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REVIEW

Role of farnesoid X receptor and bile acids in alcoholic liver disease

Sharon Manley, Wenxing Ding*

Department of Pharmacology, Toxicology and Therapeutics, the University of Kansas Medical Center, Kansas City, KS 66160, USA

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Abstract Alcoholic liver disease (ALD) is one of the major causes of liver morbidity and mortality worldwide. Chronic alcohol consumption leads to development of liver pathogenesis encompassing steatosis, inflammation, fibrosis, cirrhosis, and in extreme cases, hepatocellular carcinoma. Moreover, ALD may also associate with cholestasis. Emerging evidence now suggests that farnesoid X receptor (FXR) and bile acids also play important roles in ALD. In this review, we discuss the effects of alcohol consumption on FXR, bile acids and gut microbiome as well as their impacts on ALD. Moreover, we summarize the findings on FXR, FoxO3a (forkhead box-containing protein class O3a) and PPARα (peroxisome proliferator-activated receptor alpha) in regulation of autophagy-related gene transcription program and liver injury in response to alcohol exposure.

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Abbreviations: ADH, alcohol dehydrogenase; AF, activation function; AKT, protein kinase B; ALD, alcoholic liver disease; ALT, alanine aminotransferase; ASBT, apical sodium dependent bile acid transporter; Atg, autophagy-related; BAAT, bile acid CoA:amino acid N-acyltransferase; BACS, bile acid CoA synthetase; BSEP, bile salt export pump; CA, cholic acid; CB1R, cannabinoid receptor type 1; CDCA, chenodeoxycholic acid; CREB, cAMP response element-binding protein; CREBH, cAMP response element-binding protein, hepatocyte specific; CRTC2, CREB regulated transcription coactivator 2; CYP, cytochrome P450; DCA, deoxycholic acid; DR1, direct repeat 1; 6ECDCA, 6α-ethyl-chenodeoxycholic acid; FGF15/19, fibroblast growth factor 15/19; FGFR4, fibroblast growth factor receptor 4; FoxO3a, forkhead box-containing protein class O3a; FXR, farnesoid X receptor; GGT, gamma-glutamyltranspeptidase; HCC, hepatocellular carcinoma; IR-1, inverted repeat-1; KO, knockout; LC3, light chain 3; LRH-1, liver receptor homolog 1; LXR, liver X receptor; MRP4, multidrug resistance protein 4; NAD^+, nicotinamide adenine dinucleotide; NTCP, sodium taurocholate cotransporting polypeptide; OSTα/β, organic solute transporter α/β; PE, phosphatidylethanolamine; PPARα, peroxisome proliferator-activated receptor alpha; ROS, reactive oxygen species; RXRα, retinoid X receptor-alpha; SHP, small heterodimer partner; Sirt1, sirtuin 1; SQSTM, sequestome-1; SREBP1, sterol regulatory element-binding protein 1; TCA, taurocholic acid; TFEB, transcription factor EB; TLR4, toll-like receptor 4; TUDCA, tauro-ursodeoxycholic acid; UDCA, ursodeoxycholic acid; WAY, WAY-362450; WT, wild type

*Corresponding author. Tel.: +1 913 5889813; fax: +1 913 5887501.
E-mail address: wxding@kumc.edu (Wenxing Ding).

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1. Introduction

Alcohol consumption is ubiquitous in the United States and worldwide. In moderate amount, alcohol can be beneficial; however, excessive alcohol consumption may result in pathogenesis known as alcoholic liver disease (ALD)[1–3]. ALD encompasses a wide spectrum of morbidity initiated by simple steatosis, which may progress to more severe pathologies such as fibrosis, alcoholic hepatitis, cirrhosis, and in extreme cases, hepatocellular carcinoma (HCC)[2–4]. It is known that majority of alcoholics develop simple steatosis, but only a small cohort of the patients progress to more severe pathologies[2]. This is likely because ALD is usually associated with other risk factors including sex, obesity, genetics, and viral hepatitis[2,5–7]. Moreover, cells may adapt to the alcohol exposure and activate cellular protective mechanisms against alcohol-induced detrimental effects.

Interestingly, ALD patients also exhibit manifestations of cholestasis, a liver pathology defined by impaired flow of bile acids resulting in accumulation of hepatic bile acids[8]. Bile acids are amphipathic detergent-like molecules that are end-products of hepatic cholesterol catabolism[9]. Due to its detergent-like properties, bile acids are critical for solubilization and absorption of cholesterol, dietary lipids, and fat-soluble vitamins in the intestines[10]. Moreover, bile acids promote bile flow and cholesterol secretion from the liver[11]. Intriguingly, bile acids also function as nutrient signaling molecules through activation of farnesoid X receptor (FXR), a bile acid-sensing nuclear receptor that regulates lipid, cholesterol, and glucose metabolism[12]. FXR is highly expressed in the liver and the intestines, and is also found in kidney and adrenal glands. FXR is a key regulator for bile acid homeostasis[13–17]. FXR contains a ligand-independent transcription activation function (AF-1) region, a DNA-binding domain with two highly conserved zinc finger motifs, and a hinge region that mediates simultaneous receptor dimerization and DNA binding in the N-terminus[18]. A ligand binding domain, a dimerization interface, and a ligand-dependent AF-2 are found in the C-terminus of FXR[18]. FXR dimerizes with retinoid X receptor-alpha (RXRα), another nuclear receptor, which enables FXR to bind to an inverted repeat-1 response element (IR-1), an inverted AGGTCA sequence separated by one base pair, to initiate transcription of target genes[19,20]. FXR is critical for the transcriptional regulation of bile acid synthesis and transport genes in the liver and intestines[21–23]. Bile acids, in particular the unconjugated forms, are endogenous ligands for FXR. Moreover, synthetic ligands such as GW4064 and WAY-362450 (WAY) have been identified to be potent FXR agonists[24,25].

FXR regulates bile acid synthesis through two distinct mechanisms. In the liver, FXR up-regulates expression of small heterodimer partner (SHP), a unique nuclear receptor. SHP then interacts with liver receptor homolog-1 (LRH-1) to repress the transcription of bile acid synthesis enzymes, cytochrome P450 7A1 and 8B1 (CYP7A1 and CYP8B1), resulting in decreased bile acid synthesis[26–28]. In the intestines, bile acids activate FXR to induce the transcription and secretion of fibroblast growth factor 15/19 (FGF15/19) from the intestines into the portal vein. FGF15/19 then travels and binds to FGF receptor 4 (FGFR4) in the liver to suppress the transcription of *Cyp7a1* and in turn inhibits bile acid synthesis[21–23]. Indeed, whole body *Fxr* deficiency in mice results in increased hepatic bile acid levels and liver injury including hepatic steatosis, inflammation, and fibrosis[21,29]. Here we reviewed the emerging evidence that FXR may act as a protective factor in ALD by regulating multiple cellular and molecular pathways.

2. Alcohol consumption disrupts bile acid synthesis and enterohepatic circulation

Alcohol consumption induces hepatic metabolic changes, increases oxidative stress and alters lipid metabolism that leads to hepatotoxicity[2,4]. Interestingly, alcohol consumption has also been reported to induce cholestasis in all stages of ALD[8,30]. Dr. Lieber's group[31,32] first observed that chronic alcohol consumption results in increased bile acid pool and decreased excretion of bile acids, suggesting that alcohol consumption may affect the enterohepatic circulation. Currently, it is not clear how alcohol induces cholestasis. However, emerging evidence suggests that alcohol may down-regulate FXR, which results in increased bile acid synthesis and hepatic bile acid pool[33,34].

Taurine conjugation of bile acids can result in reduced hydrophobicity and toxicity[35]. Taurine and glycine conjugations also promote the transport of bile acids out of the hepatocytes[36]. Chronic alcohol consumption reduced levels of taurine-conjugated bile acids and increased levels of more toxic unconjugated and glycine-conjugated bile acids in rat liver, duodenum and ileum[34]. Conversely, taurine supplementation attenuated chronic alcohol-induced steatosis and lipid peroxidation possibly due to inhibition of CYP2E1 activity in rats[37]. However, it is not clear if taurine supplementation increased taurocholic acid (TCA) level. The reduced levels of taurine-conjugated and increased levels of glycine-conjugated bile acids were due to chronic alcohol-induced perturbation in expression of bile acid metabolism enzymes[34].

Accumulation of hepatic bile acids is one manifestation of ALD pathogenesis, which could be due to alcohol-induced bile acid synthesis. Acute alcohol exposure has been reported to induce bile acid biosynthesis in man and primary cultured human hepatocytes[38,39]. Chronic alcohol consumption also induced the transcription of *Cyp7a1* and *Cyp8b1* and reduced expression of FGFR4, a transcription inhibitor of CYP7A1[33,34]. Moreover, another study demonstrated that alcohol induced transcription of bile acid synthesis enzymes including *Cyp7a1*, *Cyp7b1*, *Cyp8b1*, and *Cyp27a1* by activating cAMP responsive element-binding protein (CREBH), a liver specific transcription factor and a key metabolic regulator, through alcohol-mediated stimulation of the hepatic cannabinoid receptor type 1 (CB1R)[40]. Taken together, accumulating evidence supports that alcohol consumption alters bile acid synthesis by up-regulating the expression of bile acid synthesis genes, although more studies are needed to further elucidate the mechanisms by which alcohol induces bile acid synthesis.

Chronic alcohol consumption also alters metabolic enzymes that facilitate bile acid conjugation prior to the transport of bile acids into bile canaliculi. Upon alcohol exposure, the enzyme responsible for taurine conjugation, bile acid CoA:amino acid N-acyltransferase (BAAT), was down-regulated. However, the enzyme responsible for glycine conjugation, bile acid CoA synthetase (BACS), was increased upon alcohol exposure[34]. As a result, chronic alcohol exposure alters bile acid synthesis and conjugation by up-regulating the classic pathway and decreasing BAAT-mediated taurine conjugation.

Chronic alcohol exposure also alters the enterohepatic circulation of bile acids. Alcohol exposure increases the expression of bile acid efflux transporters including the bile salt export pump (BSEP), multidrug resistance protein 4 (MRP4) and organic solute transporter α/β (OSTα/β) and decreases the expression of bile acid uptake transporter, sodium taurocholate cotransporting polypeptide (NTCP) in the liver[33,34]. In the ileum, alcohol consumption

increases the expression of bile acid transporters including OSTβ and apical sodium dependent bile acid transporter (ASBT)[34]. Furthermore, alcohol exposure decreases the expression of FGF15[34]. The transcriptional changes in the ileum may result in increased absorption of bile acids into the portal circulation. Altogether, chronic alcohol consumption increases hepatic and serum bile acid levels. Alcohol-induced accumulation of bile acids may be attributed to increased bile acid synthesis, increased bile acid absorption in the intestines, and increased bile acid efflux in the liver.

3. Role of FXR in alcohol-induced liver injury

Alcohol-induced disruption of the enterohepatic circulation has been attributed to decreased FXR activity. FXR negatively regulates the expression of CYP7A1 and CYP8B1 but positively regulates the expression of BSEP and FGF15[26,29,41]. Chronic alcohol consumption disrupted the interaction of FXR with RXRα by increasing acetylation of FXR, resulting in FXR inactivation[33]. Decreased acetylation of FXR may be due to alcohol-mediated inhibition of sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase, and activation of acetyltransferase p300[33,42,43]. Alcohol metabolism increased [NADH]:[NAD^+] ratio[44], which may reduce NAD^+-dependent SIRT1 enzymatic activity[45]. These results suggest that FXR and SIRT1 may be potential pharmacological targets for alleviating alcohol-induced cholestasis and liver injury.

WAY and 6α-ethyl-chenodeoxycholic acid (6ECDCA) are potent FXR-specific agonists[25,46]. Intriguingly, pharmacological activation of FXR by WAY and 6ECDCA attenuated chronic alcohol-induced liver injury and steatosis[33,47]. FXR regulates sterol regulatory element-binding protein 1 (SREBP1) through the SHP-liver X receptor (LXR) axis, in which SHP inhibits LXR activity resulting in decreased expression of SREBP1[48]. Indeed, FXR activation by 6ECDCA attenuated alcohol-induced steatosis by ablating SREBP1-mediated lipogenesis[47]. Furthermore, FXR activation also decreased alcohol-mediated reactive oxygen species (ROS) production[33,47]. The mechanism of how FXR activation protects against alcohol-induced oxidative stress is currently not well elucidated. However, WAY treatment decreased alcohol-mediated induction of CYP2E1, which may play a role in attenuation of alcohol-induced oxidative stress[33]. Interestingly, in human hepatocyte-derived cell lines, the proximal promoter sites of human alcohol dehydrogenase (*ADH*) isomers, *ADH1A* and *ADH1B*, have functional IR1. It has been found that FXR binds to the response elements and induces expression of ADH1A and ADH1B, resulting in increased ADH1 enzymatic activity. However, FXR did not induce ADH expression in rodent livers and hepatocytes, indicating that FXR-mediated induction of ADH may be species specific[49]. FXR may play a protective role against human ALD by inducing ADH-mediated metabolism of alcohol.

Conversely, ablation of *Fxr* exacerbated alcohol-induced liver injury in an acute alcohol model and the recent established chronic plus binge model (also called Gao-binge model)[3,4,50,51]. It was reported that Gao-binge treatment suppressed expression of lipid oxidation genes in *Fxr* knockout (KO) mice, which may contribute to exacerbated hepatic steatosis. Furthermore, Gao-binge treatment induced expression of CD14, the receptor for LPS (lipopolysaccharide), in *Fxr* KO mice with a higher degree in comparison to WT (wild type) mice. Increased CD14 expression may exacerbate alcohol-induced liver injury by increasing the sensitivity to inflammation[51]. We also observed a much higher induction of hepatic CYP2E1 after acute alcohol treatment in *Fxr* KO than that of WT mice, which may contribute to exacerbated alcohol-induced liver injury in *Fxr* KO mice[50]. Altogether, these findings suggest that FXR plays a role in protecting the liver from alcohol-induced hepatotoxicity likely by regulating lipid metabolism, sensitivity to inflammation and CYP2E1-mediated oxidative stress.

4. Bile acid modulates alcohol-induced liver injury

Bile acid accumulation in cholestatic conditions can result in hepatotoxicity. As discussed before, alcohol exposure increased bile acid hydrophobicity *via* accumulation of more toxic unconjugated bile acids. Indeed, rats feed with chronic alcohol together with chenodeoxycholic acid (CDCA), a toxic hydrophobic bile acid, increased the hydrophobicity of pooled bile acids and exacerbated alcohol-induced liver injury[52]. However, the mechanism by which CDCA increased alcohol-induced liver injury is not elucidated.

Another group demonstrated that infusion of relatively hydrophobic TCA in bile duct obstructed rats or rats with choledochocaval fistula resulted in decreased hepatic ADH and catalase activity. However, the serum ADH activity as well as microsomal alcohol oxidizing system and aldehyde dehydrogenase was greatly increased under these conditions. In contrast, hydrophilic tauroursodeoxycholic acid (TUDCA) had no effects on alcohol-metabolizing enzymes[53]. These data suggest that hydrophobic bile acids may induce leakage of cytosolic alcohol metabolizing enzymes into the serum, resulting in altered alcohol metabolism during cholestasis.

Ursodeoxycholic acid (UDCA) is a cytoprotective therapeutic hydrophilic bile acid approved to treat cholestasis. UDCA protects cholangiocytes against toxicity exerted by hydrophobic bile acids, stimulates hepatobiliary secretion, and inhibits bile acid-induced apoptosis in hepatocytes[54]. UDCA and its conjugated form, TUDCA both protected against alcohol-induced toxicity in human hepatoblastoma $HepG_2$ cells[55]. However these studies are questionable since $HepG_2$ cells may not be suitable to study alcohol hepatotoxicity due to the lack of expression of alcohol-metabolizing enzymes such as ADH and CYP2E1[56,57]. UDCA or TUDCA co-treatment with alcohol protected against alcohol-induced toxicity in ADH-containing human hepatoma cells (SK-Hep-1)[58]. Moreover, UDCA treatment reduced alcohol-induced liver injury and steatosis in rat livers[59]. UDCA and TUDCA attenuated alcohol-induced hepatotoxicity through various possible mechanisms including preservation of mitochondrial integrity, improvement of ATP synthesis, and decrease of alcohol-induced oxidative damage independent of CYP2E1 and glutathione[60–62]. Moreover, chronic alcohol consumption has been shown to inhibit production of liver prostaglandins, and UDCA treatment restored expression of prostaglandin E and increased linoleoyl-CoA desaturase activity in alcohol-treated rat livers possibly due to enhanced membrane fluidity[63]. Therefore, UDCA protects against alcohol-induced hepatotoxicity by improving mitochondrial function and attenuating oxidative stress *in vivo* although its protective effect against alcohol-induced toxicity *in vitro* is less clear.

The beneficial effects of UDCA against ALD in animal models have led to clinical trials for UDCA therapy in alcoholic cirrhosis although so far these trials yielded mixed results. In a placebo-controlled cross-over trial, the patients were administered with UDCA (15 mg/kg/d) or placebo for 4 weeks. UDCA treatment

resulted in a significant decrease in bilirubin, gamma-glutamyltranspeptidase (GGT), and alanine aminotransferase (ALT) levels[64]. These clinical data thus suggest that UDCA treatment may alleviate alcohol-induced hepatotoxicity despite continued alcohol consumption. In another study using a randomized controlled trial, UDCA (13–15 mg/kg/d) or placebo was administered for 6 months. Patients that received UDCA had decreased levels of GGT and alkaline phosphatase compared to the patients that were received placebo[65]. However, UDCA treatment also resulted in lower survival rates and increased complications[65]. It should be noted that the pilot study by Plevris et al. only included patients with initial bilirubin levels below 50 μmol/L and lower Child-Pugh score (A–B with one patient being C) in comparison to the clinical study by Pelletier et al., which consisted of patients displaying higher initial bilirubin levels (all above 50 μmol/L) and the majority of patients were Child-Pugh C. The recruitment of patients with different initial bilirubin levels may be partially contributed to the different outcomes from these two different trials. Moreover, the dose of UDCA may be inappropriate for more severe form of alcohol cirrhosis because the patients in the UDCA groups displayed dramatic increase in total bile acid levels and some patients even displayed serum bile acid levels as high as 1000 μmol/L. Therefore, the dispute in efficiency of UDCA treatment in alcoholic cirrhosis may be attributed to the severity of hepatic damage prior to the initiation of UDCA treatment. More clinical trials are needed to further determine the effects of UDCA on different stages of ALD patients.

5. Gut microbiome and ALD

Gut microbiome is one of the key players in ALD. Bacterial growth and dysbiosis are hallmarks of various liver diseases including ALD. Several groups have extensively reviewed the role of gut microbiome in ALD recently[66–68]. ALD patients exhibit bacterial overgrow along the gastrointestinal tract, which affects alcohol metabolism resulting in increased concentration of acetaldehyde[69–76]. Endotoxemia is well documented in patients with ALD. Endotoxemia increased hepatic inflammation due to activation of Kupffer cells and subsequent toll-like receptor 4 (TLR4)-mediated cytokine and chemokine production[66,77]. Alcohol exposure induces bacterial translocation and increases gut permeability that promote endotoxemia and facilitate the development of ALD[66].

Intestinal dysbiosis occurs when the composition of intestinal bacteria is altered, and is a hallmark of ALD. Patients with alcoholic cirrhosis have higher amount of *Proteobacteria*, *Prevotellaceae*, and *Veillonellaceae*, and lower amount of *Bacteroidetes* in the colon and feces compared to non-cirrhotic alcoholic patients or healthy people[78–80]. Moreover, in several animal studies, alcohol-fed animals had higher proportions of *Verrucomircobia*, *Proteobacteria*, and *Actinobacteria*, and lower proportions of *Firmicutes* including *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Lactococcus*[72,73,81]. One study has shown that *Bacteroidetes* was elevated in chronic alcohol-fed rats, whereas, another study has demonstrated that chronic alcohol feeding decreased the proportion of *Bacteroidetes*[72,73]. Currently, it is not clear how enteric dysbiosis influences the progress of ALD. Interestingly, probiotics and prebiotics including *Lactobacillius* feedings alleviated alcohol-induced liver injury and restored the gut microbiota in both animal models and alcoholic patients[66,73,82–88]. Restoring gut microbiota by dietary approach using prebiotics and probiotics emerges as a promising therapy for ALD.

Gut microbiota plays an essential role in bile acid metabolism, in which the intestinal bacteria are involved in biotransformation of bile acids through deconjugation, dehydroxylation, and reconjugation[89]. Chronic alcohol consumption increased the concentration of unconjugated bile acids along the gastrointestinal tract, especially in the small intestines (duodenum and ileum). Alcohol consumption decreased the concentration of taurine-conjugated bile acids and increased the amount of unconjugated bile acids in the intestinal tract, liver and serum[34]. The perturbed bile acid profile may be attributed to gut bacterial overgrowth, resulting in increased deconjugation of bile acids and taurine metabolism[66]. It is known that gut bacteria metabolize majority of taurine into inorganic sulfate, which results in decreased taurine bioavailability[90]. Interestingly, alcohol feeding increased the formation and excretion of a taurine metabolite, N-acetyltaurine[91]. Therefore, alcohol-induced taurine metabolism by gut bacteria may decrease taurine available for bile acid conjugation in the liver and alter the systematic bile acid profile.

Conversely, bile acids also regulate the gut flora *via* their antimicrobial activity[92]. Rats fed with a cholic acid (CA)-containing diet had increased gut *Firmicutes* to *Bacteroidetes* ratio. Moreover, these rats displayed increased level of a toxic bile acid, deoxycholic acid (DCA), in the cecum due to bacterial-mediated 7α dehydroxylation of CA. DCA is extremely toxic and selectively inhibits growth of gut bacteria including *Bacteroidetes* and *Lactobacillus*, which results in altered gut microbiota[93]. Moreover, increased abundance of *Firmicutes* promotes the growth of DCA-producing bacteria[94]. Alcohol consumption also increased DCA concentration in the gastrointestinal tract[34]. Patients with or without alcoholic cirrhosis that are active drinkers exhibited increased secondary bile acids including DCA along with decreased fecal *Bacteroidetes*[80]. Therefore, toxic DCA may play a role in alcohol-induced gut bacteria dysbiosis.

FXR activation by bile acids induced expression of genes involved in enteroprotection and inhibited bacterial overgrowth and mucosal injury[95]. Conversely, *Fxr* KO mice displayed more severe bacteria overgrowth and epithelial barrier deterioration[95]. These results suggest that FXR also has antimicrobial activity. As discussed above, alcohol consumption inhibits FXR activation, and it will be interesting to determine the role of FXR in alcohol-induced bacteria dysbiosis and gut permeability in the future.

6. Autophagy in ALD

Macroautophagy (hereafter referred to as autophagy) is an evolutionarily conserved catabolic process responsible for disposing and recycling cellular proteins and damaged/excess organelles in response to starvation and cellular stresses. The autophagy process initiates with formation of isolation membranes, which are elongated and fused to become double membrane autophagosomes[96]. Autophagosomes then fuse with the lysosomes to complete the degradation process. More than 30 autophagy-related (Atg) genes have been identified in yeast and most of them have mammalian homolog counterparts that participate in the autophagic process[97]. One of the key steps in the formation of a double membrane autophagosome is the conjugation of the microtubule associated protein 1 light chain 3 (LC3) with phosphatidylethanolamine (PE). LC3-PE translocates from the cytosol to the isolation membrane to promote the formation of

autophagosomes[98–101]. Sequestome-1 (SQSTM-1)/p62 is an autophagy receptor protein with a LC3 interacting region, which enables p62 to recruit ubiquitinated protein aggregates and deliver them to the autophagosomes for degradation[102]. p62 is also an autophagic substrate that is normally degraded under starvation-induced autophagy, and is accumulated in autophagy-deficient conditions[103–105]. Therefore, monitoring the levels of p62 has been widely used as an autophagic flux marker[100].

Autophagic degradation can be either non-selective or selective depending on the cellular conditions. Non-selective autophagy occurs in energy deficient condition such as starvation, and degrades cellular components in order to provide nutrient and energy. Selective autophagy degrades protein aggregates and excessive or damaged organelles as a protective mechanism in either nutrient-rich or poor conditions[3,106,107].

Autophagy has been shown to play an essential role in liver physiology, and deregulation of hepatic autophagy has been implicated in pathogenesis of various liver diseases including ALD[108–110]. We have demonstrated that acute alcohol treatment induced hepatic autophagy to selectively degrade damaged mitochondria and excess lipid droplets[111,112]. Acute alcohol-mediated autophagy induction required alcohol-metabolizing enzymes, *e.g.*, CYP2E1 and ADH[3,112]. More importantly, pharmacological activation of autophagy protected against alcohol-induced hepatotoxicity and steatosis. Conversely, pharmacological inhibition of autophagy exacerbated alcohol-induced hepatotoxicity[112,113]. In contrast to the acute alcohol exposure, the status of autophagy in chronic alcohol exposure is less clear and controversial, which could be reflected by the lack of reliable autophagic flux assay *in vivo* and the dynamic nature of autophagy during the long time chronic feeding conditions[3]. Nevertheless, similar to the findings from the acute alcohol exposure, pharmacological activation of autophagy also showed beneficial effects against chronic alcohol feeding-induced liver injury in mice[113]. Therefore, induction of autophagy may be a promising therapeutic option for ALD.

We recently demonstrated that alcohol also regulates autophagy at the transcriptional level[42]. Forkhead box-containing protein class O3a (FoxO3a) is a member of FoxO family of evolutionarily conserved DAF-16 like transcription factor[114–116]. Multiple post-translation modifications, which include phosphorylation, ubiquitination, acetylation, and methylation, regulate FoxO3a activity[114,116]. Protein kinase B (AKT) phosphorylates FoxO3a at serine 253, resulting in sequestration of FoxO3a in the cytoplasm, which inhibits FoxO3a-mediated transcriptional activation[117]. FoxO3a regulates transcription of genes involved in apoptosis, oxidative stress, cell-cycle transition, and DNA repair[114,117]. Moreover, FoxO3a also regulates transcription of Atg genes in skeletal muscles, cardiomyocytes, and liver[42,118–121]. Our group and others have demonstrated that FoxO3a protects against alcohol-induced hepatotoxicity and steatosis by initiating transcription of Atg and antioxidant genes[42,122,123]. Acute alcohol treatment increased expression of Atg genes in mouse livers and primary cultured hepatocytes. Mechanistically, acute alcohol treatment decreased AKT-mediated FoxO3a phosphorylation at serine 253, which resulted in nuclear accumulation of FoxO3a in mouse livers[42]. Interestingly, induction of SIRT1 activity by resveratrol promoted deacetylation of FoxO3a, which increased FoxO3a-mediated transcription of Atg genes in response to alcohol. Both acute alcohol exposure and chronic alcohol feeding induced more severe steatosis and hepatotoxicity in *FoxO3a* KO mice compared to WT mice[122]. These results suggest that FoxO3a protects against alcohol-induced hepatotoxicity likely by inducing expressions of Atg and antioxidant genes.

7. Bile acids and FXR regulate hepatic autophagy in ALD

As discussed above, bile acids are nutrient signaling molecules. Emerging evidence shows that bile acids also regulate autophagy. DCA, a hydrophobic secondary bile acid, induced accumulation of autophagosomes in cultured rodent hepatocytes and human esophageal cells as well as increased LC3-II protein expression in colon epithelial cells[124–126]. We demonstrated that bile acids inhibit completion of autophagic process in hepatocytes by decreasing Rab7-mediated fusion of autophagosomes with lysosomes in hepatocytes, a process which is independent of FXR[127]. Whole body *Fxr* KO mice had impaired hepatic autophagy as demonstrated by the elevated hepatic p62 and LC3-II levels. This is likely due to the elevated hepatic bile acid levels since hepatocyte-specific *Fxr* KO mice had normal levels of hepatic bile acids and normal autophagy. Because of the impaired hepatic autophagy in *Fxr* KO mice, it is not surprising that we further found that alcohol-induced liver injury is exacerbated in *Fxr* KO mice[50]. Intriguingly, we further found that alcohol-induced expression of Atg genes (*Atg5*, *Becn-1* and *Map1lc3b*) was abolished in *Fxr* KO mouse livers. Furthermore, alcohol-induced hepatic expression of FoxO3a target genes (*MnSod*, *p21*, and *FoxO3a*) in WT mice was also suppressed in *Fxr* KO mice[50]. These results suggest that FXR is associated with acute alcohol-induced FoxO3a activation and autophagy in mouse livers. The decreased FoxO3a-mediated expression of Atg genes in acute alcohol-treated mouse livers was likely due to the secondary effects such as increased hepatic AKT activation in *Fxr* KO mice, but not due to the direct FXR-FoxO3a interaction since we failed to detect such an interaction in either FXR and FoxO3a over-expressed cultured cells or in alcohol-treated mouse livers[50].

FXR is a nutrient-sensing nuclear receptor that is activated in fed state by bile acids returning to the liver, whereas peroxisome proliferator-activated receptor alpha (PPARα) is activated by fatty acids during fasting[128–133]. Therefore, FXR may also be involved in regulating autophagy in response to nutrient abundance especially in postprandial period. Indeed, two recent studies from Moore's group and Kemper's group[134,135] independently demonstrated that activation of FXR ablated nutrient starvation-induced autophagy *in vitro* and *in vivo*. Pharmacological activation of FXR by GW4064 attenuated the expression of Atg genes in fasted, but not fed mouse livers[134,135]. Mechanistically, FXR inhibited transcription of Atg genes by two distinct but complementary mechanisms. First, FXR and PPARα compete for binding to the shared direct repeat 1 (DR1) sites, with opposite transcriptional outcomes on the expression of autophagy genes[135]. Second, FXR disrupts the interaction of cAMP response element binding protein (CREB) with its co-activator, CREB-regulated transcription coactivator 2 (CRTC2), resulting in decreased expression of Atg genes in fed state[134]. These findings suggest that FXR negatively regulates autophagy by nutrient status. These observations seem to conflict with our findings that *Fxr* KO mice had impaired hepatic autophagy. However, it should be noted that *Fxr* KO mice had increased hepatic bile acids, inflammation and cell death as well as altered various signaling pathways such as AKT that may regulate autophagy[29,136,137]. The impaired hepatic autophagy in *Fxr* KO mice could be due to the secondary factors as a result of the loss of FXR. Indeed, *Fxr* KO mice have increased hepatic accumulation of p62, inflammation and developed spontaneous liver tumors, which are very similar to the autophagy-deficient mouse livers[127,136–140]. Therefore, while FXR may inhibit autophagy in response to the nutrient status, FXR is not the only

regulator of hepatic autophagy. It is also possible that the chronic loss (or inhibition) of FXR or acute activation of FXR may have different impacts on hepatic autophagy. As discussed above, chronic alcohol consumption has been shown to inhibit FXR activity. Currently, the effect of chronic alcohol exposure on FXR-mediated repression of autophagy gene transcription has not been elucidated. However, one would assume that chronic alcohol exposure might increase the expression of Atg genes due to inhibition of FXR and subsequent impair FXR-mediated repression of Atg gene transcription. Liver-specific *Fxr* KO mice fed with alcohol will be a very useful model to further test this hypothesis, because these mice would avoid the problems such as increased bile acids and liver injury in whole body *Fxr* KO mice.

Chronic alcohol-treated *Pparα* KO mice exhibited hepatomegaly and increased hepatocyte proliferation, enhanced mitochondrial damage, liver injury and inflammation[141]. Chronic alcohol feeding decreased the DNA-binding affinity of PPARα, which was reversed by treatment with WY14643, a PPARα agonist[142]. Pharmacological activation of PPARα by WY14643 protected against chronic alcohol-induced hepatotoxicity and steatosis in mice[142,143]. These results are consistent with the notion that PPARα promotes autophagy although the expression of autophagy genes and autophagy activity were not determined in these studies.

As discussed above, PPARα and FXR compete for DR1 binding to regulate the expression of Atg genes and FXR negatively regulates transcription factor EB (TFEB), a regulator of lysosomal biogenesis[134]. PPARα is an important downstream mediator of TFEB in response to nutrient starvation[144]. Chronic alcohol feeding did not affect CREB phosphorylation or nuclear translocation, but chronic alcohol feeding (four weeks) plus a single alcohol binge increased phosphorylated CREB and decreased nuclear CREB levels in rat livers[145]. Whether and how TFEB, FXR, CREB and PPARα are integrated or independently participate in the regulation of Atg genes expression after alcohol exposure are currently unknown. It seems that the regulation of autophagy at the transcriptional level after alcohol exposure could be very complex and different from starvation conditions. Nevertheless, future studies on deciphering these complex transcriptional factor-mediated regulations on autophagy after alcohol exposure may offer some novel promising approaches for treating ALD.

8. Conclusions and perspectives

ALD is one of the major causes of liver morbidity and mortality worldwide. Currently, there is no effective treatment for ALD. The last treatment proposed for ALD was in the 1970s, in which corticosteroids were used to treat alcoholic steatohepatitis. Therefore, developing novel pathophysiological-targeted therapies is urgently needed. FXR has emerged as a novel possible therapeutic target in ALD. Recent evidence demonstrated that alcohol exposure impairs FXR activation, which results in increased bile acid synthesis and pool. Moreover, FXR protects against alcohol-induced gut bacteria dysbiosis and overgrowth as well as the accumulation of toxic bile acids such as DCA. Increased bile acids may inhibit the completion of autophagic degradation in hepatocytes. Therefore, restoring bile acid homeostasis is important for

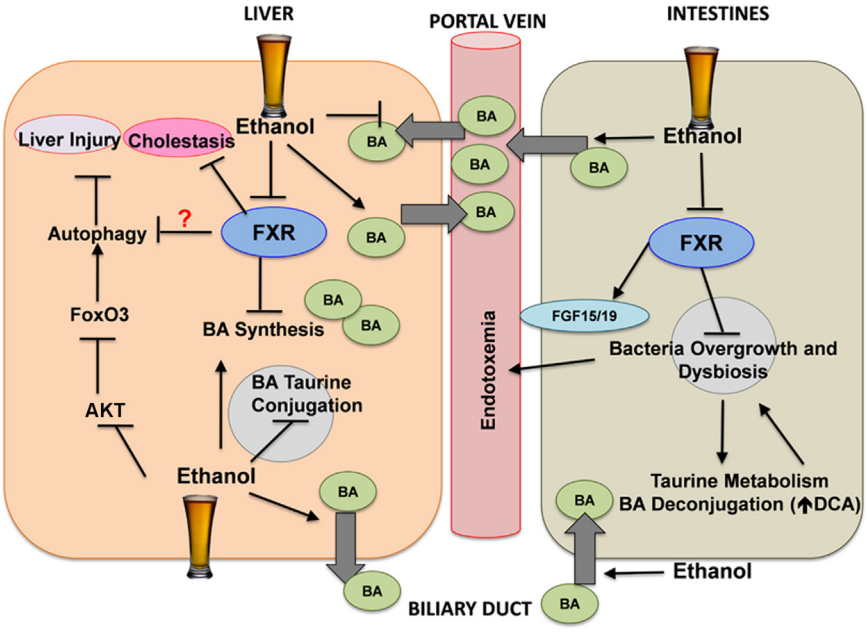

Figure 1 Schematic diagram of the cellular and molecular events of alcohol exposure on FXR, enterohepatic circulation, gut microbiome and autophagy. Alcohol treatment inhibits FXR in the liver, which results in increased bile acid synthesis. Moreover, alcohol exposure decreases taurine conjugation of bile acids and increases efflux of bile acids out of the hepatocytes into the portal vein and bile duct. Acute alcohol exposure also induces autophagy by inhibiting AKT, which results in FoxO3a activation and FoxO3a-mediated up-regulation of Atg genes. Alcohol increases the uptake of bile acids into the enterocytes and promotes efflux of bile acids into the portal circulation from the intestines. Alcohol exposure also inhibits intestinal FXR activation, which leads to decreased FGF15/19 expression and promotes bacteria overgrowth and dysbiosis. Increased abundance of intestinal bacteria promotes taurine metabolism and bile acid deconjugation. Finally, increased levels of unconjugated bile acids including DCA exacerbate alcohol-induced dysbiosis. FXR may negatively regulate autophagy and cholestasis, and autophagy protects against ALD.

the autophagy function, which is an important protective mechanism against ALD. Paradoxically, FXR may inhibit the induction of autophagy in response to nutrient starvation. However, the effect of alcohol exposure on FXR-mediated repression of autophagy needs to be further deciphered. Moreover, other transcription factors including FoxO3a, PPARα, CREB and TFEB have been implicated in the regulation of Atg gene expression. Therefore, elucidating the mechanism of how transcriptional factor-mediated regulation on autophagy after alcohol exposure may lead to generate promising therapeutic target for ALD in the future. In summary, the FXR-bile acid axis may be a promising therapeutic target for ALD. More studies are needed to further examine the role of FXR and bile acids in alcohol-induced hepatotoxicity and steatosis. The molecular and cellular events of alcohol on FXR, bile acids, gut microbiome and autophagy are summarized in Fig. 1.

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