The bile acid receptor TGR5 and cholestasis

Grégory Merlen, Valeska Bidault-Jourdainne, Isabelle Doignon, Isabelle Garcin, Thierry Tordjmann[*]

INSERM U.1193, Faculté des Sciences d'Orsay, Université Paris Saclay, 91400 Orsay, France

*Correspondence: Thierry Tordjmann, INSERM U.1193, Faculté des Sciences d'Orsay, Université Paris Saclay, Bât. Henri Moissan, 17 Avenue des Sciences, 91400 Orsay, France. thierry.tordjmann@universite-paris-saclay.fr

Abstract

During liver injury and cholestasis, the mechanisms allowing the organ to protect itself with the aim of maintaining biliary homeostasis are not completely understood. Central to their biological roles, bile acids (BAs) and their receptors constitute a signaling network with multiple molecular and cellular impacts on both liver repair and protection from BA overload. BA signal through nuclear [mainly farnesoid X receptor (FXR)] and membrane [mainly G protein-coupled BA receptor 1 (GPBAR-1), aka Takeda G protein-coupled receptor 5 (TGR5)] receptors, in which activation elicits a wide array of biological responses. So far, most of the studies have been focused on FXR signaling as hepato-protective, TGR5 being less explored to this regard. While the liver faces massive and potentially harmful BA overload during cholestasis, it is crucial to understand that BAs induce also protective responses contributing not only to reduce the inflammatory burden, but also to spare liver cells and their repair capacities. Based on the available literature, the TGR5 BA receptor protects the liver in the cholestatic context and counteracts BA overload with the aim of restoring biliary homeostasis mainly through the control of inflammatory processes, biliary epithelial barrier permeability, and BA pool composition. Mouse experimental models of cholestasis reveal that the lack of TGR5 was associated with exacerbated inflammation and necrosis, leaky biliary epithelium, and excessive BA pool hydrophobicity, resulting in biliary cell and parenchymal insult, and compromising optimal restoration of biliary homeostasis and liver repair. There are thus widely opened translational perspectives with the aim of targeting TGR5-related signaling or biological responses to trigger protection of the cholestatic liver.

Keywords

Liver, bile acids, cholestasis, Takeda G protein-coupled receptor 5 (TGR5), hepatoprotection

Introduction

Bile acids (BAs) are synthesized from cholesterol (CT) in hepatocytes (primary BA), transported in the bile canaliculus lumen, flow through the biliary tree towards the duodenum, progress in the intestine, and are finally massively reabsorbed in the ileum, going back to the liver through the portal circulation,

accomplishing the enterohepatic cycle (EHC). A small percentage of BAs (2–5%) escape from reabsorption and keep on flowing through the colon where they are transformed by the gut microbiota into more hydrophobic secondary BA (more toxic) as compared with primary BA. Thanks to their high hydrophobicity, secondary BAs passively cross the colon epithelium, joining other BA in the so-called "BA pool". Integrity of the EHC is central to biliary homeostasis during which BAs are almost confined in the gut-liver axis, with only BA traces spilling over into the general circulation [1, 2]. If ileal BA reabsorption would be defective, exacerbated BA loss in the feces would occur. On the opposite, during cholestasis in case of insufficient trans-hepatocyte BA flow (reduced sinusoidal and/or canalicular BA transport) or when bile duct obstruction occurs, BA overload (hepatic and systemic) will appear. In the biliary tree, the gallbladder (GB) in addition to its physiological role of bile reservoir, also has a critical impact on bile composition [3], and is particularly enriched in the BA Takeda G protein-coupled receptor 5 (TGR5) receptor [4, 5]. Precise mechanisms by which BAs induce hepatic injury remain controversial, with reports on both direct and indirect BA cytotoxicity [6–8]. Direct BA-induced cell death mechanisms include plasma membrane damage (for high concentrations of hydrophobic BA), mitochondrial injury [9], and reactive oxygen species (ROS) production [10], as well as induction of apoptosis [11]. Reported indirect mechanisms mainly focus on the impact of BA on inflammatory processes [12], but the precise machinery linking BA and inflammation is still difficult to precisely depict, with BA species and targeted cell types having a crucial impact [7].

BA overload in liver pathophysiology

When bile secretion or bile flow is defective (i.e. in case of cholestasis), the organism is overloaded with BA which cannot be secreted or excreted in bile. Such BA overload also takes place when the balance between BA return from the gut and BA uptake in the liver is altered. This type of imbalance is observed when part of the liver is either removed [partial hepatectomy (PH)] or injured [13, 14]. In that non-cholestatic setting, BA secretion is normal or even increased, while the remnant hepatocyte mass is too reduced to cope with the important BA load coming back from the gut: this results in a significant accumulation of BA in the systemic blood and consequently in the liver and in the whole organism (referred as a "BA spillover" or "BA overload"). More precisely, systemic BA concentration will rise from 2–5 μmol/L in basal conditions, to 200–500 μmol/L or even more during cholestasis [15] or in the first minutes and hours after PH as reported in mice and rats [13, 16, 17], but also in humans [2, 13]. With that said, it can be envisioned to consider BA overload as a possible consequence of any type of significant liver injury, and not only as a specific hallmark of cholestasis per se [2, 18, 19].

Another important aspect of BA overload is that it surprisingly has not only deleterious but also positive influence on liver repair processes [14, 19–21]. Thus, BA should be envisioned as double edge swords, signaling in hepatocytes for their protection and favoring their proliferation on the one hand, whilst being deleterious for those cells on the other hand [14].

BA receptors and signaling

Since specific nuclear and membrane BA receptors have been discovered, BA signaling is known to be coupled mainly with the nuclear receptor farnesoid X receptor (FXR), and the G protein-coupled BA receptor 1 (GPBAR-1, or TGR5) [22]. Although FXR has been amply studied in the cholestatic and hepatoprotection contexts, this is not the case for TGR5 which has been less explored; only recently TGR5 has proved to be hepatoprotective [14, 16, 23, 24]. As a whole, TGR5 has been more reported in non-hepatic cells and tissues than in the liver [25, 26], in which its functions are relatively less described [23, 27]. Importantly, secondary BAs [deoxycholic acid (DCA) and lithocholic acid (LCA)] either conjugated or not to taurine or glycine, exhibit the highest affinity for TGR5 [median effective concentrations (EC_{50}): 0.5–1 μmol/L], over the primary BA cholic acid (CA) and chenodeoxycholic acids (CDCAs) [25, 28]. In line, hydrophilic BAs like ursodeoxycholic acid (UDCA) display very weak affinities for TGR5 (EC_{50}: 36 μmol/L) [25, 29, 30]. Interestingly, TGR5 is also activated by hyocholic acid (HCA) in the pig, with potential therapeutic impact on glucose metabolism [31]. When activated,

TGR5 classically induces cyclic adenosine monophosphate (cAMP) production, although it can also interfere with intracellular calcium signaling [32, 33]. A number of studies showed an association of TGR5 activation with epidermal growth factor receptor (EGFR) transactivation [34–37] and ROS production [32, 38], providing evidence that TGR5 is able to modulate cell proliferation and apoptosis [39]. TGR5 is also reported as a regulator of energy homeostasis and inflammation, and as such has the potential to be a therapeutic target in the context of metabolic syndrome, with a particular interest in diabetes and obesity-related non-alcoholic steatohepatitis (NASH) [40–44]. As to TGR5 expression in the liver, it is weak or not significant in hepatocytes at least in rodents, but TGR5 has repeatedly been reported as highly concentrated in the GB and biliary tract [5, 45], as well as in endothelial and Kupffer cells [46].

TGR5 and bile secretion

The role of TGR5 on bile secretion is not really established, i.e. the TGR5 impact on transport of BA, phospholipid, and ion in bile has not been fully explored (Figure 1) [47–49]. Authors including us reported in TGR5-knockout (KO) as compared with wild-type (WT) mice a smaller BA pool size, a more hydrophobic BA composition, and a slightly reduced bile flow; intriguingly the effects of TGR5 agonists on bile flow were only slight or not obvious [16, 47–49]. In the rat, TGR5 stimulation was choleretic through BA-independent effects [50] (Figure 2). In mice, we did not find any direct effect of TGR5 stimulation on bile flow [4]. Noteworthy, neither BA synthesis nor canalicular bile secretion is expected to be directly impacted by TGR5 because this receptor expression is very weak in hepatocytes. Slight TGR5 hepatocyte expression has been recently reported [45], with a possible functional impact on glucose metabolism, but consequences on bile secretion have not been specifically explored. We recently found that in the lack of TGR5, the expression of BA synthesis enzymes and BA transporters messenger RNAs (mRNAs) was not changed as compared with WT mice [16]. In the same line, hepatocyte-specific TGR5-KO and WT mice exhibited similar responses to PH [4], while BA overload was exacerbated, and bile secretion adaptation was altered post-PH in total TGR5-KO mice [16]. Conversely, as TGR5 expression in cholangiocytes is high, this points to a possible impact of this receptor and related signaling on the ductular contribution to bile production. Indeed, TGR5 regulates Cl⁻ secretion in human GB [51] and other epithelial cells through an impact on cystic fibrosis transmembrane regulator (CFTR) (Figure 1) [52]. However, since these reports, no study quantifying the physiological contribution of TGR5 to ductular bile secretion has been published.
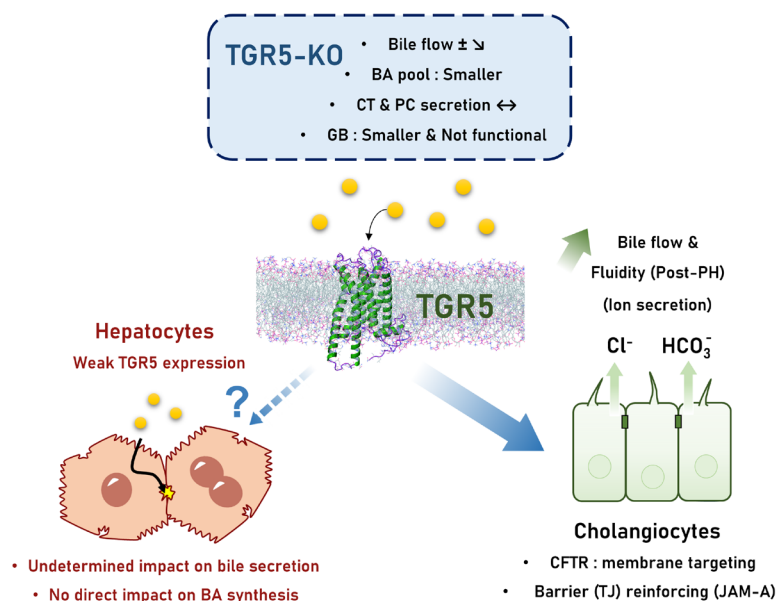

Figure 1. TGR5 control on bile secretion. Bile secretion in TGR5-KO mice exhibit only mild abnormalities, including a discrete (if any) bile flow reduction, a smaller BA pool, and a smaller and non-functional GB. Direct effects related to TGR5 on hepatocyte bile secretion are undetermined although unlikely because of a very weak expression of this receptor. TGR5 mainly operates its control on bile secretion/bile flow through the regulation of cholangiocyte processes: ion (chloride and bicarbonate) secretion, and tight junction (TJ) barrier reinforcing. JAM-A: junctional adhesion molecule-A; PC: phosphatidylcholine; ±: still discussed; ?: unknown mechanism; full line arrow: documented effect; dashed line arrow: uncertain effect
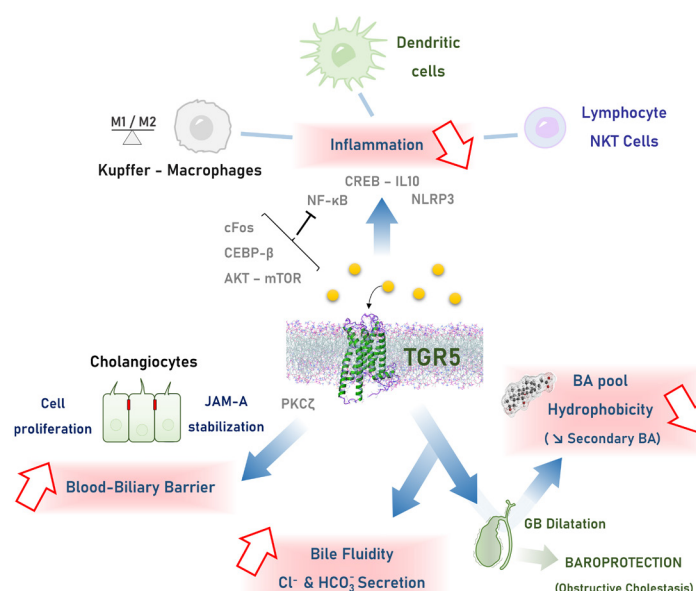
Figure 2. Mechanisms of TGR5-mediated hepato-protection. TGR5 engagement is mainly associated with: 1. reduction of inflammation in the liver, through multiple signaling pathways in monocytes-macrophages, lymphocytes and dendritic cells; 2. biliary epithelial barrier reinforcing by promoting cholangiocyte proliferation and TJ strengthening through stabilization of the JAM-A protein; 3. modulation of bile composition, through 2 main mechanisms converging on making a less toxic bile: a. stimulation of chloride and bicarbonate transport in bile, reducing BA protonation and protecting cells from BA toxicity; b. GB dilatation, favoring BA reabsorption reported as the cholecysto-hepatic shunt, reducing the amount of hydrophobic secondary BA. CEBP-β: CCAAT/enhancer-binding protein beta; CREB: cAMP response element-binding protein; IL10: interleukin 10; mTOR: mechanistic target of rapamycin; NF-κB: nuclear factor-kappa B; NKT: natural killer T; NLRP3: Nod-like receptor family pyrin domain containing 3; PKCζ: protein kinase C-zeta

TGR5-dependent protective responses during cholestasis

During cholestasis, but also after liver injury or immediately after PH [19, 20], it has been well described that hepatocytes handle excessively raised intracellular BA concentrations by using so-called "adaptive reactions" [53, 54] that are at least in part FXR-dependent. This adaptation is performed by the modulation of gene transcription for membrane BA transporters, as well as for BA synthesis and conjugation enzymes [16, 54], with aim of counteracting intracellular BA overload [53]. In case of significant parenchymal loss in the context of cholestasis, hepatocyte cell cycle progression can also be boosted through FXR-dependent mechanisms [20], contributing to replenish a functional hepatocyte mass that will progressively restore a full BA handling capacity to the liver. Also in enterocytes of the ileum, fibroblast growth factor 15 (FGF15) induction through BA-induced FXR activation has been identified as an important suppressor of hepatocyte BA synthesis [55], which is protective during BA overload and cholestasis [56]. Besides these FXR-dependent processes during cholestasis, TGR5-elicited hepato-protective pathways have been also reported, as described in the following (Figure 2).

Anti-inflammatory effects

One of the most frequently reported mechanisms of TGR5-mediated liver protection involves its anti-inflammatory properties (Figure 2), and the bulk of related studies in this field is growing [57]. As pointed out by several papers, BAs induce pro-inflammatory effects and this can be considered as crucial for mechanisms of liver injury during cholestasis [7, 12, 58, 59]. During cholestasis, a primary event would be that BAs activate cytokine production in hepatocytes, and neutrophils would secondarily contribute to liver injury [59]. Although the role of macrophages and Kupffer cells during cholestasis remains controversial, the anti-inflammatory action of TGR5 during cholestasis likely occurs at least in part in these cells, downstream an initiating effect of BA in hepatocytes (Figure 1) [5, 45, 46, 60]. In line, early and recent studies reported that TGR5 activation reduces the effect of lipopolysaccharide (LPS) on cytokine gene induction in rat Kupffer cells and mouse macrophages [60, 61]. Consequently, anti-inflammatory effects of TGR5 have been reported as protective in several rodent experimental models (see below) [40, 61–65]. From a signaling perspective in macrophages, TGR5 stimulation blunts NF-κB activation through mechanisms depending on cAMP production, involving an inhibition

of Ikappa beta (IκB) phosphorylation and p65 nuclear translocation [61]. Anti-inflammatory effects of TGR5 engagement also involve impact on the AKT-mTOR complex 1 pathway, on CEBP-β induction, and on a complex modulation of the NLRP3 inflammasome activity [66–69]. We previously observed that cytokine induction was exacerbated in TGR5-KO as compared to WT mice after either PH or bile duct ligation (BDL), and that it enhanced cholestasis [70], favored hepatocyte necrosis, and delayed regeneration [16]. This was later confirmed by others [39, 71, 72], and reproduced by us [4, 5] in the BDL model. In line, TGR5-KO mice harbored exacerbated sensitivity to LPS-induced hepatic inflammation, while treatment with a TGR5 agonist improved WT mice [61]. In the more chronic *Mdr2*-KO mice model of primary sclerosing cholangitis (PSC), specific TGR5 stimulation alone did not improve the phenotype, in contrast with a dual FXR/TGR5 agonist [49]. As a whole in the lack of TGR5, mice harbored an excessive susceptibility to pro-inflammatory stimuli, not only in the liver but also outside this organ [64].

As a whole, TGR5 engagement during cholestasis or after liver injury likely tunes Kupffer cells and macrophage activation, with an impact on hepatocyte protection and proliferation allowing compensation of parenchymal cell loss [73, 74]. In line, it has been reported that TGR5 stimulation stabilized the M1/M2 ratio of macrophage populations towards an anti-inflammatory phenotype [75, 76]. Importantly, translational data suggested that such TGR5-dependent anti-inflammatory responses may also occur in human patients with liver failure, with an impact on clinical outcome [77]. In the same line, it has been recently reported that during PSC in humans, as well as in the *Mdr2*-KO murine model, TGR5 downregulation in biliary epithelial cells contributed to the pro-inflammatory phenotype observed in cholangiocytes during this disease [78].

Biliary epithelial barrier

During cholestatic liver diseases, especially when obstructive components occur within their pathophysiological course, the biliary epithelium integrity is obviously key to protect parenchymal cells (hepatocytes) from BA-induced injury. This may involve not only the replacement of dead cells but also the strengthening of barrier function [79]. In fact, the "blood-biliary barrier (BBB)" [80], namely the separation between blood and bile, is mainly formed by both epithelial liver cell types, hepatocytes and cholangiocytes, and especially involves their respective TJs that seal adjacent cells (Figure 1). Hepatocytes, due to their physiological position along the biliary path, are obviously frontally exposed to BA, but they share this frontline position in the BBB with the downstream layer of cholangiocytes that shields parenchyma from BA-induced damage. In the biliary epithelium as well as in any lining epithelial sheet, TJs constitute a regulated barrier to the diffusion of molecules in the paracellular space, allowing selective exchanges of small molecules (especially ions) between apical and basolateral microenvironments [81]. TJs are composed of occludin, claudins, and JAMs, which constitute the main core of plasma membrane proteins which associate with cytoplasmic actin-binding proteins [including zonula occludens (ZO) proteins] [81, 82]. In contiguous cells, extracellular domains of the transmembrane proteins interact with their counterparts and seal plasma membranes. Paracellular permeability is obviously a regulated process, although the mechanisms involved still remain poorly explored. It is reported that occludin, JAM-A, and claudin-4 phosphorylation can modulate paracellular permeability, although this aspect has been poorly studied in liver epithelial cells [83, 84]. In the liver, the role of selected TJ proteins expressed in hepatocytes in the regulation of bile secretion has been explored [85–87], in contrast with TJ proteins expressed in cholangiocytes of which pathophysiological impact is not strongly and specifically delineated. It is hypothesized that if inter-cholangiocyte TJs would be altered, bile duct walls would leak, resulting in bile-induced parenchymal injury. However, strong evidence confirming this pathophysiological view is still lacking [88–90], with a few exceptions. Indeed, severe liver disease is observed in patients with neonatal ichtyosis and sclerosing cholangitis (NISCH) syndrome, a primary (mutational) claudin-1 defect [91, 92], or with the more recently described mutations in the TJ-associated protein *ZO-2* gene [93]. In line, TJP2 inactivation in both hepatocytes and cholangiocytes in mice resulted in altered biliary homeostasis, with exacerbated cholestatic features upon CA diet [94]. But still, the balanced influence of inter-cholangiocyte and inter-hepatocyte TJ protein

defects during these diseases is not defined, in part because TJ protein repertoires differ in hepatocytes and cholangiocytes [95]. In mice also, double inactivation of *ZO-1* and *ZO-2* genes in hepatocytes resulted in profound alteration in hepatic tissue architecture and functions leading to lethality by 6 weeks of age [96]. During inflammatory cholestatic diseases, hepatic TJ can also be secondarily altered [88, 89, 97] by cytokines and endotoxin [97]. Interestingly, double deletion of *β-* and *γ-catenins* genes in hepatocytes in mice resulted in complete disorganization of TJ proteins and structure. Those mice displayed a complete loss of hepatocyte BBB, and as a consequence severe cholestasis-induced parenchymal injury [98]. Crucial impact of TJ integrity for liver recovery after cholestatic injury in the 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) mouse model has also been reported, pointing out that vaso-intestinal peptide (VIP) secreted by periportal mesenchymal cells and cholangiocyte VIP receptor 1 were involved in a crucial crosstalk for TJP expression in this experimental setting [99]. As a whole, the literature points to a crucial hepatoprotective role of TJ in the liver, although it is better documented in mice than in human patients, and more specifically addressed in hepatocytes than in cholangiocytes.

The regulatory role of BA on paracellular permeability is mostly known as deleterious, because BAs enhance paracellular passage in intestinal [100] and respiratory epithelial cells [101], but signal transduction mechanisms and in particular the BA receptors likely involved were not reported. However, other reports established that TGR5-dependent BA signaling reinforces barriers in several murine tissues, i.e. intestinal, endothelial, retinal, and blood-brain barriers [102–105]. Presumably, BAs exert their effects on paracellular permeability through multiple pathways, possibly enhancing or reducing it as a function of the pathophysiological context and depending on the interested cell types. As the biliary tract is naturally exposed to high concentrations of BA, searching for an impact of BA signaling on cholangiocyte paracellular permeability in physiological and cholestatic conditions is thus highly relevant. In a recent study, we found that BA-induced TGR5 engagement protected the liver parenchyme against BA overload during cholestasis through a reduction of paracellular permeability [5]. More precisely TGR5 activation induces the PKCζ-dependent phosphorylation of the TJ protein JAM-A, which results in a protective modulation of the TJ barrier function (Figure 1). This was shown in two mice experimental models of cholestasis [BDL and 1 naphthyl isothiocyanate (ANIT) intoxication], and importantly, we provided data from patients with primary biliary cirrhosis (PBC) and PSC suggesting that the TJ protein JAM-A may be protective in the biliary epithelium during human cholestatic diseases, rising the therapeutic challenge of targeting this protein and TGR5 related pathways in the future.

Bile composition

Although scarce literature is available, it is suggested that the impact of TGR5 on bile composition operates mainly through cholangiocyte-dependent processes (Figure 2). As evoked above, the TGR5 effect on both CFTR and the anion exchanger 2 (AE2) mediates chloride/bicarbonate exchange across the apical membrane, promoting the formation of the so-called "bicarbonate umbrella". Consequently, the alkaline environment enables the reduction of BA protonation and thus protects the cholangiocytes (bile ducts) and hepatocytes (liver parenchyma) from BA cytotoxicity in liver injury and cholestatic contexts [49, 106].

This might constitute an adaptive mechanism enhancing bile secretion and fluidity through which TGR5 would protect the liver from BA toxicity during repair [16] or cholestasis. TGR5 is also expressed on cholangiocyte cilia and may regulate bile flow through an effect on this organelle [107, 108], via a coupling with HCO_3^- secretion in bile [109]. Finally, TGR5 may modulate water handling in the biliary epithelium, although no direct evidence has been reported yet, while further studies would be necessary to eventually extend data published in kidney epithelial cells [110, 111].

It is also emphasized that the BA pool composition itself including its hydrophobicity is crucial to liver homeostasis [112, 113]. Excessive BA pool hydrophobicity is indeed reported to be noxious for liver repair and cholestatic contexts [56, 114–118]. This is best exemplified in the *BSEP/abcb11$^{-/-}$* mice, which exhibit non-progressive mild cholestasis [119], contrasting with the severe human progressive familial intrahepatic cholestasis type 2 (PFIC2, severe progressive cholestasis in children) phenotype. This discrepancy is, at least in part, due to the less hydrophobic (less toxic) BA pool observed in *Bsep$^{-/-}$*

mice, as it is enriched in hyperhydroxylated BA [120]. These BA pool features are viewed as protective against cholestatic liver injury not only in mice [119], but also in children with progressive intrahepatic cholestasis [121]. Nice proof of concept of this protection has been reported in double *Mdr2*$^{-/-}$ and *Bsep*$^{-/-}$ mice, in which hyper-hydrophilic BA prevented liver damage caused by the lack of biliary phospholipids [122]. Recent studies also pointed out that an efficient activation of the BA receptors (entirely dependent on the BA pool composition) had a strong impact on alcoholic and non-alcoholic liver diseases [65, 123]. In line, Cyp8b1 inhibition, reducing BA pool hydrophobicity, prevented steatosis in high-fat diet-fed mice [124]. It has also been emphasized in a murine NASH preclinical model that the BA pool composition was dramatically altered, with poor signaling power on both FXR and TGR5, and that DCA diet complementation prevented liver disorders in this model [125]. Recently, the BA metabolizing enzyme Cyp2c70 which converts CDCA (a hydrophobic primary BA) into the more hydrophilic muricholic acid (MCA), has been identified as a master regulator of the BA pool composition in mice [126]. This enzyme is lacking in humans, explaining in part why the BA pool is more hydrophobic in humans than in mice. Interestingly, *Cyp2c70*$^{-/-}$ mice exhibit a human-like more hydrophobic BA pool as compared with control animals, associated with liver inflammation [127] and altered FXR signaling [128]. More recently, it was reported that a cholangiopathy with biliary fibrosis developed in female *Cyp2c70*$^{-/-}$ mice, a phenotype reversed upon UDCA treatment [129], reinforcing the fact that BA pool hydrophobicity had a crucial pathophysiological impact on liver disease outcome.

Others and we reported that BA pool composition was more hydrophobic in TGR5-KO than in WT mice [4, 16, 47, 48, 130], as measured in bile, plasma, liver, and feces [4, 16]. More precisely, MCA the most hydrophilic primary BA in mice, and the MCA/CA ratio, were strongly reduced, whereas secondary (more hydrophobic) BAs were significantly overrepresented in TGR5-KO as compared with WT mice [16]. Our recent study explored different mechanistic hypotheses to support this phenotype, linking TGR5 with BA pool composition [4]. First, we did not find any significant direct impact of TGR5 on BA synthesis. Second, based on gut microbiota transfer experiments, we provided data showing that despite the lack of TGR5 was associated with an intestinal dysbiosis, this latest was not responsible for the observed more hydrophobic BA pool. This is in contrast with data indicating that FXR-mediated modification of the gut microbiota resulted in reshaping the BA pool (LCA enrichment) towards TGR5 stimulation and improved glucose tolerance through intestinal glucagon-like peptide-1 (GLP-1) secretion [131]. We finally focused on TGR5-mediated impact on the GB, as it is reported as the tissue displaying the highest TGR5 expression, at least in mice [132], suggesting that this receptor may have a physiological impact on GB functions, including GB relaxation as reported [48] and potentially BA reabsorption also known as the cholecysto-hepatic shunt [3]. This shunt is expected to allow a short circuit by which BAs from the GB return directly to the liver via the portal circulation without passing through the intestine [3], thereby restricting the amount of toxic secondary BA in the pool. Importantly, we found that in the lack of TGR5, defective GB dilatation resulted in reduced cholecysto-hepatic shunt, building a more hydrophobic BA pool. We also put forward TGR5-dependent hepato-protective properties of GB dilatation in the setting of obstructive cholestasis [4]. We showed that after cholecystectomy (as compared with spared GB), BDL mice had more severe necrotico-inflammatory liver injury, more bile duct dilatation, and reduced survival rate. Importantly this phenotype was not found in TGR5-KO mice, suggesting that TGR5-dependent GB dilatation was crucial in hepatoprotection upon obstructive cholestasis [4].

Therapeutic perspectives

In liver diseases in general, BAs as well as their analogues and derivatives begin to be viewed as potential or patent therapeutic agents. Indeed, BAs are now well known for their signaling properties on nuclear or transmembrane receptors that modulate several cellular functions as explained above [28, 133, 134]. In this perspective, FXR-activating therapies have been developed for their benefit in non-alcoholic fatty liver disease (NAFLD)/NASH, metabolic syndrome-related liver diseases, and cholestatic liver diseases (i.e. in PBC) [133]. Besides these approaches, preclinical mouse experimental data also suggest that TGR5 might be a potential target for liver disease treatments. Unfortunately, encouraging mice data have not yet been

translated towards human clinics. The multi-organ TGR5 expression and the related potential of unwanted intra- and extra-hepatic effects are challenging liver researchers and pharmaceutical industry in their quest for TGR5-mediated hepato-protective therapies.

Conclusions

BAs are particularly powerful molecules because during the course of cholestasis and liver injury, they contribute both to injure liver cells and to launch protection and repair reactions. Central to this duality, BAs generate corresponding signaling pathways through the binding to two main receptors, FXR and TGR5. Compiled literature strongly supports a hepato-protective role of TGR5 in mice during cholestasis and liver injury, through a combination of several regulatory actions of this receptor on inflammation, cholangiocyte secretion, biliary epithelial barrier permeability, and BA pool composition. Targeting these pathways should be considered, expecting for direct or indirect reduction of BA-induced tissue injury while optimizing liver regeneration potential. Future studies should delineate in depth TGR5-dependent mechanisms controlling these processes in the biliary epithelium and other liver and non-liver cells, with the aim of setting-up TGR5-based therapies in hepato-biliary medicine.

Abbreviations

BAs: bile acids

BBB: blood-biliary barrier

BDL: bile duct ligation

CA: cholic acid

cAMP: cyclic adenosine monophosphate

CFTR: cystic fibrosis transmembrane regulator

FXR: farnesoid X receptor

GB: gallbladder

JAM-A: junctional adhesion molecule-A

KO: knockout

MCA: muricholic acid

NASH: non-alcoholic steatohepatitis

PH: partial hepatectomy

PSC: primary sclerosing cholangitis

TGR5: Takeda G protein-coupled receptor 5

TJ: tight junction

WT: wild-type

ZO: zonula occludens

Declarations

Author contributions

TT: Writing—original draft. GM, VBJ, ID and IG: Writing—review & editing.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical approval

Not applicable.

Copyright

References

1. Dawson PA, Karpen SJ. Intestinal transport and metabolism of bile acids. J Lipid Res. 2015;56:1085–99.

2. Hoekstra LT, van Lienden KP, Schaap FG, Chamuleau RAFM, Bennink RJ, van Gulik TM. Can plasma bile salt, triglycerides, and apoA-V levels predict liver regeneration? World J Surg. 2012;36:2901–8.

3. Debray D, Rainteau D, Barbu V, Rouahi M, El Mourabit H, Lerondel S, et al. Defects in gallbladder emptying and bile acid homeostasis in mice with cystic fibrosis transmembrane conductance regulator deficiencies. Gastroenterology. 2012;142:1581–91.e6.

4. Bidault-Jourdainne V, Merlen G, Glénisson M, Doignon I, Garcin I, Péan N, et al. TGR5 controls bile acid composition and gallbladder function to protect the liver from bile acid overload. JHEP Rep. 2021;3:100214.

5. Merlen G, Kahale N, Ursic-Bedoya J, Bidault-Jourdainne V, Simerabet H, Doignon I, et al. TGR5-dependent hepatoprotection through the regulation of biliary epithelium barrier function. Gut. 2020;69:146–57.

6. Maillette de Buy Wenniger L, Beuers U. Bile salts and cholestasis. Dig Liver Dis. 2010;42:409–18.

7. Woolbright BL, Jaeschke H. Inflammation and cell death during cholestasis: the evolving role of bile acids. Gene Expr. 2019;19:215–28.

8. Merlen G, Bidault-Jourdainne V, Kahale N, Glenisson M, Ursic-Bedoya J, Doignon I, et al. Hepatoprotective impact of the bile acid receptor TGR5. Liver Int. 2020;40:1005–15.

9. Yerushalmi B, Dahl R, Devereaux MW, Gumpricht E, Sokol RJ. Bile acid-induced rat hepatocyte apoptosis is inhibited by antioxidants and blockers of the mitochondrial permeability transition. Hepatology. 2001;33:616–26.

10. Reinehr R, Graf D, Häussinger D. Bile salt-induced hepatocyte apoptosis involves epidermal growth factor receptor-dependent CD95 tyrosine phosphorylation. Gastroenterology. 2003;125:839–53.

11. Miyoshi H, Rust C, Roberts PJ, Burgart LJ, Gores GJ. Hepatocyte apoptosis after bile duct ligation in the mouse involves Fas. Gastroenterology. 1999;117:669–77.

12. Allen K, Jaeschke H, Copple BL. Bile acids induce inflammatory genes in hepatocytes: a novel mechanism of inflammation during obstructive cholestasis. Am J Pathol. 2011;178:175–86.

13. Doignon I, Julien B, Serrière-Lanneau V, Garcin I, Alonso G, Nicou A, et al. Immediate neuroendocrine signaling after partial hepatectomy through acute portal hyperpressure and cholestasis. J Hepatol. 2011;54:481–8.

14. Merlen G, Ursic-Bedoya J, Jourdainne V, Kahale N, Glenisson M, Doignon I, et al. Bile acids and their receptors during liver regeneration: "dangerous protectors". Mol Aspects Med. 2017;56:25–33.

15. Jansen PLM, Ghallab A, Vartak N, Reif R, Schaap FG, Hampe J, et al. The ascending pathophysiology of cholestatic liver disease. Hepatology. 2017;65:722–38.

16. Péan N, Doignon I, Garcin I, Besnard A, Julien B, Liu B, et al. The receptor TGR5 protects the liver from bile acid overload during liver regeneration in mice. Hepatology. 2013;58:1451–60.

17. Naugler WE. Bile acid flux is necessary for normal liver regeneration. PLoS One. 2014;9:e97426.

18. Hoekstra LT, Rietkerk M, van Lienden KP, van den Esschert JW, Schaap FG, van Gulik TM. Bile salts predict liver regeneration in rabbit model of portal vein embolization. J Surg Res. 2012;178:773–8.

19. Meng Z, Wang Y, Wang L, Jin W, Liu N, Pan H, et al. FXR regulates liver repair after CCl$_4$-induced toxic injury. Mol Endocrinol. 2010;24:886–97.

20. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J, et al. Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration. Science. 2006;312:233–6.

21. Bhushan B, Borude P, Edwards G, Walesky C, Cleveland J, Li F, et al. Role of bile acids in liver injury and regeneration following acetaminophen overdose. Am J Pathol. 2013;183:1518–26.

22. Keitel V, Stindt J, Häussinger D. Bile acid-activated receptors: GPBAR1 (TGR5) and other G protein-coupled receptors. In: Fiorucci S, Distrutti E, editors. Bile acids and their receptors.Cham: Springer International Publishing; 2019. pp. 19–49.

23. Keitel V, Häussinger D. Role of TGR5 (GPBAR1) in liver disease. Semin Liver Dis. 2018;38:333–9.

24. Jourdainne V, Péan N, Doignon I, Humbert L, Rainteau D, Tordjmann T. The bile acid receptor TGR5 and liver regeneration. Dig Dis. 2015;33:319–26.

25. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem. 2003;278:9435–40.

26. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun. 2002;298:714–9.

27. Pols TWH, Noriega LG, Nomura M, Auwerx J, Schoonjans K. The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. J Hepatol. 2011;54:1263–72.

28. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. Nat Rev Drug Discov. 2008;7:678–93.

29. Sato H, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, et al. Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies. J Med Chem. 2008;51:1831–41. Erratum in: J Med Chem. 2008;51:4849.

30. Carino A, Biagioli M, Marchianò S, Fiorucci C, Zampella A, Monti MC, et al. Ursodeoxycholic acid is a GPBAR1 agonist and resets liver/intestinal FXR signaling in a model of diet-induced dysbiosis and NASH. Biochim Biophys Acta Mol Cell Biol Lipids. 2019;1864:1422–37.

31. Zheng X, Chen T, Jiang R, Zhao A, Wu Q, Kuang J, et al. Hyocholic acid species improve glucose homeostasis through a distinct TGR5 and FXR signaling mechanism. Cell Metab. 2021;33:791–803.e7.

32. Hong J, Behar J, Wands J, Resnick M, Wang LJ, DeLellis RA, et al. Role of a novel bile acid receptor TGR5 in the development of oesophageal adenocarcinoma. Gut. 2010;59:170–80.

33. Keitel V, Görg B, Bidmon HJ, Zemtsova I, Spomer L, Zilles K, et al. The bile acid receptor TGR5 (Gpbar-1) acts as a neurosteroid receptor in brain. Glia. 2010;58:1794–805.

34. Jensen DD, Godfrey CB, Niklas C, Canals M, Kocan M, Poole DP, et al. The bile acid receptor TGR5 does not interact with β-arrestins or traffic to endosomes but transmits sustained signals from plasma membrane rafts. J Biol Chem. 2013;288:22942–60.

35. Yasuda H, Hirata S, Inoue K, Mashima H, Ohnishi H, Yoshiba M. Involvement of membrane-type bile acid receptor M-BAR/TGR5 in bile acid-induced activation of epidermal growth factor receptor and mitogen-activated protein kinases in gastric carcinoma cells. Biochem Biophys Res Commun. 2007;354:154–9.

36. Casaburi I, Avena P, Lanzino M, Sisci D, Giordano F, Maris P, et al. Chenodeoxycholic acid through a TGR5-dependent CREB signaling activation enhances cyclin D1 expression and promotes human endometrial cancer cell proliferation. Cell Cycle. 2012;11:2699–710.

37. Nagathihalli NS, Beesetty Y, Lee W, Washington MK, Chen X, Lockhart AC, et al. Novel mechanistic insights into ectodomain shedding of EGFR ligands amphiregulin and TGF-α: impact on gastrointestinal cancers driven by secondary bile acids. Cancer Res. 2014;74:2062–72.

38. Cao W, Tian W, Hong J, Li D, Tavares R, Noble L, et al. Expression of bile acid receptor TGR5 in gastric adenocarcinoma. Am J Physiol Gastrointest Liver Physiol. 2013;304:G322–7.

39. Reich M, Deutschmann K, Sommerfeld A, Klindt C, Kluge S, Kubitz R, et al. TGR5 is essential for bile acid-dependent cholangiocyte proliferation *in vivo* and *in vitro*. Gut. 2016;65:487–501.

40. Pols TWH, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, et al. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. Cell Metab. 2011;14:747–57.

41. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab. 2009;10:167–77.

42. McGavigan AK, Garibay D, Henseler ZM, Chen J, Bettaieb A, Haj FG, et al. TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. Gut. 2017;66:226–34.

43. Ding L, Sousa KM, Jin L, Dong B, Kim BW, Ramirez R, et al. Vertical sleeve gastrectomy activates GPBAR-1/TGR5 to sustain weight loss, improve fatty liver, and remit insulin resistance in mice. Hepatology. 2016;64:760–73.

44. Gillard J, Picalausa C, Ullmer C, Adorini L, Staels B, Tailleux A, et al. Enterohepatic Takeda G-protein coupled receptor 5 agonism in metabolic dysfunction-associated fatty liver disease and related glucose dysmetabolism. Nutrients. 2022;14:2707.

45. Holter MM, Chirikjian MK, Govani VN, Cummings BP. TGR5 signaling in hepatic metabolic health. Nutrients. 2020;12:2598.

46. Keitel V, Reinehr R, Gatsios P, Rupprecht C, Görg B, Selbach O, et al. The G-protein coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells. Hepatology. 2007;45:695–704.

47. Vassileva G, Golovko A, Markowitz L, Abbondanzo SJ, Zeng M, Yang S, et al. Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation. Biochem J. 2006;398:423–30.

48. Li T, Holmstrom SR, Kir S, Umetani M, Schmidt DR, Kliewer SA, et al. The G protein-coupled bile acid receptor, TGR5, stimulates gallbladder filling. Mol Endocrinol. 2011;25:1066–71.

49. Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, et al. Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver injury in the $Mdr2^{-/-}$ ($Abcb4^{-/-}$) mouse cholangiopathy model by promoting biliary HCO_3^- output. Hepatology. 2011;54:1303–12.

50. Roda A, Pellicciari R, Gioiello A, Neri F, Camborata C, Passeri D, et al. Semisynthetic bile acid FXR and TGR5 agonists: physicochemical properties, pharmacokinetics, and metabolism in the rat. J Pharmacol Exp Ther. 2014;350:56–68.

51. Keitel V, Cupisti K, Ullmer C, Knoefel WT, Kubitz R, Häussinger D. The membrane-bound bile acid receptor TGR5 is localized in the epithelium of human gallbladders. Hepatology. 2009;50:861–70.

52. Hendrick SM, Mroz MS, Greene CM, Keely SJ, Harvey BJ. Bile acids stimulate chloride secretion through CFTR and calcium-activated Cl^- channels in Calu-3 airway epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2014;307:L407–18.

53. Geier A, Wagner M, Dietrich CG, Trauner M. Principles of hepatic organic anion transporter regulation during cholestasis, inflammation and liver regeneration. Biochim Biophys Acta. 2007;1773:283–308.

54. Csanaky IL, Aleksunes LM, Tanaka Y, Klaassen CD. Role of hepatic transporters in prevention of bile acid toxicity after partial hepatectomy in mice. Am J Physiol Gastrointest Liver Physiol. 2009;297:G419–33.

55. Uriarte I, Fernandez-Barrena MG, Monte MJ, Latasa MU, Chang HCY, Carotti S, et al. Identification of fibroblast growth factor 15 as a novel mediator of liver regeneration and its application in the prevention of post-resection liver failure in mice. Gut. 2013;62:899–910.

56. Modica S, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, et al. Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. Gastroenterology. 2012;142:355–65.e4.

57. Bertolini A, Fiorotto R, Strazzabosco M. Bile acids and their receptors: modulators and therapeutic targets in liver inflammation. Semin Immunopathol. 2022;44:547–64.

58. Cai SY, Ouyang X, Chen Y, Soroka CJ, Wang J, Mennone A, et al. Bile acids initiate cholestatic liver injury by triggering a hepatocyte-specific inflammatory response. JCI Insight. 2017;2:e90780.

59. Li M, Cai SY, Boyer JL. Mechanisms of bile acid mediated inflammation in the liver. Mol Aspects Med. 2017;56:45–53.

60. Keitel V, Donner M, Winandy S, Kubitz R, Häussinger D. Expression and function of the bile acid receptor TGR5 in Kupffer cells. Biochem Biophys Res Commun. 2008;372:78–84.

61. Wang YD, Chen WD, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) in mice. Hepatology. 2011;54:1421–32.

62. Xiao H, Sun X, Liu R, Chen Z, Lin Z, Yang Y, et al. Gentiopicroside activates the bile acid receptor Gpbar1 (TGR5) to repress NF-kappaB pathway and ameliorate diabetic nephropathy. Pharmacol Res. 2020;151:104559.

63. Guo C, Qi H, Yu Y, Zhang Q, Su J, Yu D, et al. The G-protein-coupled bile acid receptor Gpbar1 (TGR5) inhibits gastric inflammation through antagonizing NF-κB signaling pathway. Front Pharmacol. 2015;6:287.

64. Su J, Zhang Q, Qi H, Wu L, Li Y, Yu D, et al. The G-protein-coupled bile acid receptor Gpbar1 (TGR5) protects against renal inflammation and renal cancer cell proliferation and migration through antagonizing NF-κB and STAT3 signaling pathways. Oncotarget. 2017;8:54378–87.

65. Iracheta-Vellve A, Calenda CD, Petrasek J, Ambade A, Kodys K, Adorini L, et al. FXR and TGR5 agonists ameliorate liver injury, steatosis, and inflammation after binge or prolonged alcohol feeding in mice. Hepatol Commun. 2018;2:1379–91.

66. Hao H, Cao L, Jiang C, Che Y, Zhang S, Takahashi S, et al. Farnesoid X receptor regulation of the NLRP3 inflammasome underlies cholestasis-associated sepsis. Cell Metab. 2017;25:856–67.e5.

67. Gong Z, Zhou J, Zhao S, Tian C, Wang P, Xu C, et al. Chenodeoxycholic acid activates NLRP3 inflammasome and contributes to cholestatic liver fibrosis. Oncotarget. 2016;7:83951–63.

68. Guo C, Xie S, Chi Z, Zhang J, Liu Y, Zhang L, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity. 2016;45:944. Erratum for: Immunity. 2016;45:802–16.

69. Perino A, Pols TWH, Nomura M, Stein S, Pellicciari R, Schoonjans K. TGR5 reduces macrophage migration through mTOR-induced C/EBPβ differential translation. J Clin Invest. 2014;124:5424–36.

70. Spirlì C, Nathanson MH, Fiorotto R, Duner E, Denson LA, Sanz JM, et al. Proinflammatory cytokines inhibit secretion in rat bile duct epithelium. Gastroenterology. 2001;121:156–69.

71. Rao J, Yang C, Yang S, Lu H, Hu Y, Lu L, et al. Deficiency of TGR5 exacerbates immune-mediated cholestatic hepatic injury by stabilizing the β-catenin destruction complex. Int Immunol. 2020;32:321–34. Erratum in: Int Immunol. 2022;34:53.

72. Yang H, Luo F, Wei Y, Jiao Y, Qian J, Chen S, et al. TGR5 protects against cholestatic liver disease via suppressing the NF-κB pathway and activating the Nrf2/HO-1 pathway. Ann Transl Med. 2021;9:1158.

73. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol. 2004;5:836–47.

74. Michalopoulos GK. Principles of liver regeneration and growth homeostasis. In: Terjung R, editor. Comprehensive Physiology. John Wiley & Sons, Ltd; 2013. pp. 485–513.

75. Högenauer K, Arista L, Schmiedeberg N, Werner G, Jaksche H, Bouhelal R, et al. G-protein-coupled bile acid receptor 1 (GPBAR1, TGR5) agonists reduce the production of proinflammatory cytokines and stabilize the alternative macrophage phenotype. J Med Chem. 2014;57:10343–54.

76. Biagioli M, Carino A, Cipriani S, Francisci D, Marchianò S, Scarpelli P, et al. The bile acid receptor GPBAR1 regulates the M1/M2 phenotype of intestinal macrophages and activation of GPBAR1 rescues mice from murine colitis. J Immunol. 2017;199:718–33.

77. Leonhardt J, Haider RS, Sponholz C, Leonhardt S, Drube J, Spengler K, et al. Circulating bile acids in liver failure activate TGR5 and induce monocyte dysfunction. Cell Mol Gastroenterol Hepatol. 2021;12:25–40.

78. Reich M, Spomer L, Klindt C, Fuchs K, Stindt J, Deutschmann K, et al. Downregulation of TGR5 (GPBAR1) in biliary epithelial cells contributes to the pathogenesis of sclerosing cholangitis. J Hepatol. 2021;75:634–46.

79. Sato K, Meng F, Giang T, Glaser S, Alpini G. Mechanisms of cholangiocyte responses to injury. Biochim Biophys Acta Mol Basis Dis. 2018;1864:1262–9.

80. Lee NP. The blood-biliary barrier, tight junctions and human liver diseases. In: Cehng, CY, editor. Biology and regulation of blood-tissue barriers. New York, NY: Springer New York; 2013. pp. 171–85.

81. Van Itallie CM, Anderson JM. Architecture of tight junctions and principles of molecular composition. Semin Cell Dev Biol. 2014;36:157–65.

82. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. Cold Spring Harb Perspect Biol. 2009;1:a002584.

83. Shigetomi K, Ikenouchi J. Regulation of the epithelial barrier by post-translational modifications of tight junction membrane proteins. J Biochem. 2018;163:265–72.

84. Iden S, Misselwitz S, Peddibhotla SSD, Tuncay H, Rehder D, Gerke V, et al. aPKC phosphorylates JAM-A at Ser285 to promote cell contact maturation and tight junction formation. J Cell Biol. 2012;196:623–39.

85. Matsumoto K, Imasato M, Yamazaki Y, Tanaka H, Watanabe M, Eguchi H, et al. Claudin 2 deficiency reduces bile flow and increases susceptibility to cholesterol gallstone disease in mice. Gastroenterology. 2014;147:1134–45.e10.

86. Boyer JL. The hepatobiliary paracellular pathway: a paradigm revisited. Gastroenterology. 2014;147:965–8.

87. Tanaka H, Imasato M, Yamazaki Y, Matsumoto K, Kunimoto K, Delpierre J, et al. Claudin-3 regulates bile canalicular paracellular barrier and cholesterol gallstone core formation in mice. J Hepatol. 2018;69:1308–16.

88. Sakisaka S, Kawaguchi T, Taniguchi E, Hanada S, Sasatomi K, Koga H, et al. Alterations in tight junctions differ between primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology. 2001;33:1460–8.

89. Fickert P, Fuchsbichler A, Wagner M, Zollner G, Kaser A, Tilg H, et al. Regurgitation of bile acids from leaky bile ducts causes sclerosing cholangitis in *Mdr2* (*Abcb4*) knockout mice. Gastroenterology. 2004;127:261–74.

90. Pradhan-Sundd T, Vats R, Russell JO, Singh S, Michael AA, Molina L, et al. Dysregulated bile transporters and impaired tight junctions during chronic liver injury in mice. Gastroenterology. 2018;155:1218–32.e24.

91. Hadj-Rabia S, Baala L, Vabres P, Hamel-Teillac D, Jacquemin E, Fabre M, et al. *Claudin-1* gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. Gastroenterology. 2004;127:1386–90. Erratum in: Gastroenterology. 2005;128:524.

92. Grosse B, Cassio D, Yousef N, Bernardo C, Jacquemin E, Gonzales E. Claudin-1 involved in neonatal ichthyosis sclerosing cholangitis syndrome regulates hepatic paracellular permeability. Hepatology. 2012;55:1249–59.

93. Sambrotta M, Thompson RJ. Mutations in *TJP2*, encoding zona occludens 2, and liver disease. Tissue Barriers. 2015;3:e1026537.

94. Xu J, Kausalya PJ, Van Hul N, Caldez MJ, Xu S, Ong AGM, et al. Protective functions of ZO-2/Tjp2 expressed in hepatocytes and cholangiocytes against liver injury and cholestasis. Gastroenterology. 2021;160:2103–18.

95. Rahner C, Stieger B, Landmann L. Structure-function correlation of tight junctional impairment after intrahepatic and extrahepatic cholestasis in rat liver. Gastroenterology. 1996;110:1564–78.

96. Itoh M, Terada M, Sugimoto H. The zonula occludens protein family regulates the hepatic barrier system in the murine liver. Biochim Biophys Acta Mol Basis Dis. 2021;1867:165994.

97. Müller T, Beutler C, Picó AH, Otten M, Dürr A, Al-Abadi H, et al. Increased T-helper 2 cytokines in bile from patients with IgG4-related cholangitis disrupt the tight junction–associated biliary epithelial cell barrier. Gastroenterology. 2013;144:1116–28.

98. Pradhan-Sundd T, Zhou L, Vats R, Jiang A, Molina L, Singh S, et al. Dual catenin loss in murine liver causes tight junctional deregulation and progressive intrahepatic cholestasis. Hepatology. 2018;67:2320–37.

99. Sato A, Kakinuma S, Miyoshi M, Kamiya A, Tsunoda T, Kaneko S, et al. Vasoactive intestinal peptide derived from liver mesenchymal cells mediates tight junction assembly in mouse intrahepatic bile ducts. Hepatol Commun. 2020;4:235–54.

100. Raimondi F, Santoro P, Barone MV, Pappacoda S, Barretta ML, Nanayakkara M, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am J Physiol Gastrointest Liver Physiol. 2008;294:G906–13.

101. Su KC, Wu YC, Chen CS, Hung MH, Hsiao YH, Tseng CM, et al. Bile acids increase alveolar epithelial permeability via mitogen-activated protein kinase, cytosolic phospholipase A_2, cyclooxygenase-2, prostaglandin E_2 and junctional proteins. Respirology. 2013;18:848–56.

102. Cipriani S, Mencarelli A, Chini MG, Distrutti E, Renga B, Bifulco G, et al. The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis. PLoS One. 2011;6:e25637. Erratum in: PLoS One. 2013;8.

103. Kida T, Omori K, Hori M, Ozaki H, Murata T. Stimulation of G protein–coupled bile acid receptor enhances vascular endothelial barrier function via activation of protein kinase A and Rac1. J Pharmacol Exp Ther. 2014;348:125–30.

104. Zhu L, Wang W, Xie TH, Zou J, Nie X, Wang X, et al. TGR5 receptor activation attenuates diabetic retinopathy through suppression of RhoA/ROCK signaling. FASEB J. 2020;34:4189–203.

105. Liang H, Matei N, McBride DW, Xu Y, Tang J, Luo B, et al. Activation of TGR5 protects blood brain barrier via the BRCA1/Sirt1 pathway after middle cerebral artery occlusion in rats. J Biomed Sci. 2020;27:61. Erratum in: J Biomed Sci. 2020;27:71.

106. Hohenester S, de Buy Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Oude Elferink RP, et al. A biliary HCO_3^- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. Hepatology. 2012;55:173–83.

107. Keitel V, Ullmer C, Häussinger D. The membrane-bound bile acid receptor TGR5 (Gpbar-1) is localized in the primary cilium of cholangiocytes. Biol Chem. 2010;391:785–9.

108. Masyuk AI, Huang BQ, Radtke BN, Gajdos GB, Splinter PL, Masyuk TV, et al. Ciliary subcellular localization of TGR5 determines the cholangiocyte functional response to bile acid signaling. Am J Physiol Gastrointest Liver Physiol. 2013;304:G1013–24.

109. Gradilone SA, Masyuk AI, Splinter PL, Banales JM, Huang BQ, Tietz PS, et al. Cholangiocyte cilia express TRPV4 and detect changes in luminal tonicity inducing bicarbonate secretion. Proc Natl Acad Sci U S A. 2007;104:19138–43.

110. Li S, Qiu M, Kong Y, Zhao X, Choi HJ, Reich M, et al. Bile acid G protein-coupled membrane receptor TGR5 modulates aquaporin 2–mediated water homeostasis. J Am Soc Nephrol. 2018;29:2658–70.

111. Han M, Li S, Xie H, Liu Q, Wang A, Hu S, et al. Activation of TGR5 restores AQP2 expression via the HIF pathway in renal ischemia-reperfusion injury. Am J Physiol Renal Physiol. 2021;320:F308–21.

112. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome–mediated bile acid metabolism regulates liver cancer via NKT cells. Science. 2018;360:eaan5931.

113. Carulli N, Bertolotti M, Carubbi F, Concari M, Martella P, Carulli L, et al. Review article: effect of bile salt pool composition on hepatic and biliary functions. Aliment Pharmacol Ther. 2000;14:14–8.

114. Park YJ, Qatanani M, Chua SS, LaRey JL, Johnson SA, Watanabe M, et al. Loss of orphan receptor small heterodimer partner sensitizes mice to liver injury from obstructive cholestasis. Hepatology. 2008;47:1578–86.

115. García-Rodríguez JL, Barbier-Torres L, Fernández-Álvarez S, Gutiérrez-de Juan V, Monte MJ, Halilbasic E, et al. SIRT1 controls liver regeneration by regulating bile acid metabolism through farnesoid X receptor and mammalian target of rapamycin signaling. Hepatology. 2014;59:1972–83.

116. Padrissa-Altés S, Bachofner M, Bogorad RL, Pohlmeier L, Rossolini T, Böhm F, et al. Control of hepatocyte proliferation and survival by Fgf receptors is essential for liver regeneration in mice. Gut. 2015;64:1444–53.

117. Gilgenkrantz H, Tordjmann T. Bile acids and FGF receptors: orchestrators of optimal liver regeneration. Gut. 2015;64:1351–2.

118. Fickert P, Fuchsbichler A, Marschall HU, Wagner M, Zollner G, Krause R, et al. Lithocholic acid feeding induces segmental bile duct obstruction and destructive cholangitis in mice. Am J Pathol. 2006;168:410–22.

119. Fuchs CD, Paumgartner G, Wahlström A, Schwabl P, Reiberger T, Leditznig N, et al. Metabolic preconditioning protects *BSEP/ABCB11$^{-/-}$* mice against cholestatic liver injury. J Hepatol. 2017;66:95–101.

120. Hrycay E, Forrest D, Liu L, Wang R, Tai J, Deo A, et al. Hepatic bile acid metabolism and expression of cytochrome P450 and related enzymes are altered in *Bsep$^{-/-}$* mice. Mol Cell Biochem. 2014;389:119–32.

121. Lee CS, Kimura A, Wu JF, Ni YH, Hsu HY, Chang MH, et al. Prognostic roles of tetrahydroxy bile acids in infantile intrahepatic cholestasis. J Lipid Res. 2017;58:607–14.

122. Wang R, Sheps JA, Liu L, Han J, Chen PSK, Lamontagne J, et al. Hydrophilic bile acids prevent liver damage caused by lack of biliary phospholipid in *Mdr2$^{-/-}$* mice. J Lipid Res. 2019;60:85–97.

123. Kong B, Zhang M, Huang M, Rizzolo D, Armstrong LE, Schumacher JD, et al. FXR deficiency alters bile acid pool composition and exacerbates chronic alcohol induced liver injury. Dig Liver Dis. 2019;51:570–6.

124. Chevre R, Trigueros-Motos L, Castaño D, Chua T, Corlianò M, Patankar JV, et al. Therapeutic modulation of the bile acid pool by *Cyp8b1* knockdown protects against nonalcoholic fatty liver disease in mice. FASEB J. 2018;32:3792–802.

125. Gillard J, Clerbaux LA, Nachit M, Sempoux C, Staels B, Bindels LB, et al. Bile acids contribute to the development of non-alcoholic steatohepatitis in mice. JHEP Rep. 2021;4:100387.

126. Takahashi S, Fukami T, Masuo Y, Brocker CN, Xie C, Krausz KW, et al. Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. J Lipid Res. 2016;57:2130–7.

127. Honda A, Miyazaki T, Iwamoto J, Hirayama T, Morishita Y, Monma T, et al. Regulation of bile acid metabolism in mouse models with hydrophobic bile acid composition. J Lipid Res. 2020;61:54–69.

128. de Boer JF, Verkade E, Mulder NL, de Vries HD, Huijkman N, Koehorst M, et al. A human-like bile acid pool induced by deletion of hepatic *Cyp2c70* modulates effects of FXR activation in mice. J Lipid Res. 2020;61:291–305.

129. de Boer JF, de Vries HD, Palmiotti A, Li R, Doestzada M, Hoogerland JA, et al. Cholangiopathy and biliary fibrosis in *Cyp2c70*-deficient mice are fully reversed by ursodeoxycholic acid. Cell Mol Gastroenterol Hepatol. 2021;11:1045–69.

130. Donepudi AC, Boehme S, Li F, Chiang JYL. G-protein-coupled bile acid receptor plays a key role in bile acid metabolism and fasting-induced hepatic steatosis in mice. Hepatology. 2017;65:813–27.

131. Pathak P, Xie C, Nichols RG, Ferrell JM, Boehme S, Krausz KW, et al. Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. Hepatology. 2018;68:1574–88.

132. Maruyama T, Tanaka K, Suzuki J, Miyoshi H, Harada N, Nakamura T, et al. Targeted disruption of G protein-coupled bile acid receptor 1 (*Gpbar1/M-Bar*) in mice. J Endocrinol. 2006;191:197–205.

133. Donkers JM, Roscam Abbing RLP, van de Graaf SFJ. Developments in bile salt based therapies: a critical overview. Biochem Pharmacol. 2019;161:1–13.

134. Li T, Chiang JYL. Bile acid-based therapies for non-alcoholic steatohepatitis and alcoholic liver disease. Hepatobiliary Surg Nutr. 2020;9:152–69.