the complicated interaction between the gut microbiota and host, studies on single gut symbioses are urgently required to define the exact functions of a given bacterium in causing disease or maintaining health.

It is important to note that, to date, most findings on the crosstalk among BAs, gut microbiota and metabolic diseases are from rodent models. However, the BA composition of rodents and humans is different. In mice, the majority of CDCA is converted to α-muricholic acid (MCA) and β-MCA, and accordingly, CA and MCAs are the major primary BAs in the mouse BA pool. Therefore, it is not well known whether alterations of BAs in mice also occur in humans. It is noteworthy that inconsistent results have been seen in a few human studies. Thus the differences in BA composition between humans and mice need to be considered when interpreting data from mice studies. Additionally, it is of particular importance to elucidate how a human gut microbiota influences BA composition. To improve the clinical trial of drug candidates, testing on large animals such as monkeys in preclinical trial might be helpful for the medical translation.

Contributors

Li-hua JIN drafted and revised the manuscript. Zhi-peng FANG and Min-jie FAN drafted the manuscript. Wen-dong HUANG revised the manuscript. All authors approved the final manuscript.

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Compliance with ethics guidelines

Li-hua JIN, Zhi-peng FANG, Min-jie FAN, and Wen-dong HUANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：胆汁学：从研究到临床

概　要：胆汁酸广为人知的功能是帮助脂类的消化和吸收。近几年的大量研究表明：胆汁酸除了在胆固醇代谢平衡、肝肠循环以及调节肝脏再生等方面的作用以外，还可以作为一类肠道激素调节机体的生理和病理反应，包括糖脂代谢、能量消耗、炎症、肿瘤、心血管疾病和神经系统疾病等。胆汁酸与肠道菌群关系密切，这可能也是其在各器官中发挥重要功能的原因之一。胆汁酸调节的信号主要通过其特异的胆酸核受体与G蛋白偶联的胆酸膜受体进行调节。研究表明一些药理制剂或者外科减肥手术在代谢性疾病中的疗效也是通过靶向胆汁酸信号实现的。本文主要总结了胆汁学最新的研究进展，重点关注了与胆汁酸信号通路调节相关的转化医学研究进展。

关键词：胆汁酸；肠道菌群；法尼醇受体；G蛋白偶联胆汁酸受体；代谢性疾病