SMGr🍃up

Transforming Growth Factor β and Cholestasis

Chung-Davidson YW[1] and Li W[1,2]*

[1]Department of Fisheries and Wildlife, Michigan State University, USA

[2]Department of Physiology, Michigan State University, USA

*Corresponding author: Weiming Li, Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, 480 Wilson Road, East Lansing, MI 48824, USA, Tel: +1-517-432-6705; Fax: +1-517-432-1699; Email: liweim@msu.edu

ABSTRACT

Cholestasis is an impairment of bile formation and bile flow, manifested by secretory defects of hepatocytes or cholangiocytes (or both), or obstruction of bile ducts. Many adaptations occur during cholestasis, including decreases in bile acid synthesis, induction of bile acid detoxification systems, recruitment of alternative export pumps, and increases in urinary bile acid excretion. These adaptive changes are mediated by specific nuclear receptors that can be activated by accumulated bile acids, proinflammatory cytokines(including transforming growth factor β, TGFβ), drugs and hormones. Other mechanisms involved in the pathogenesis of cholestasis can include changes in transporter protein membrane dynamics, alteration in cell polarity, disruption of cell junctions, and perturbations of cell membranes and cytoskeletons. Here we briefly describe the mechanisms of bile formation and cholestatic pathogenesis followed by reviewing the effects of TGFβ on bile formation and different hepatobiliary components altered during cholestasis. Recent studies have shown that TGFβ represses *cyp7a1* gene expression in human hepatocytes via Smad3-dependent inhibition of hepatocyte nuclear factor 4α (HNF4α) and histone deacetylase (HDAC) remodeling of *cyp7a1* chromatin. TGFβ/Smad3 signaling also alters phospholipid and bile

acid metabolism following hepatic inflammation with biliary injury. In addition, TGFβ1 represses expression of multiple genes involved in bile acid Phase II detoxification. In the hepatobiliary system, TGFβ1 is an important soluble factor involved in the regulation of myofibroblast extracellular matrix synthesis, and is overexpressed in hepatic fibrous areas in adults. TGFβ also induces the dissolution of tight junctions during epithelial-to-mesenchymal transition in other organs. However, the effect of TGFβ upon the tight junction and permeability in the liver has not been studied. Further research is also required to investigate potential effects of TGFβ on the bile acid transporter systems.

Keywords: Smad; Cyp7a1; Tight junction; Detoxification; Fibrosis

Abbreviations: BA-Biliary Atresia; BSEP-Bile Salt Export Pump; CYP7A1-Cholesterol 7α-hydroxylase; FGF15-Fibroblast Growth Factor 15; FXR-Farnesoid X Receptor; HDAC-Histone deacetylase; HNF4α-Hepatocyte Nuclear Factor 4α; HSCs-Hepatic Stellate Cells; IL-1β-interleukine-1β; NTCP-Na$^+$-Taurocholate Cotransporter; OATP-Organic Anion Transporting Protein; PBC-Primary Biliary Cirrhosis; PSC-Primary Sclerosing Cholangitis; SHP-1-Small Heterodimer Protein-1; Smad-Mother Against Decapentaplegic Homolog; TGFβ-Transforming Growth Factor β; TGFβRI-Type I TGFβ Receptor; TGFβRII-type II TGFβ Receptor; TNFα-Tumor Necrosis Factor α

BILE FORMATION AND CHOLESTASIS

Cholestasis is an impairment of bile formation and bile flow [1,2]. Bile acids are synthesized and secreted by hepatocytes with highly polarized plasma membranes, which can be separated into the basolateral (facing the sinusoid and the intercellular space) and the canalicular domains [3]. Bile acid homeostasis is the result of coordinated feedback and feed-forward regulation of genes involved in bile acid synthesis, detoxification and transport [4]. Bile is secreted across the canalicular domain [3]. The canalicular lumen is incompletely sealed by tight junctions as the only anatomical barrier between the blood and the bile. These tight junctions have some permeability to water and inorganic ions and the paracellular pathway is a major site of electrolyte and fluid entry into the bile. Bile formation requires the coordinationof transportoccurring at the basolateral membrane (mostly uptake processes), within the hepatocyte (intracellar transport) and across the canalicular membrane (secretory processes) [3].

Bile flow is an osmotic process driven by active secretion of solutes into the bile canalicular space between adjacent hepatocytes that are sealed from blood by tight junctions, followed by passive influx of water and electrolytes by trans- or para-cellular pathways [5]. The main portion of the bile flow is bile salt-dependent and the remainder is bile salt-independent with glutathione as an important contributor [6,7]. Cholangiocytes also contribute significantly to bile formation by secretin-stimulated secretion of HCO$_3^-$ and Cl$^-$, which accounts for up to 40% of human bile flow [8,9].

Most bile salts are recycled by enterohepatic circulation,a process that reabsorbs and circulates bile salts from the intestinal lumen back to the liver via the portal system for uptake and re-secretion into the bile [5]. Three transport events are important for this process: sinusoidal uptake, intracellular transport and canalicular secretion. Hepatocyte bile salt uptake is mediated via the Na^+-taurocholate cotransporter (NTCP) localized at the sinusoidal membrane [10-12]. In most cases, canalicular secretion is the rate-limiting step and involves a member of the ATP-binding cassette (ABC) transporter superfamily, bile salt export pump(BSEP/ABCB11) [13-16]. Other proteins involved in the sodium-independent hepatic uptake of bile salts are organic anion transporting proteins OATP2 (OATP-C, LST1) and OATP8 [17-20]. Genetic cholestasis can be caused by mutations in any of the genes encoding these bile acid transporters [5]. A reduction of bile flow can be established without changes in serum markers, and down-regulation of bile acid transporters may be a cause or a consequence of cholestasis [5].

In general, the expression of the key enzymes and transporters involved in cholesterol and bile salt metabolism is under transcriptional control of nuclear hormone receptors. For example, CYP7A1 (cholesterol 7α-hydroxylase), the rate limiting enzyme in the neutral pathway for bile salt synthesis, and its downstream enzyme CYP8B, are under the transcriptional control of FXR (farnesoid X receptor or NR1H4). Various bile salts and their taurine-conjugates bind and activate FXR. FXR complexes with the retinoid X-receptor and the resulting heterodimers interact with a highly conserved inverse repeat-1 in the promoter region of SHP-1 (small heterodimer protein-1). SHP-1 thensuppresses the transcription of *cyp7a1*and *cyp8b* by binding to a transcription factor called liver receptor homolog 1 [21,22]. SHP-1 also suppresses NTCP expression [23]. In contrast, FXR up-regulates the expression of bile salt export from hepatocytes via BSEP [24,25]. Bile acid-activated FXR also induces intestinal expression of fibroblast growth factor 15 (FGF15), which may be transported to the liver to inhibit *cyp7a1* gene expression [26,27].

Cholestasis can result from secretory defects of hepatocyte or cholangiocytes (or both), or from obstruction of bile ducts by lesions, stones or tumors, and may be caused by multiple mechanisms in conditions such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), or other cholestatic diseases [28]. Cholestasis is a frequently observed clinical condition that can lead to nutritional problems related to malabsorption of dietary fat and fat-soluble vitamins, and irreversible liver damage caused by accumulation of toxic compounds [5]. However, the treatment for cholestasis is often hampered by insufficient knowledge of underlying causes and difficulties in distinguishing the primary events from secondary consequences [5]. Cholestasis is usually classified into "extrahepatic" and "intrahepatic" forms. Extrahepatic cholestasis refers to the obstruction of large bile ducts outside the liver. Intrahepatic cholestasisoccurs inside the liver, either in the parenchymal cells (hepatocellular cholestasis) or in the canaliculi/intrahepatic bile ductules and/or portal ducts (ductular/ductal cholestasis) [5,29]. Cholestasis can also be categorized into inflammation-induced cholestasis and genetic cholestasis according to the inducer of cholestasis [29].

Inflammatory cytokines produced in response to various infections and non-infectious stimuli are potent inducers of intrahepatic cholestasis. Inflammatory cytokines inhibit the expression and function of hepatocellular transport systems normally mediating hepatic uptake and biliary excretion of bile salts and various non-bile salt organic anions such as bilirubin. These cytokine effects are reversible and bile secretory function can be restored upon removal of the inflammatory stimuli [29]. Clinically, inflammation-induced cholestasis occurs in the setting of intra- and extra-hepatic infections, drug- and alcohol-induced liver injury, total parenteral nutrition, non-metastatic neoplastic disorders (lymphomas or solid tumors) and postoperatively (after major cardiothoracic or abdominal surgery). Although clinically this comprises a broad range of etiologies, the common theme is that cholestasis generally results from systemic and/ or intrahepatic release of proinflammatory cytokines that are potent inhibitors of hepatocellular bile secretion [29].

Conversely, high levels of bile acids during cholestasis can cause liver inflammation and secretion of a broad array of cytokines from Kupffer cells. Bile acids induce proinflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukine1β (IL-1β), which repress *cyp7a1* gene expression via c-Jun/JNK pathway [30-32]. Interestingly, SHP-1 also suppresses the TGFβ pathway [33-35]. In this review, we will discuss the involvement of TGFβ in bile acid synthesis, tight junction dissolution, and fibrosis in the bile duct (Figure 1).
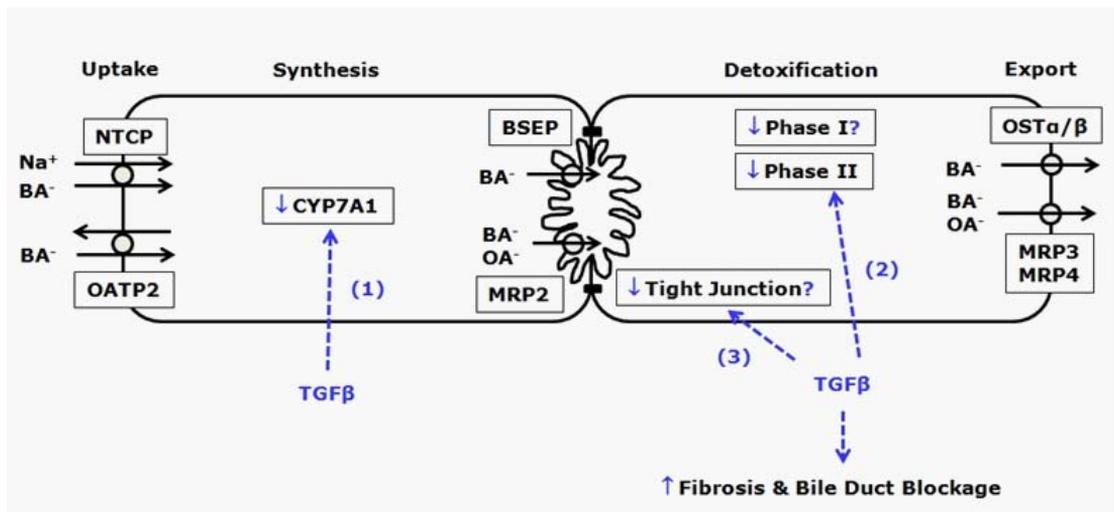

Figure 1: Transforming growth factor β (TGF β)is involved in many hepatobiliary functions. All hepatocytes have bile acid uptake, synthesis, detoxification and export systems. To simplify the diagram, these functions are depicted in two adjacent hepatocytes. Bile acids (BA-) are taken up by the Na+/taurocholate cotransporter (NTCP) and organic anion transporting proteins (OATP2/OATP1B1) at the basolateral membrane of hepatocytes. BA- is exported into the bile by the canalicular bile salt export pump (BSEP) and the canalicular conjugate export pump (MRP2). At the basolateral membrane of hepatocytes, MRP3, MRP4, and the heteromeric organic solute transporter (OST) α/β provide an alternative excretion route for BA- and

other anions (OA-), such as bilirubin, into the blood. During cholestasis, several adaptive mechanisms and hepatocellular changes occur: (1) downregulation of transport systems and bile acid synthesis, (2) increased Phase I (i.e., hydroxylation) and Phase II (i.e., sulfation, glucuronidation) detoxification of bile acids, and (3) disruption of cytoskeletons, tight junctions, and vesicle localization and transportin the hepatocytes. TGFβ is involved in repression of bile acid synthesis and Phase II detoxification, and may also play a role in reduction of Phase I detoxification and dissolution of the tight junctions. In addition, TGFβ is involved in the fibrosis and blockage of the bile ducts. This figure is modified from [4,69].

TGFβ AND BILE ACID SYNTHESIS

The TGFβ signaling pathway is well delineated. It starts withTGFβ binding to type II TGFβ receptor (TGFβRII), which subsequently recruits, phosphorylates and activates type I TGFβ receptor (TGFβRI). The activated TGFβRI in turn phosphorylates and activates downstream transcription factors signal mother against decapentaplegic (Smad) homologs, Smad2 and Smad3 (either homodimers or heterodimers). Smad 2 and Smad3 bind with cofactor Smad4, translocate to the nucleus, bind to DNA and other transcription factors, and regulate gene transcription [36,37].

In primary hepatic culture and cell lines, TGFβ inhibits *cyp7a1* gene expression and bile acid synthesis via Smad3 signaling [32,38-40]. TGFβ and downstream Smad3 inhibited *cyp7a1* promoter activity and mRNA expression by inhibiting DNA-binding activity of HNF4α. Tricostatin A, aHDAC inhibitor, partially blocked TGFβ1 inhibition of *cyp7a1* mRNA expression. TGFβ1 also decreased histone 3 acetylation in *cyp7a1* chromatin. TGFβ1 treatment and adenovirus *smad3* reduced HNF4α binding, but increased the recruitment of Smad3, HDAC1, and a repressor mSin3A to the *cyp7a1* chromatin [39]. Therefore, TGFβ represses *cyp7a1* gene expression in human hepatocytes via Smad3-dependent inhibition of HNF4α and HDAC remodeling of *cyp7a1* chromatin. TGFβ1/Smad3 signaling may reduce bile acid synthesis in the liver and prevent hepatocyte injury in cholestatic liver disease [39].

Surprisingly, some primary hepatocytes from certain donors showed no inhibition in *cyp7a1* expression after TGFβ1 treatment [39]. It is possible that the endogenous TGFβ1 levels may vary widely among different donor livers because of certain pathological conditions such as fatty liver, fibrosis, inflammation, and proliferation. Under these conditions, the TGFβ1/Smad3 signaling may already be activated or inactivated. In addition, activated TGFβ1/Smad3 pathway can be inhibited by a feedback mechanism. Smad3 can induce Ski and Sno oncoproteins, which then interact with Smad3 and inhibit TGFβ1/Smad3 signaling [41,42]. Furthermore, TGFβ1 signaling is blocked during liver proliferation to allow DNA synthesis [43]. It is possible that the observed increase or lack ofeffect of *cyp7a1* activity and mRNA expression is due to liver regeneration in response to injury. The complex interplay of cytokines and growth factors induced during liver injury may modulate the rate of bile acid synthesis to maintain lipid homeostasis in the liver [39].

Effects of TGFβ on *cyp7a1* gene expression have also been observed in bile duct-ligated (BDL) animal models. During cholestatic liver injury, hepatic nonparenchymal cells including Kupffer cells (hepatic macrophages) and hepatic stellate cells (HSCs) release proinflammatory cytokines (IL-1β and TNFα) and growth factors (hepatic growth factor and TGFβ) to hepatocytes. These cytokines have been shown to inhibit bile acid synthesis through repression of *cyp7a1* transcription [32,38,39]. Inhibition of bile acid synthesis and stimulation of bile secretion are adaptive responses to protect hepatocytes from injury. Paradoxically, CYP7A1 activity and its mRNA expression are stimulated in bile duct-ligated rat and mouse livers [28,44,45].TGFβ1 is increased in the livers of BDL rats and mice, and in patients with liver fibrosis [28,45,46]. TGFβ1-activated Smad3 stimulates the rat *cyp7a1* gene. Smad3 directly binds to the rat *cyp7a1* promoter and acts in synergy with FoxO1 and HNF4α to induce rat *cyp7a1* promoter activity. Smad3 and FoxO1 may crosstalk and synchronize with insulin inhibition and TGFβ1 activation of CYP7A1 in diabetes and liver fibrosis. On the other hand, TNFα antagonizes TGFβ1 activation of *cyp7a1* gene transcription. Therefore, these cell-signaling pathways may crosstalk in response to extracellular signals to regulate bile acid synthesis and maintain lipid homeostasis [40].

TGFβ-SMAD3 SIGNALING PATHWAY MEDIATES BILE ACID AND PHOSPHOLIPID METABOLISM

In addition to affecting bile acid synthesis, TGFβ also influences the metabolism of bile acids and phospholipids.TGFβ is activated as a result of cholestasis and regulates hepatic phospholipid and bile acid homeostasis through SMAD3 activation in lithocholic acid-induced experimental intrahepatic cholestasis. Injection of lithocholic acid induced expression of TGFβ1 and its receptors TGFBRI and TGFBRII in the liver, and increased TGFβ immunoreactivity around the portal vein. Serum metabolomics also indicated increased bile acids and decreased lysophosphatidylcholine after lithocholic acid injection. TGFβ-SMAD3 signaling appears to alter phospholipid and bile acid metabolism following hepatic inflammation with biliary injury [47]. However, TGFβ protein levels measured by an enzyme-linked immunoassay are not e   levated in the blood or bile of patients with PSC, which is a chronic cholestatic disorder of unknown etiology characterized by progressive fibrosis and stricturing biliary tract [48].

TGFβ AND BILE DUCT FIBROTIC BLOCKAGE

Hepatic fibrosis can be induced by TGFβ and is a major complication of chronic liver diseases such as chronic viral hepatitis or alcoholic liver diseases in adults, chronic cholestasis, or metabolic diseases in infancy. Biliary atresia (BA)and paucity of interlobular bile ducts are the two main causes of neonatal cholestasis leading to hepatic fibrosis [49]. TGFβ1, an important soluble factor involved in the regulation of myofibroblast extracellular matrix synthesis, was overexpressed in hepatic fibrous areas in adults [50,51]. In a few cases, TGFβ1 expression was located in areas of ductular proliferation characteristic of BA [52]. Interestingly, TGFβ1 was diffusely present in hepatocytes of children [49]. However, TGFβ1 was rarely found in healthy adult livers [51], but

was found in fibrotic septae in diseased liver [50,51]. TGFβ1 was also highly expressed in tumor hepatocytes [50]. In some BA patients, the TGFβ1 signal was enhanced at the periphery of biliary structures where inflammatory cells were present [49]. In chronic biliary disease, TGFβ1 was mainly found in mesenchymal cells [49]. Cholangiocytes have also been found to overexpress TGFβ2 in adult cholestatic liver disease [51].

The TGFβ superfamily promotes collagen synthesis by transforming HSCs into myofibroblasts, upregulating type I collagen gene transcription and increasing its mRNA stability [48,53]. TGFβ also increases the synthesis of other extracellular matrix proteins and inhibitors of metalloproteinase, and directly inhibits type I collagenase [53].High levels of TGFβ1 are often found in cholestatic livers. Bile acids in cholestatic levels can directly stimulate HSCs proliferation through protein kinase C-dependent induction of the epidermal growth factor receptor [54]. Bile acids can also induce thrombospondin-1, an activator of latent TGFβ1, in hepatocytes and contribute to HSCs proliferation and transdifferentiation [55]. Thus, bile acids promote liver fibrosis through activation of HSCs. Inhibition of bile acid is important in reducing both hepatocyte injury and HSCs activation in the fibrogenic process [39]. Corticosteroids, potent inhibitors of TGFβ, had therapeutic benefit on the course of PSC [56-57].

TGFβ AND PHASE I/II DETOXIFICATION ENZYMES AND TIGHT JUNCTIONS

Some of the adaptive mechanisms in response to cholestasis are also regulated by TGFβ. TGFβ1 represses expression of multiple genes involved in bile acid Phase II detoxification, which is correlated with a decrease in cellular glutathione and an increase in cellular reactive oxygen species [58]. TGFβ1 also inhibits xenobiotic-induced Phase I detoxification enzymes such as CYP1 isozymes [59,60]. However, the effect of TGFβ and bile acid Phase I detoxification CYP3 isozymes has not been studied.

It is well known that TGFβ induces the loss of tight junctions during epithelial-to-mesenchymal transition [61]. The epithelial-to-mesenchymal transition is a striking example of cellular plasticity that involves the dissolution of epithelial tight junctions, modulation of adherens junctions, reorganization of the actin cytoskeleton, loss of apical-basal polarity, and induction of a mesenchymal gene-expression program [62,63]. The mechanism appears to involve regulation of the polarity protein Par6 [64]. TGFβ1 treatment decreased tight junction proteins zona occludens protein 1 and occludin mRNA and protein levels in human renal proximal tubular cells [65]. The TGFβ1-mediated Rho/ROCK signaling pathway plays a role in the dissolution of tight junctions during epithelial-mesenchymal transition [65]. IL-1β and TGFβ also disassembled the tight junctions at the placental barrier [66]. TGFβ3 accelerated endocytosis of tight junction proteins for degradation and altered the permeability of the blood-testis barrier [67]. TGFβ3 treatment also promoted tight junction loss, and reorganization and cleavage of the adherens junction protein E-cadherin in mammary epithelial cells [68]. However, the effect of TGFβ upon the tight junction and permeability in the liver has not been studied.

CONCLUSION

It is likely that TGFβ involves in many aspects of hepatobiliary functions that are altered during cholestasis such as bile acid synthesis, metabolismand detoxification.Several research areas can be fruitful for cholestatic research such as exploring possible regulatory roles of TGFβ in bile acid transporter systems, Phase I detoxification system, tight junction dissolution, cytoskeleton organization, and transporter protein targeting, localization and reuptake.

ACKNOWLEDGEMENT

References

1. Trauner M, Meier PJ, Boyer JL. Molecular pathogenesis of cholestasis. N Engl J Med. 1998; 339: 1217-1227.

2. Trauner M, Boyer JL. Bile salt transporters: molecular characterization, function, and regulation. Physiol Rev. 2003; 83: 633-671.

3. Erlinger S. What is cholestasis in 1985? J Hepatol. 1985; 1: 687-693.

4. Zollner G, Trauner M. Mechanisms of cholestasis. Clin Liver Dis. 2008; 12: 1-26.

5. Koopen NR, Müller M, Vonk RJ, Zimniak P, Kuipers F. Molecular mechanisms of cholestasis: causes and consequences of impaired bile formation. Biochim Biophys Acta. 1998; 1408: 1-17.

6. Ballatori N, Truong AT. Relation between biliary glutathione excretion and bile acid-independent bile-flow. Am J Physiol. 1989; 256: G22-G30.

7. Ballatori N, Truong AT. Glutathione as a primary osmotic driving force in hepatic bile formation. Am J Physiol. 1992; 263: G617-G624.

8. Alavaro D, Mennone A, Boyer JL. Role of kinases and phosphatases in the regulation of fluid secretion and Cl⁻/HCO3⁻ exchange in cholangiocytes. Am J Physiol. 1997; 273: G303-G313.

9. Schlenker T, Romac JM, Sharara AI, Roman RM, Kim SJ, LaRusso N, et al. Regulation of biliary secretion through apical purinergic receptors in cultured rat cholangiocytes. Am J Physiol. 1997; 273: G1108-G1117.

10. Hagenbuch B, Meier PJ. Molecular cloning, chromosomal localization, and functional characterization of a human liver Na⁺/bile acid cotransporter. J Clin Invest. 1994; 93: 1326-1331.

11. Meier PJ. Molecular mechanisms of hepatic bile salt transport from sinusoidal blood into bile. Am J Physiol. 1995; 269: G801-G812.

12. Meier PJ, Eckhardt U, Schroeder A, Hagenbuch B, Stieger B. Substrate specificity of sinusoidal bile acid and organic anion uptake systems in rat and human liver. Hepatol. 1997; 26: 1667-1677.

13. Dean M, Alikmets R. Evolution of ATP-binding cassette transporter genes. Curr Opin Genet Dev. 1995; 5: 779-785.

14. Gatmaitan ZC, Arias IM. ATP-dependent transport systems in the canalicular membrane of the hepatocyte. Physiol Rev. 1995; 75: 261-275.

15. Müller M, Jansen PL. Molecular aspects of hepatobiliary transport. Am J Physiol. 1997; 272: G1285-G1303.

16. Vore M. Canalicular transport: discovery of ATP-dependent mechanisms. Toxicol Appl Pharmacol. 1993; 118: 2-7.

17. Kouzuki H, Suzuki H, Stieger B, Meier PJ, Sugiyama Y. Characterization of the transport properties of organic anion transporting polypeptide 1 (oatp1) and Na⁺/taurocholate cotransporting polypeptide (Ntcp): comparative studies on the inhibitory effect of their possible substrates in hepatocytes and cDNA-transfected COS-7 cells. J Pharmacol Exp Ther. 2000; 292: 505-511.

18. Cui Y, Konig J, Leier I, Buchholz U, Keppler D. Hepatic uptake of bilirubin and its conjugates by the human organic anion transporter SLC21A6. J Biol Chem. 2001; 276: 9626-9630.

19. Konig J, Cui Y, Nies AT, Keppler D. A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. Am J Physiol Gastrointest Liver Physiol. 2000; 278: G156-G164.

20. Kullak-Ublick GA, Ismair MG, Stieger B, Landmann L, Huber R, Pizzagalli F, et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. Gastroenterology. 2001; 120: 525-533.

21. Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell. 2000; 6: 507-515.

22. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell. 2000; 6: 517-526.

23. Denson LA, Strum E, Echevarria W, Zimmerman TL, Makishima M, Mangelsdorf DJ, et al. The orphan nuclear receptor, shp, mediates bile acid-induced inhibition of the rat bile acid transporter, ntcp. Gastroenterology□. 2001; 121: 140-147.

24. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ, et al. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell. 2000; 102: 731-744.

25. Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, Suchy FJ. Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. J Biol Chem. 2001; 276: 28857-28865.

26. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab. 2005; 2: 217-225.

27. Holt JA, Luo G, Billin AN, Bisi J, McNeill YY, Kozarsky KF, et al. Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. Genes Dev. 2003; 17: 1581-1591.

28. Beuers U, Trauner M, Jansen P, Poupon R. New paradigms in the treatment of hepatic cholestasis: from UDCA to FXR, PXR and beyond. J Hepatol. 2015; 62: S25-S37.

29. Trauner M, Fickert P, Stauber RF. Inflammation-induced cholestasis. J Gastroenterol Hepatol. 1999; 14: 946-959.

30. De Fabiani E, Mitro N, Anzulovich AC, Pinelli A, Galli G, Crestani M, et al. The negative effects of bile acids and tumor necrosis factor-alpha on the transcription of cholesterol 7 alpha-hydroxylase gene (CYP7A1) converge to hepatic nuclear factor-4: a novel mechanism of feedback regulation of bile acid synthesis mediated by nuclear receptors. J Biol Chem. 2001; 276: 30708-30716.

31. Gupta S, Stravitz RT, Dent P, Hylemon PB. Down-regulation of cholesterol 7 alpha-hydroxylase (CYP7A1) gene expression by bile acids in primary rat hepatocytes is mediated by the c-Jun N-terminal kinase pathway. J Biol Chem. 2001; 276: 15816-15822.

32. Li T, Jahan A, Chiang JY. Bile acids and cytokines inhibit the human cholesterol 7α-hydroxylase gene via the JNK/c-jun pathway in human liver cells. Hepatol. 2006; 43: 1202-1210.

33. Chanda D, Lee CH, Kim YH, Noh JR, Kim DK, Park JH, et al. Fenofibrate differentially regulates plasminogen activator inhibitor-1 gene expression via adenosine monophosphate-activated protein kinase-dependent induction of orphan nuclear receptor small heterodimer partner. Hepatol. 2009; 50: 880-892.

34. Cheng CC, Lin NN, Lee YF, Wu LY, Hsu HP, Lee WJ, et al. Effects of Shugan-Huayu powder, a traditional Chinese medicine, on hepatic fibrosis in rat model. Chin J Physiol. 2010; 53: 223-233.

35. Jung GS, Jeon JH, Choi YK, Jang SY, Park SY, Kim MK, et al. Small heterodimer partner attenuates profibrogenic features of hepatitis C virus-infected cells. Liver Int. 2015; 35:2233-2245.

36. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signaling from cell membrane to nucleus through SMAD proteins. Nature. 1997; 390: 465-471.

37. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signaling. Nature. 2003; 425: 577-584.

38. Song KH, Ellis E, Strom S, Chiang JY. Hepatocyte growth factor signaling pathway inhibits cholesterol 7α-hydroxylase and bile acid synthesis in human hepatocytes. Hepatol. 2007; 46: 1993-2002.

39. Li T, Chiang JYL. A novel role of transforming growth factor β1 in transcriptional repression of human cholesterol 7α-hydroxylase gene. Gastroenterol. 2007; 133: 1660-1669.

40. Li T, Ma H, Chiang JYL. TGFβ1, TNFα, and insulin signaling crosstalk in regulation of the rat cholesterol 7α-hydroxylase gene expression. J Lipid Res. 2008; 49: 1981-1989.

41. Luo K, Stroschein SL, Wang W, Chen D, Martens E, Zhou S, et al. The Ski oncoprotein interacts with the Smad proteins to repress TGF-beta signaling. Genes Dev. 1999; 13: 2196-2206.

42. Stroschein SL, Wang W, Zhou S, Zhou Q, Luo K. Negative feedback regulation of TGF-beta signaling by the SnoN oncoprotein. Science. 1999; 286: 771-774.

43. Taub R. Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol. 2004; 5: 836-847.

44. Dueland S, Reichen J, Everson GT, Davis RA. Regulation of cholesterol and bile acid homeostasis in bile-obstructed rats. Biochem J. 1991; 280: 373-377.

45. Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, et al. Hepatoprotection by the farnesoid X receptor agonist GW4064 in rat models of intra-and extrahepatic cholestasis. J Clin Invest. 2003; 112: 1678-1687.

46. Napoli J, Prentice D, Niinami C, Bishop GA, Desmond GA, McCaughan GW., et al. Sequential increases in the intrahepatic expression of epidermal growth factor, basic fibroblast growth factor, and transforming growth factor beta in a bile duct ligated rat model of cirrhosis. Hepatol. 1997; 26: 624-633.

47. Matsubara T, Tanaka N, Sato M, Kang DW, Krausz KW, Flanders KC, et al. TGF-β-SMAD3 signaling mediates hepatic bile acid and phospholipid metabolism following lithocholic acid-induced liver injury. J Lipid Res. 2012; 53: 2698-2707.

48. Watt JK, Hawkins K, Zhang M, Lipschitz J, Sandha G, et al. Transforming growth factor-β (TGF-β) protein levels are not elevated in the blood or bile of patients with primary sclerosing cholangitis: a pilot study. Digest Dis Sci. 2004; 49: 5-8.

49. Lamireau T, Le Bail B, Boussarie L, Fabre M, Vergnes P, Bernard O, et al. Expression of collagens type I and IV, osteonectin and transforming growth factor beta-1 (TGFβ1) in biliary atresia and paucity of intrahepatic bile ducts during infancy. J Hepatol. 1999; 31: 248-255.

50. Bédossa P, Peltier E, Terris B, Franco D, Poynard T. Transforming growth factor-beta 1 (TGF-β1) and TGF-β1 receptors in normal, cirrhotic, and neoplastic human livers. Hepatol. 1995; 21: 760-766.

51. Milani S, Herbst H, Schuppan D, Stein H, Surrentil C. Transforming growth factors β1 and β2are differentially expressed in fibrotic liver disease. Am J Pathol. 1991; 139: 1221-1229.

52. Malizia G, Brunt EM, Peters MG, Rizzo A, Broekelman TJ, McDonald JA, et al. Growth factor and procollagen type I gene expression in human liver disease. Gastroenterol. 1995; 108: 145-156.

53. Bissell DM, Roulot D, George J. Transforming growth factor-β and the liver. Hepatol. 2001; 34: 859-867.

54. Svegliati-Baroni G, Ridolfi F, Hannivoort R, Saccomanno S, Homan M, De Minicis S, et al. Bile acids induce hepatic stellate cell proliferation via activation of the epidermal growth factor receptor. Gastroenterol. 2005; 128: 1042-1055.

55. Myung SL, Yoon JH, Gwak GY, Kim W, Yang JI, Lee SH, et al. Bile acids-mediated thrombospondin-1 induction in hepatocytes leads to transforming growth factor-beta-dependent hepatic stellate cell activation. Biochem Biophys Res Comm. 2007; 353: 1091-1096.

56. Johnson GK, Greenen JE, Venu RP, Schmaltz MJ, Hogan WJ. Endoscopic treatment of biliary tract strictures in sclerosing cholangitis: a larger series and recommendations for treatment. Gastrointest Endosc. 1991; 37: 38-43.

57. Lee YM, Kaplan MM. Primary sclerosing cholangitis. New Engl J Med. 1995; 332: 923-933.

58. Bakin AV, Stourman NV, Sekhar KR, Rinehart C, Yan X, Meredith MJ, et al. Smad3-ATF3 signaling mediates TGF-β3 suppression of genes encoding Phase II detoxifying proteins. Free Rad Biol Med. 2005; 38: 375-387.

59. Abdel-Razzak Z, Corcos L, Fautrel A, Campion J-P, Guillouzo A. Transforming growth factor-β1 down-regulates basal and polycyclic aromatic hydrocarbon-induced cytochromes P-450 1A1 and 1A2 in adult human hepatocytes in primary culture. Mol Pharmacol. 1994; 46: 1100-1110.

60. Müller GF, Döhr O, El-Bahay C, Kahl R, Abel J. Effect of transforming growth factor-β1 on cytochrome P450 expression: inhibition of CYP1 mRNA and protein expression in primary rat hepatocyte. Arch Toxicol. 2000; 74: 145-152.

61. Miettinen PJ, Ebner R, Lopez AR, Derynck R. TGF-beta induced transdifferentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. J Cell Biol. 1994; 127: 2021-2036.

62. Grünert S, Jechlinger M, Beug H. Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. Nat Rev Mol Cell Bio. 2003; 4: 657-665.

63. Shook D, Keller R. Mechanisms, mechanics and function of epithelial–mesenchymal transitions in early development. Mech Dev. 2003; 120: 1351-1383.

64. Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL, et al. Regulation of the polarity protein Par6 by TGFβ receptors controls epithelial cell plasticity. Science. 2005; 307: 1603-1609.

65. Zhang K, Zhang H, Xiang H, Liu J, Liu Y, Zhang X, et al. TGF-β1 induces the dissolution of tight junctions in human renal proximal tubular cells: role of the Rho/ROCK signaling pathway. Int J Mol Med. 2013; 32: 464-468.

66. Tossetta G, Paolinelli F, Avellini C, Salvolini E, Ciarmela P, Lorenzi T, et al. IL-1β and TGF-β weaken the placental barrier through destruction of tight junctions: an in vivo and in vitro study. Placenta. 2014; 35: 509-516.

67. Wong EW, Mruk DD, Lee WM, Cheng CY. Regulation of blood-testis barrier dynamics by TGFβ3 is a Cdc42-dependent protein trafficking event. Proc Natl Acad Sci U S A. 2010; 107: 11399-11404.

68. Fornetti J, Flanders KC, Henson PM, Tan AC, Borges VF, Schedin P, et al. Mammary epithelial cell phagocytosis downstream of TGF-β3 is characterized by adherens junction reorganization. Cell Death Diff. 2016; 23: 185-196.

69. Wagner M, Zollner G, Trauner M. New molecular insights into the mechanisms of cholestasis. J Hepatol. 2009; 51: 565-580.