DOI: 10.55415/deep-2022-0052.v2

Molecular mechanism of transporter regulation and their impairment in intrahepatic cholestasis

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Abstract: Intrahepatic cholestasis (IC) is a liver disease caused by bile formation and excretion disorders due to structural and functional abnormalities of hepatocytes and/or bile capillary. It is commonly caused by hepatitis virus, alcohol, drug-induced liver damage, autoimmune liver disease and heredity. If patients with IC do not receive effective treatment, IC can progress to liver fibrosis, cirrhosis and ultimately liver failure. However, the mechanism of IC is still poorly understood. It is thought that IC is closely related to changes in the transcription, function and localization of hepatocellular transport proteins. To better understand the molecular mechanism of transport proteins in IC, we reviewed the roles of these transport proteins and discussed the underlying regulation mechanism of them in IC, aiming to provide a valuable reference for understanding its pathogenesis and developing effective drug therapies.

Keywords: Intrahepatic cholestasis, transport proteins, localization, regulation.

Introduction

Intrahepatic cholestasis (IC) is characterized by damage to hepatocytes or intrahepatic bile ducts and the accumulation of bile components in the serum[1]. IC can be caused by inflammatory disorders, drugs, heredity and environment, mainly including primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC) and intrahepatic cholestasis of pregnancy (ICP)[2, 3]. Any functional disturbance of the bile secretory process may be leading to IC, which is associated with intracellular accumulation of toxic bile constituents and consecutive cholestatic liver cell

DOI: 10.55415/deep-2022-0052.v2

damage[4]. The main pathogenic mechanisms of IC may include deregulation of bile secretion, impaired cell membrane fluidity, inflammatory responses, change of hepatocyte tight junctions and transporters [5].

It has currently been known that many transporters expressed in hepatocytes and cholangiocytes are involved in bile formation and excretion. The secretion of bile is the hepatocellular transport processes, mainly occurring across the canalicular membrane of hepatocytes. Disturbances of the function, expression and/or localization of transporters lead to the intracellular accumulation of toxic bile acids, promoting cholestatic liver injury[6]. Alterations of hepatobiliary transporter function are important risk factors for an individual's susceptibility to develop IC[7]. Mutations of transporter genes can cause hereditary cholestatic liver disease [8]. Mutations in MDR3 and BSEP can cause an array of cholestatic syndromes, including progressive and benign forms of familial IC, intrahepatic cholestasis of pregnancy [9, 10], are a well-established cause of inherited cholestatic syndromes[11, 12]. Furthermore, genetically determined functional changes in hepatobiliary transport systems have been demonstrated as the cause of acquired cholestatic syndromes, such as ICP and drug-induced cholestasis[7]. The transcriptions and expressions of transporters are regulated by complex networks. On one hand, transporters are regulated by multiple nuclear receptors (NRs) on the transcriptional level[13]. On the other hand, transporters after transcription and translation can also be regulated by various protein kinases (PKs) such as protein kinase B (Akt) and protein kinases C (PKCs)[14-16]. The first line treatment of IC is ursodeoxycholic (UDCA), however, approximately 40% of patients have inadequate response [17]. Thus, we herein reviewed the molecular mechanism of transporter dysregulation under IC, aiming to provide a valuable reference for understanding its pathogenesis and developing effective drug therapies.

1. Physiology of hepatobiliary transport and bile formation

Hepatic uptake and efflux processes involved in bile formation are maintained by distinct transport systems. After canalicular secretion, bile composition undergoes further modification in the canalicular, involving reabsorption and secretion processes

DOI: 10.55415/deep-2022-0052.v2

maintained by apical and basolateral transport system in cholangiocytes. Figure 1 shows a scheme of hepatocellular and bile ductular transport proteins involved in uptake and efflux of bile compounds (e.g., bile acids).

Bile, mainly including bile acids (BAs), cholesterol, bilirubin, bile pigment, phosphatidylcholine (PC), water and inorganic salts, etc., and plays crucial role in the digestion and absorption of lipids and lipid-soluble drugs. Bile production starts at the canaliculus of hepatocytes and bile is modified downstream by cholangiocytes[18, 19]. Bile formation is a fundamental physiological process that is formed by the active transport of BAs and other solutes across the canalicular membrane[20]. BAs, as the most important components of bile, are synthesized from cholesterols[21]. BA synthesis requires 17 enzymatic reactions. In the classical synthetic pathway, metabolism of cholesterols into 7α-hydroxycholesterol via 7α-hydroxylase (CYP7A1) is a key step. The vast majority of primary BAs, such as cholic acid (CA) and deoxycholic acid (DCA), are metabolized immediately into taurine- and glycine-binding BAs. They are transported into the canaliculus by hepatic transporters, then mixed with other components to forming bile[22].

BAs in bile enter the intestine through the contraction of the gallbladder. Under the action of intestinal flora, CA and DCA are transformed into ursodeoxycholic acid (UDCA) and lithocholic acid (LCA), respectively, and all taurine- and glycine-binding BAs are deamidated in the terminal ileum and colon. In the whole process of enterohepatic circulation, 90% of BAs are reabsorbed by intestinal epithelium, and the rest is discharged from human body through feces[23]. In the small intestine, some free BAs and glycine-binding BAs can be reabsorbed passively, but most of them are actively absorbed in the terminal ileum by apical sodium-dependent bile acid transporter (ASBT, SLC10A2) and organic solute transporter α/β (OSTα/β, SLC51a/b) on the basement membrane[24]. BAs in the hepatic portal vein, together with bilirubin and a large variety of other organic anions are mostly reabsorbed from the blood into the liver through uptake transporters, and the rest are eliminated from the kidneys through the blood circulation. It is estimated that BAs can generally be eliminated after 20 enterohepatic circulations. Therefore, BAs uptake disorders, obstructed BAs efflux and bile duct injury in the liver can all lead to cholestasis [25].

DOI: 10.55415/deep-2022-0052.v2

1.1 Liver transporters involved in bile formation and excretion

The secretion and excretion of bile depend on complex hepatobiliary transport systems and cholangiocytes. Many transporters are expressed on the basolateral or canalicular membrane of hepatocyte, mainly including ATP-binding cassette (ABC) and solute carrier family (SLC) transporters. ABC transporters are considered to be responsible for efflux of substrates, while SLC transporters mediate uptake of substrates into cells. These transporters play an important role in bile formation and the biliary excretion of xenobiotics. In the process of bile flow obstruction, cholestasis is attributed to the expression and functional abnormalities of various transporters[26]. The transporters in cholangiocytes can take in the electrolytes and water into the blood forming a "biliary-liver" cycle, and excrete them into the bile duct further promoting bile flow (Figure 1). Therefore, transporters play important roles in the secretion and excretion of bile in cholestasis.

1.1.1 SLC transporters

SLC transporters include Na^+-taurocholate co-transport polypeptide (NTCP, SLC10A1), organic anion transporter polypeptides (OAPTs, SLCO), OSTα/β and ASBT. On the basolateral membrane of hepatocytes, BAs are mainly taken up by the NTCP and OATPs into hepatocytes. Besides, NTCP also transports steroidal hormones and a variety of drugs. Repression and translocation of NTCP contribute to the etiopathogenesis of IC[27]. OATPs uptake other cholephilic compounds, including glucuronidated bilirubin, exogenous organic anions, leukotrienes, estrogen-conjugates (e.g. estrone-3-sulfate or estradiol-17-β-d-glucuronide), thyroid hormones, mycotoxins, and numerous xenobiotics[28−30]. Human OATP1A and rat OATP2 mediate the uptake of bulky organic cations; small organic cations are taken up by the organic cation transporter 1 (OCT1, SLC22A1)[31].

1.1.2 ABC transporters

In hepatocytes, the bile excretion mainly occurs at the canalicular membrane predominantly through the ABC transporters. ABC transporters are a superfamily of membrane proteins that mediate diverse ATP-driven transport processes, mainly including bile salt export pump (BSEP, ABCB11), multidrug resistance protein 2

DOI: 10.55415/deep-2022-0052.v2

(MRP2, ABCC2), P-glycoprotein (P-gp/MDR1, ABCB1), breast cancer drug resistance protein (BCRP, ABCG2), multidrug resistance protein 3 (MDR3, ABCB4), etc[32, 33]. BSEP and MRP2, two main transporters on the canalicular membrane, can excrete BAs into the bile duct. Monoanionic bile salts are mainly excreted in the canalicular pole by BSEP[34]. In contrast, canalicular efflux of divalent, sulfated or glucuronidated bile salts, glutathione, glucuronidated bilirubin is mediated by MRP2[33]. In addition, PC, cholesterol and other compounds are excreted into the canaliculus through ATP binding cassette subfamily B member 4 (MDR2, ABCB4), ATP-binding cassette sub-family G member 5 /8 (ABCG5/G8), ATP binding cassette subfamily B member 1 (MDR1, ABCB1) and MRP2[35].

Cooperation of transporters is critical to maintain the homeostasis of bile (Figure 2). In the early stage of acute and chronic cholestasis, the "NTCP-BSEP axis" is blocked, leading to accumulation of BAs in hepatocytes and spontaneously activating "OATPs-MRP2 axis" to accelerate the excretion of BAs. It maintains bile homeostasis, delays rapid increase of intracellular BAs concentrations within a short time, and alleviates hepatocyte and their structural and functional damages[36].

1.2 Transporters in intrahepatic cholangiocytes

During bile flow formation and excretion, the intrahepatic bile duct secretes electrolytes Cl^{-1} and HCO_3^{-1} into bile by several transporters or channels expressed on the cholangiocyte membrane to synergistically regulate the fluidity and pH of bile in the bile duct [37]. They mainly include cystic fibrosis transmembrane conductance regulator (CTFR), anion exchanger 2 (AE-2) and aquaporin-1 (AQP1). Their dysfunction directly leads to abnormal secretion of inorganic salts and water, the change of bile compositions and flow[38]. Impairment of cholangiocyte transporters and aquaporin (AQP) leads to a "toxic" bile due to both lack of the "HCO^{3-} umbrella" and an increase intraluminal levels of damaging BAs[39]. CTFR transports intracellular Cl^{-1} to the outside of the plasma membrane[40]. Subsequently, Cl^{-1} on the plasma membrane secondarily drives Cl^{-1}-HCO_3^{-1} transporter AE-2, and actively secretes HCO_3^{-1} to bile[41], while AQP1 transports water molecules into bile[42]. Genetic abnormalities of CFTR attenuate bile hydration, accumulate toxic BAs, damage cholangiocytes, and induce cholestasis that ultimately progresses into cystic

DOI: 10.55415/deep-2022-0052.v2

fibrosis[43]. Abnormal function and expression of AE-2 are also associated with PBC[44].

2. Transcription regulation of hepatic transporters by NRs

NRs form a family of 48 members, and play important roles in bile acid homeostasis, lipid metabolism and mechanisms involved in fibrosis and inflammation. Several of the adaptive changes in cholestasis are mediated via NRs because biliary compounds retained during cholestasis (e.g., bile acids, bilirubin, oxysterols, hormones, drugs) act as NR ligands and coordinately affect target gene expression[45, 46]. It has currently been confirmed that NRs involved in the maintenance of BA homeostasis in IC, mainly including farnesol X receptor (FXR, NR1H4), pregnane X receptor (PXR, NR1I2), constitutive androgen receptor (CAR, NR1I3), liver X receptor α (LXRa, NR1H3) and vitamin D receptor (VDR, NR1I1)[13]. In addition, other NRs, including liver receptor homolog-1 (LRH-1, NR5A2) and peroxisome proliferator-activated receptors (PPARs, NR1Cs) (Table 1), play important roles in IC[47]. Changes in transporter regulation comprise a complex interacting network of several ligand-activated NRs as well as liver-enriched hepatocyte nuclear factors.

2.1 FXR

FXR is a main NR involved in regulating the expressions of transporters and maintaining BA homeostasis during the pathogenesis of cholestasis[48]. In the early stage of chronic cholestasis, FXR is rapidly activated to form an FXR-RXR (retinoid X receptor, NR2B1) dimer with RXR. Then, the dimer binds the inverted repeat 1 (IR-1) of the target gene promoter, to significantly up-regulate expression levels of BSEP and MRP2, thereby accelerating BA excretion. In the meantime, FXR can induce the expression of a nuclear orphan receptor “small heterodimer partner” (SHP), then inhibit the functions of other NRs such as liver X receptor (NR1H3) and hepatocyte nuclear factor 4α (HNF4α), and ultimately suppress the expressions of CYP7A1/CYP8B1 and NTCP in hepatocytes, thereby reducing the synthesis and intake of BAs as well as indirectly accelerating BA clearance[49]. Similar to NTCP, OATP1B1 and ASBT are also negatively regulated by FXR involving SHP interaction with HNF4[50]. In addition, FXR directly promotes cellular bile clearance via directly inducing canalicular BSEP and MRP2[51, 52].

DOI: 10.55415/deep-2022-0052.v2

In another regulatory pathway for the inhibition of BA synthesis, intestinal FXR can induce the expression of an intestinal hormone-like peptide, fibroblast growth factor 15/19 (murine: FGF15; human: FGF19). FXR induces the expression of FGF15/19 through the activation of hepatic FGF receptor 4 (FGFR4), then activates the intracellular stress-activated Jun N-terminal kinase (JNK) pathway to inhibit the CYP7A1 activity leading to reducing BA synthesis[53]. Thus the FXR-FGF19 pathway, as a typical negative feedback regulation mechanism, plays a critical role in the pathogenesis of cholestatic diseases[54]. Taken together, FXR may be a promising therapeutic target for novel drug development in IC.

2.2 PXR and CAR

In addition to FXR, recent studies have revealed that PXR and CAR are key NRs for regulating many adaptive responses in IC. They coordinate protective hepatic responses to toxic stimuli induced by endogenous compounds (BAs, bilirubin) and xenobiotics[55, 56]. As sensors of toxic byproducts, they are central in the detoxifying pathways involving phases I/II detoxification and transporters[55, 57, 58]. Studies found that the levels of PXR and CAR were reduced in IC[59], while PXR polymorphisms was associated with increased susceptibility to ICP[60]. MRP3/4 expression was upregulated via PXR and CAR to alleviate cholestatic liver injury[61, 62]. In addition, FXR can also induce BA export and metabolism via transcriptionally activating PXR[63]. Taken together, they may be therapeutic benefits for cholestasis patients in the future.

2.3 VDR

VDR, expressed in the intestine, kidney and liver, can be also activated by BAs. Recent reports found that VDR can regulate BA transporters and its polymorphic variants may affect individual susceptibility and the quality of life in patients with IC, such as PBC, ICP[64, 65]. However, the direct impact of VDR polymorphisms on the pathogenesis of IC is unclear. Loss of VDR exacerbates cholestatic liver injury by disruption of biliary epithelial cell junctions in mice[66]. VDR can increase ASBT mRNA expression and promoter activity[67]. Moreover, VDR seems to play an indirect role in bile acid homeostasis. Furthermore, vitamin D intake might relieves

DOI: 10.55415/deep-2022-0052.v2

biliary fibrosis in ABCB knockout mice and improve cholestatic disease[68]. Therefore, VDR may represent a therapeutic target in cholestatic diseases.

2.4 LRH-1

LRH-1, as transcription factor for bile salt synthesis, is mainly expressed in liver, intestine, exocrine pancreas, and reproductive tissue[69]. It binds to DNA in its monomeric form and regulates other NRs and the transcription of genes involved in the biosynthesis and transport of BAs, including CYP7A1[70], BSEP, MRP2, ASBT, NTCP, MRP3 and MDR2[71−74]. LRH-1 induces the expression of CYP7A1, BSEP[72] and ASBT[74]. In addition, deletion of LRH-1 significantly reduced the expression of FXP and SHP as well as transporters (i.e., NTCP, BSEP, MRP3, MRP2, MDR2)[71]. However, the effect of LRH-1 still need to be fully elucidated in IC.

2.5 PPARα

PPARα, ligand-activated nuclear receptor, plays a central role in maintaining cholesterol, lipid and bile acid homeostasis by regulating genes involved bile acid synthesis, and transport. PPARα primarily down-regulates BA synthesis through inhibition of the BA-synthesizing enzymes (i.e., CYP7A1, CYP27A1)[75]. In addition, PPARα induces biliary phospholipid output by activating canalicular MDR3[76]. PPARα activators directly induce canalicular MDR2 thereby inducing biliary phospholipid output[77]. In addition, ASBT expression in cholangiocytes and intestine is induced by PPARα[78], resulting in increased BAs absorption from the intestine and bile ducts. Bezafibrate, a dual PPAR and PXR agonist, could increase the expression of NTCP, MDR1, MDR3, and MRP2 to protecte from cholestatic liver injury[79]. Agonists of PPARα are promising therapeutic approaches in IC.

3. Localization regulation of hepatic transporters

Accurate expressions and localization of transporters on the plasma membrane require interactions among various proteins between the membrane and cytoskeleton. Meanwhile, protein kinases (PKs) regulate this complex process. PKs contain serine (Ser), threonine (Thr) and tyrosine (Tyr) residues or lysine (Lys), histidine (His) and arginine (Arg) residues. Various PKs, such as PKB (Akt) and PKC, can regulate the

DOI: 10.55415/deep-2022-0052.v2

localization of hepatic bile transporters after transcription, and activation of PI3K/Akt signaling causes sustained internalization of MRP2 and BSEP, eventually leading to cholestasis[80, 81]. Relying on second messages, PKs can initiate signal cascades, regulate the phosphorylation and dephosphorylation of hepatobiliary transport system and corresponding crosslink proteins or scaffolding proteins, change the membrane localization of transporters and rapidly adjust the bile composition, subsequently promoting cholestasis and/or exerting choleretic effects.

3.1 PKB (Akt)

The Ser/Thr kinase PKB (Akt) has been widely accepted as a cell growth factor regulating the functions of multiple downstream anti-apoptotic proteins[82]. Akt is regard as the characteristic target protein and terminal effector of PI3K[83, 84]. Beuers et al.[85] found that in TLCA-induced cholestasis model, wortmannin (WM), PI3K-specific inhibitor, reduced Akt activity and attenuated cholestasis, suggesting a causal link between the two events. Besides, in $E_2$17G- and TLCA-induced cholestasis model, the anti-cholestatic effect of Akt inhibitor (Calbiochem 124005) was similar to that of PI3K inhibitor (LY294002), and identical anti-cholestatic outcomes were achieved by combining WM with Calbiochem 124005 or cPKC inhibitor (G6976)[86]. In addition, the activation of PI3K and Akt contributes to sustain internalization of transporters and the consequent impairment of their activity. Thus, the PI3K/Akt pathway is mainly responsible for cholestasis.

3.2 PKC

PKC is a group of PKs mediating the function of targeted proteins through phosphorylating serine and threonine amino acid residues. Ten subtypes of PKC have been found in tissue of mammals, which could be divided into three groups: conventional PKC (cPKC), including α, βI, βII and γ subtypes; novel PKC (nPKC), including δ, ε, η and θ subtypes; atypical PKC (aPKC), including ι (also known as λ in mice) and ζ subtypes[87]. The activation of several PKC subtypes in the liver, such as aPKCζ and nPKCδ, depends on PI3K[88−90]. However, the activation of cPKCs does not rely on PI3K[86, 91, 92], and oxidative stress can mediate the activation of cPKCs and nPKCs[93]. In the past, the activation of PKCs has been found to induce

DOI: 10.55415/deep-2022-0052.v2

cholestasis[94], inhibit cAMP-induced intake of taurocholate (TC) and decrease MRP3- and OATP-mediated organic solute transport[95–97]. Different PKCs are activated by various compounds, exerting pro-cholestatic, anti-cholestatic and choleretic effects. Accordingly, it is reasonable to assume that different compounds work differently by affecting various subtypes of PKCs and corresponding signaling pathways (Figure 3).

3.2.1 The roles of PKC subtypes in IC

Both cPKCα and nPKCδ participate in the pathogenesis of cholestasis. In $E_2$17G-induced cholestasis model, cPKCα participates in pathogenesis by activating downstream signal estrogen receptor (ERα)[98]. cPKCα mediates NTCP retrieval induced by phorbol myristate acetate (PMA) and taurochenodeoxycholate acid (TCDCA)[99, 100]. And TUDCA exerts its post-translational anticholestatic effect mainly by a cooperative cPKCα-/PKA-dependent mechanism in the experimental model of TLCA-induced cholestasis[101]. A recent study showed that nPKCδ was activated by cAMP and involved in cAMP-mediated NTCP and MRP2 translocation in hepatocytes[102]. Different from cPKCα, the effects of nPKCδ are associated with its phosphorylation sites that are activated by different signals. Studies found that activation of nPKCδ may lead to cholestatic effects via Tyr phosphorylation, while its activation may lead to anti-cholestatic effects via Thr phosphorylation[103, 104]. Consistent with this hypothesis, the activation of nPKCδ by cAMP and GCDCA is associated with Thr and not Tyr phosphorylation in rat hepatocytes[90, 105]. Nevertheless, the effects and mechanism underlying differential phosphorylation of nPKCδ in IC still need validation.

nPKCε and aPKCζ are two important PKC subtypes. In primary hepatocyte, TLCA can activate nPKCε and induce MRP2 endocytosis. Knock down of nPKCε reverses TLCA-induced internalization of MRP2[106]. And cAMP and TUDCA reverses TLCA-induced cholestasis and MRP2 retrieval by inhibiting nPKCε[85, 107, 108]. MRP2 retrieval induced by ethacrynic acid (EA) is also mediated via nPKCε in rat[109]. In contrast to nPKCε, cAMP promotes the delivery and localization of NTCP towards the basement membrane via the PI3K/aPKCζ pathway[110]. Since aPKCζ, BSEP and MRP2 are all expressed on the membrane of hepatocytes[111], aPKCζ may

DOI: 10.55415/deep-2022-0052.v2

also be involved in the canalicular localization of the two transporters, which should be further studied.

Conclusion

Transporters participate in the transmembrane transport of bile and their disorders play an important role in the pathogenesis of cholestasis. In the past decades, the essential roles of hepatic transporters in the pathogenesis of cholestasis have been gradually revealed. However, studies on the molecular mechanism for cholestasis have been mainly limited to the transcriptional, expression and functional abnormalities of individual or several transporters, so their conclusions are often one-sided. Notably, abnormal transporter localization can also cause cholestasis. The localization of hepatocyte transporters has been studied, but it is not comprehensive. In addition, there are few studies on the location and function of the cholangiocyte transporters. Overall, the pathogenesis of cholestasis induced by abnormal bile transport is a complex network comprising multiple transporters that synergistically complete bile secretion and excretion from the liver. Furthermore, given the compensatory protective mechanism of the human body, transporters exert distinctly different effects on acute and chronic cholestasis, thus allowing us to unravel the underlying molecular mechanism.

NRs, as transcription factors, regulate transporter genes required for hepatobiliary transport, as well as the phases I and II metabolizing enzymes involved in processing of their substrates. Impaired NR signaling may affect the expression of transporters, and genetic variants of NR-encoding genes are associated with susceptibility and progression of IC. In addition, altered localization of transporters participate in pathogenesis of IC. These changes in transporter localization are highly regulated post-translational events requiring various cellular signaling pathways, such as PKB (Akt) and PKC. Atypical PKCζ may mediate choleretic effects by inserting NTCP into the plasma membrane and nPKCε may mediate cholestatic effects by retrieving MRP2 from the plasma membrane[16]. On the other hand, cPKCα and nPKCδ may be involved in choleretic, cholestatic and anticholestatic effects by inserting, retrieving and inhibiting retrieval of transporters, respectively. Thus, we herein review the molecular mechanism that transporters are regulated through various proteins such as NRs and PKCs proteins in cholestasis, aiming to provide a valuable reference for

DOI: 10.55415/deep-2022-0052.v2

understanding its pathogenesis and developing effective drug therapies. Nevertheless, considerable in-depth studies are still in need to comprehensively clarify the network consisting of regulatory mechanism for cholestasis-related transporters.

Abbreviations

BA, bile acid; CA, cholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid; LCA, lithocholic acid; TUDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; ABC, ATP-binding cassette; SLC, solute carrier; ABCB11, bile salt export pump, BSEP; CAR, constitutive androgen receptor; CYP7A1, cholesterol 7α-hydroxylase; FXR, farnesol X receptor; GR, glucocorticoid receptor; LXR, liver X receptor; ABCB4, multidrug resistance protein 3; ABCC2, multidrug resistance protein 2; ABCC3, multidrug resistance protein 3; ABCC4, multidrug resistance protein 4; NTCP, Na+-taurocholate co-transport polypeptide; OSTα/β, organic solute transporter α/β; PC, phosphatidylcholine; PXR, pregnane X receptor; PPARα, peroxisome proliferator-activated receptor α; PPARγ, peroxisome proliferator-activated receptor γ; SHP, small heterodimer partner; SULT, sulfotransferase; UGT, UDP-glucuronyltransferase; PI3K, phosphoinositide-3-kinase; PKC, protein kinase C; CTFR, cystic fibrosis transmembrane conductance regulator; AE-2, Cl^{-1}-HCO_3^{-1} transporter; APQ-1, aquaporin-1;

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (NO.82073939).

DECLARATION OF COMPETING INTERESTS

The authors have no conflict of interests related to this publication.

AUTHOR CONTRIBUTIONS

Xiping Li and Yue Zu, as co-first authors, had made contributions to concept, design the manuscript, data analysis and interpretation and wrote the manuscript or revised it critically.

Guodong Li and Dong Xiang read the manuscript and proposed comments on manuscript revision.

Chengliang Zhang and Dong Liu were responsible for the integrity of the work as a

whole, and finally approved the version to be published.

All authors participated in this manuscript and agreed to publish.

References

[1] Amirneni S, Haep N, Gad MA, Soto-Gutierrez A, Squires JE, Florentino RM: Molecular overview of progressive familial intrahepatic cholestasis. World J Gastroenterol 2020, 26(47):7470-84. https://doi.org/10.3748/wjg.v26.i47.7470.

[2] Xiao J, Li Z, Song Y, Sun Y, Shi H, Chen D, et al.: Molecular pathogenesis of intrahepatic cholestasis of pregnancy. Can J Gastroenterol Hepatol 2021, 2021:6679322. https://doi.org/10.1155/2021/6679322.

[3] Trauner M, Meier PJ, Boyer JL: Molecular pathogenesis of cholestasis. N Engl J Med 1998, 339(17):1217-27. https://doi.org/10.1056/NEJM199810223391707.

[4] Pauli-Magnus C, Meier PJ: Hepatocellular transporters and cholestasis. J Clin Gastroenterol 2005, 39(4 Suppl 2):S103-10. https://doi.org/10.1097/01.mcg.0000155550.29643.7b.

[5] Zu Y, Yang J, Zhang C, Liu D: The pathological mechanisms of Estrogen-Induced cholestasis: Current perspectives. Front Pharmacol 2021, 12:761255. https://doi.org/10.3389/fphar.2021.761255.

[6] Piatek K, Kurzawinska G, Magielda J, Drews K, Barlik M, Malewski Z, et al.: The role of ABC transporters' gene polymorphism in the etiology of intrahepatic cholestasis of pregnancy. Ginekol Pol 2018, 89(7):393-7. https://doi.org/10.5603/GP.a2018.0067.

[7] Pauli-Magnus C, Meier PJ: Pharmacogenetics of hepatocellular transporters. Pharmacogenetics 2003, 13(4):189-98. https://doi.org/10.1097/00008571-200304000-00003.

[8] Thompson R, Jansen PL: Genetic defects in hepatocanalicular transport. Semin Liver Dis 2000, 20(3):365-72. https://doi.org/10.1055/s-2000-9384.

[9] Jacquemin E: Role of multidrug resistance 3 deficiency in pediatric and adult liver disease: One gene for three diseases. Semin Liver Dis 2001, 21(4):551-62. https://doi.org/10.1055/s-2001-19033.

[10] Rosmorduc O, Hermelin B, Poupon R: MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. Gastroenterology 2001, 120(6):1459-67. https://doi.org/10.1053/gast.2001.23947.

[11] Noe J, Kullak-Ublick GA, Jochum W, Stieger B, Kerb R, Haberl M, et al.: Impaired expression and function of the bile salt export pump due to three novel ABCB11 mutations in intrahepatic cholestasis. J Hepatol 2005, 43(3):536-43. https://doi.org/10.1016/j.jhep.2005.05.020.

[12] Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, et al.: A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Genet 1998, 20(3):233-8. https://doi.org/10.1038/3034.

[13] Halilbasic E, Baghdasaryan A, Trauner M: Nuclear receptors as drug targets in cholestatic liver diseases. Clin Liver Dis 2013, 17(2):161-89. https://doi.org/10.1016/j.cld.2012.12.001.

[14] Abdullah M, Amélie M, Marc LV, Bruno S, Claire D, Yannick P, et al.: Protein kinases C-Mediated regulations of drug transporter activity, localization and expression. Int J Mol Sci 2017, 18(4).

[15] Marcelo GR, Ismael RB, Gisel SM, Fernando AC, Enrique JSP: Dynamic localization of hepatocellular transporters: Role in biliary excretion and impairment in cholestasis. Curr Med Chem 2019, 26(7).

[16] Anwer MS: Role of protein kinase C isoforms in bile formation and cholestasis. Hepatology 2014, 60(3):1090-7. https://doi.org/10.1002/hep.27088.

[17] Woolbright BL, Jaeschke H: Therapeutic targets for cholestatic liver injury. Expert Opin Ther Targets 2016, 20(4):463-75. https://doi.org/10.1517/14728222.2016.1103735.

[18] Eshmuminov D, Schuler MJ, Becker D, Bautista BL, Mueller M, Hagedorn C, et al.: Bile formation in long-term ex situ perfused livers. Surgery 2021, 169(4):894-902. https://doi.org/10.1016/j.surg.2020.11.042.

[19] Boyer JL, Soroka CJ: Bile formation and secretion: An update. J Hepatol 2021. https://doi.org/10.1016/j.jhep.2021.02.011.

[20] Kullak-Ublick GA, Stieger B, Meier PJ: Enterohepatic bile salt transporters in normal physiology and liver disease. Gastroenterology 2004, 126(1):322-42. https://doi.org/10.1053/j.gastro.2003.06.005.

[21] Cuperus FJ, Claudel T, Gautherot J, Halilbasic E, Trauner M: The role of canalicular ABC

DOI: 10.55415/deep-2022-0052.v2

transporters in cholestasis. Drug Metab Dispos 2014, 42(4):546-60. https://doi.org/10.1124/dmd.113.056358.
[22] Javitt NB: Hepatic bile formation: Bile acid transport and water flow into the canalicular conduit. Am J Physiol Gastrointest Liver Physiol 2020, 319(5):G609-18. https://doi.org/10.1152/ajpgi.00078.2020.
[23] Liu X, Wang Y: [an overview of bile acid synthesis and its physiological and pathological functions]. Yi Chuan 2019, 41(5):365-74. https://doi.org/10.16288/j.yczz.19-011.
[24] Hofmann AF: The enterohepatic circulation of bile acids in mammals: Form and functions. Front Biosci (Landmark Ed) 2009, 14:2584-98. https://doi.org/10.2741/3399.
[25] Rodrigues AD, Lai Y, Cvijic ME, Elkin LL, Zvyaga T, Soars MG: Drug-induced perturbations of the bile acid pool, cholestasis, and hepatotoxicity: Mechanistic considerations beyond the direct inhibition of the bile salt export pump. Drug Metab Dispos 2014, 42(4):566-74. https://doi.org/10.1124/dmd.113.054205.
[26] Strazzabosco M, Spirli C, Okolicsanyi L: Pathophysiology of the intrahepatic biliary epithelium. J Gastroenterol Hepatol 2000, 15(3):244-53. https://doi.org/10.1046/j.1440-1746.2000.02091.x.
[27] Irak K, Bayram M, Cifci S, Acar Z, Kazezoglu C, Yorulmaz E, et al.: Role of serum organic solute transporter alpha/beta and sodium taurocholate cotransporting polypeptide in intrahepatic cholestasis of pregnancy. Ginekol Pol 2021, 92(11):767-73. https://doi.org/10.5603/GP.a2021.0063.
[28] Bohan A, Boyer JL: Mechanisms of hepatic transport of drugs: Implications for cholestatic drug reactions. Semin Liver Dis 2002, 22(2):123-36. https://doi.org/10.1055/s-2002-30099.
[29] Ito K, Suzuki H, Horie T, Sugiyama Y: Apical/basolateral surface expression of drug transporters and its role in vectorial drug transport. Pharm Res 2005, 22(10):1559-77. https://doi.org/10.1007/s11095-005-6810-2.
[30] Beaudoin JJ, Bezencon J, Sjostedt N, Fallon JK, Brouwer K: Role of organic solute transporter Alpha/Beta in hepatotoxic bile acid transport and drug interactions. Toxicol Sci 2020, 176(1):34-5. https://doi.org/10.1093/toxsci/kfaa052.
[31] Van Montfoort JE, Muller M, Groothuis GM, Meijer DK, Koepsell H, Meier PJ: Comparison of "type I" and "type II" organic cation transport by organic cation transporters and organic anion-transporting polypeptides. J Pharmacol Exp Ther 2001, 298(1):110-5.
[32] Thoeni C, Waldherr R, Scheuerer J, Schmitteckert S, Roeth R, Niesler B, et al.: Expression analysis of ATP-Binding cassette transporters ABCB11 and ABCB4 in primary sclerosing cholangitis and variety of pediatric and adult cholestatic and noncholestatic liver diseases. Can J Gastroenterol Hepatol 2019, 2019:1085717. https://doi.org/10.1155/2019/1085717.
[33] Akita H, Suzuki H, Ito K, Kinoshita S, Sato N, Takikawa H, et al.: Characterization of bile acid transport mediated by multidrug resistance associated protein 2 and bile salt export pump. Biochim Biophys Acta 2001, 1511(1):7-16. https://doi.org/10.1016/s0005-2736(00)00355-2.
[34] Suchy FJ, Ananthanarayanan M: Bile salt excretory pump: Biology and pathobiology. J Pediatr Gastroenterol Nutr 2006, 43 Suppl 1:S10-6. https://doi.org/10.1097/01.mpg.0000226385.71859.5f.
[35] Ben SA, Bruneau A, Mareux E, Lapalus M, Delaunay JL, Gonzales E, et al.: Molecular regulation of canalicular ABC transporters. Int J Mol Sci 2021, 22(4). https://doi.org/10.3390/ijms22042113.
[36] Cuperus FJ, Claudel T, Gautherot J, Halilbasic E, Trauner M: The role of canalicular ABC transporters in cholestasis. Drug Metab Dispos 2014, 42(4):546-60. https://doi.org/10.1124/dmd.113.056358.
[37] Marinelli RA, Vore M, Javitt NB: Hepatic bile formation: Canalicular osmolarity and paracellular and transcellular water flow. J Pharmacol Exp Ther 2019, 371(3):713-7. https://doi.org/10.1124/jpet.119.261115.
[38] Hatano R, Akiyama K, Tamura A, Hosogi S, Marunaka Y, Caplan MJ, et al.: Knockdown of ezrin causes intrahepatic cholestasis by the dysregulation of bile fluidity in the bile duct epithelium in mice. Hepatology 2015, 61(5):1660-71. https://doi.org/10.1002/hep.27565.
[39] Trauner M, Fickert P, Halilbasic E, Moustafa T: Lessons from the toxic bile concept for the pathogenesis and treatment of cholestatic liver diseases. Wien Med Wochenschr 2008, 158(19-20):542-8. https://doi.org/10.1007/s10354-008-0592-1.
[40] Wang S, Yue H, Derin RB, Guggino WB, Li M: Accessory protein facilitated CFTR-CFTR interaction, a molecular mechanism to potentiate the chloride channel activity. Cell 2000, 103(1):169-79. https://doi.org/10.1016/s0092-8674(00)00096-9.
[41] Thornell IM, Bevensee MO: Regulators of Slc4 bicarbonate transporter activity. Front Physiol 2015, 6:166. https://doi.org/10.3389/fphys.2015.00166.

DOI: 10.55415/deep-2022-0052.v2

[42] Banales JM, Prieto J, Medina JF: Cholangiocyte anion exchange and biliary bicarbonate excretion. World J Gastroenterol 2006, 12(22):3496-511. https://doi.org/10.3748/wjg.v12.i22.3496.
[43] Herrmann U, Dockter G, Lammert F: Cystic fibrosis-associated liver disease. Best Pract Res Clin Gastroenterol 2010, 24(5):585-92. https://doi.org/10.1016/j.bpg.2010.08.003.
[44] Salas JT, Banales JM, Sarvide S, Recalde S, Ferrer A, Uriarte I, et al.: Ae2a,b-deficient mice develop antimitochondrial antibodies and other features resembling primary biliary cirrhosis. Gastroenterology 2008, 134(5):1482-93. https://doi.org/10.1053/j.gastro.2008.02.020.
[45] Wagner M, Zollner G, Trauner M: New molecular insights into the mechanisms of cholestasis. J Hepatol 2009, 51(3):565-80. https://doi.org/10.1016/j.jhep.2009.05.012.
[46] Zollner G, Marschall HU, Wagner M, Trauner M: Role of nuclear receptors in the adaptive response to bile acids and cholestasis: Pathogenetic and therapeutic considerations. Mol Pharm 2006, 3(3):231-51. https://doi.org/10.1021/mp060010s.
[47] Gonzalez-Sanchez E, Firrincieli D, Housset C, Chignard N: Nuclear receptors in acute and chronic cholestasis. Dig Dis 2015, 33(3):357-66. https://doi.org/10.1159/000371688.
[48] Stofan M, Guo GL: Bile acids and FXR: Novel targets for liver diseases. Front Med (Lausanne) 2020, 7:544. https://doi.org/10.3389/fmed.2020.00544.
[49] 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(1 Suppl):S25-37. https://doi.org/10.1016/j.jhep.2015.02.023.
[50] Eloranta JJ, Kullak-Ublick GA: Coordinate transcriptional regulation of bile acid homeostasis and drug metabolism. Arch Biochem Biophys 2005, 433(2):397-412. https://doi.org/10.1016/j.abb.2004.09.019.
[51] 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(31):28857-65. https://doi.org/10.1074/jbc.M011610200.
[52] Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, et al.: Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor. J Biol Chem 2002, 277(4):2908-15. https://doi.org/10.1074/jbc.M109326200.
[53] Masaaki M, Yumi S, Hitomi M, Kouichi Y, Yasushi Y: Fibroblast growth factor 19 treatment ameliorates disruption of hepatic lipid metabolism in farnesoid x receptor (Fxr)-Null mice. Biological and Pharmaceutical Bulletin 2011, 34(12).
[54] Zhou X, Cao L, Jiang C, Xie Y, Cheng X, Krausz KW, et al.: PPARalpha-UGT axis activation represses intestinal FXR-FGF15 feedback signalling and exacerbates experimental colitis. Nat Commun 2014, 5:4573. https://doi.org/10.1038/ncomms5573.
[55] Xu C, Huang M, Bi H: PXR- and CAR-mediated herbal effect on human diseases. Biochim Biophys Acta 2016, 1859(9):1121-9. https://doi.org/10.1016/j.bbagrm.2016.02.009.
[56] Suh JI: [Role of PXR and CAR in cholestasis]. Korean J Hepatol 2006, 12(1):5-15.
[57] Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, Mackenzie KI, Latour A, et al.: The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. Proc Natl Acad Sci U S A 2001, 98(6):3369-74. https://doi.org/10.1073/pnas.051551698.
[58] Kakizaki S, Takizawa D, Tojima H, Horiguchi N, Yamazaki Y, Mori M: Nuclear receptors CAR and PXR; Therapeutic targets for cholestatic liver disease. Front Biosci (Landmark Ed) 2011, 16:2988-3005. https://doi.org/10.2741/3893.
[59] Zollner G, Wagner M, Fickert P, Silbert D, Gumhold J, Zatloukal K, et al.: Expression of bile acid synthesis and detoxification enzymes and the alternative bile acid efflux pump MRP4 in patients with primary biliary cirrhosis. Liver Int 2007, 27(7):920-9. https://doi.org/10.1111/j.1478-3231.2007.01506.x.
[60] Castano G, Burgueno A, Fernandez GT, Pirola CJ, Sookoian S: The influence of common gene variants of the xenobiotic receptor (PXR) in genetic susceptibility to intrahepatic cholestasis of pregnancy. Aliment Pharmacol Ther 2010, 31(5):583-92. https://doi.org/10.1111/j.1365-2036.2009.04210.x.
[61] Chai J, Luo D, Wu X, Wang H, He Y, Li Q, et al.: Changes of organic anion transporter MRP4 and related nuclear receptors in human obstructive cholestasis. J Gastrointest Surg 2011, 15(6):996-1004. https://doi.org/10.1007/s11605-011-1473-2.
[62] Karpen SJ: Exercising the nuclear option to treat cholestasis: CAR and PXR ligands. Hepatology 2005, 42(2):266-9. https://doi.org/10.1002/hep.20833.
[63] Jung D, Mangelsdorf DJ, Meyer UA: Pregnane X receptor is a target of farnesoid X receptor. J Biol Chem 2006, 281(28):19081-91. https://doi.org/10.1074/jbc.M600116200.

DOI: 10.55415/deep-2022-0052.v2

[64] Wolski H, Kurzawinska G, Ozarowski M, Drews K, Barlik M, Piatek K, et al.: FokI vitamin D receptor polymorphism as a protective factor in intrahepatic cholestasis of pregnancy. Ginekol Pol 20209, 1(12):719-25. https://doi.org/10.5603/GP.a2020.0135.
[65] Kempinska-Podhorecka A, Wunsch E, Jarowicz T, Raszeja-Wyszomirska J, Loniewska B, Kaczmarczyk M, et al.: Vitamin d receptor polymorphisms predispose to primary biliary cirrhosis and severity of the disease in polish population. Gastroenterol Res Pract 2012, 2012:408723. https://doi.org/10.1155/2012/408723.
[66] Firrincieli D, Zuniga S, Rey C, Wendum D, Lasnier E, Rainteau D, et al.: Vitamin D nuclear receptor deficiency promotes cholestatic liver injury by disruption of biliary epithelial cell junctions in mice. Hepatology 2013, 58(4):1401-12. https://doi.org/10.1002/hep.26453.
[67] Chen X, Chen F, Liu S, Glaeser H, Dawson PA, Hofmann AF, et al.: Transactivation of rat apical sodium-dependent bile acid transporter and increased bile acid transport by 1alpha,25-dihydroxyvitamin D3 via the vitamin D receptor. Mol Pharmacol 2006, 69(6):1913-23. https://doi.org/10.1124/mol.105.020792.
[68] Hochrath K, Stokes CS, Geisel J, Pollheimer MJ, Fickert P, Dooley S, et al.: Vitamin D modulates biliary fibrosis in ABCB4-deficient mice. Hepatol Int 2014, 8(3):443-52. https://doi.org/10.1007/s12072-014-9548-2.
[69] Fayard E, Auwerx J, Schoonjans K: LRH-1: An orphan nuclear receptor involved in development, metabolism and steroidogenesis. Trends Cell Biol 2004, 14(5):250-60. https://doi.org/10.1016/j.tcb.2004.03.008.
[70] Out C, Hageman J, Bloks VW, Gerrits H, Sollewijn GM, Bos T, et al.: Liver receptor homolog-1 is critical for adequate up-regulation of Cyp7a1 gene transcription and bile salt synthesis during bile salt sequestration. Hepatology 2011, 53(6):2075-85. https://doi.org/10.1002/hep.24286.
[71] Lee YK, Moore DD: Liver receptor homolog-1, an emerging metabolic modulator. Front Biosci 2008, 13:5950-8. https://doi.org/10.2741/3128.
[72] Song X, Kaimal R, Yan B, Deng R: Liver receptor homolog 1 transcriptionally regulates human bile salt export pump expression. J Lipid Res 2008, 49(5):973-84. https://doi.org/10.1194/jlr.M700417-JLR200.
[73] Bohan A, Chen WS, Denson LA, Held MA, Boyer JL: Tumor necrosis factor alpha-dependent up-regulation of Lrh-1 and Mrp3(Abcc3) reduces liver injury in obstructive cholestasis. J Biol Chem 2003, 278(38):36688-98. https://doi.org/10.1074/jbc.M304011200.
[74] Chen F, Ma L, Dawson PA, Sinal CJ, Sehayek E, Gonzalez FJ, et al.: Liver receptor homologue-1 mediates species- and cell line-specific bile acid-dependent negative feedback regulation of the apical sodium-dependent bile acid transporter. J Biol Chem 2003, 278(22):19909-16. https://doi.org/10.1074/jbc.M207903200.
[75] Post SM, Duez H, Gervois PP, Staels B, Kuipers F, Princen HM: Fibrates suppress bile acid synthesis via peroxisome proliferator-activated receptor-alpha-mediated downregulation of cholesterol 7alpha-hydroxylase and sterol 27-hydroxylase expression. Arterioscler Thromb Vasc Biol 2001, 21(11):1840-5. https://doi.org/10.1161/hq1101.098228.
[76] Ghonem NS, Ananthanarayanan M, Soroka CJ, Boyer JL: Peroxisome proliferator-activated receptor alpha activates human multidrug resistance transporter 3/ATP-binding cassette protein subfamily B4 transcription and increases rat biliary phosphatidylcholine secretion. Hepatology 2014, 59(3):1030-42. https://doi.org/10.1002/hep.26894.
[77] Kok T, Bloks VW, Wolters H, Havinga R, Jansen PL, Staels B, et al.: Peroxisome proliferator-activated receptor alpha (PPARalpha)-mediated regulation of multidrug resistance 2 (Mdr2) expression and function in mice. Biochem J 2003, 369(Pt 3):539-47. https://doi.org/10.1042/BJ20020981.
[78] Jung D, Fried M, Kullak-Ublick GA: Human apical sodium-dependent bile salt transporter gene (SLC10A2) is regulated by the peroxisome proliferator-activated receptor alpha. J Biol Chem 2002, 277(34):30559-66. https://doi.org/10.1074/jbc.M203511200.
[79] Honda A, Ikegami T, Nakamuta M, Miyazaki T, Iwamoto J, Hirayama T, et al.: Anticholestatic effects of bezafibrate in patients with primary biliary cirrhosis treated with ursodeoxycholic acid. Hepatology 2013, 57(5):1931-41. https://doi.org/10.1002/hep.26018.
[80] Andermatten RB, Ciriaci N, Schuck VS, Di Siervi N, Razori MV, Miszczuk GS, et al.: Sphingosine 1-phosphate receptor 2/adenylyl cyclase/protein kinase a pathway is involved in taurolithocholate-induced internalization of Abcc2 in rats. Arch Toxicol 2019, 93(8):2279-94. https://doi.org/10.1007/s00204-019-02514-6.
[81] Boaglio AC, Zucchetti AE, Sanchez PE, Pellegrino JM, Ochoa JE, Mottino AD, et al.: Phosphoinositide 3-kinase/protein kinase B signaling pathway is involved in estradiol

DOI: 10.55415/deep-2022-0052.v2

17beta-D-glucuronide-induced cholestasis: Complementarity with classical protein kinase C. Hepatology 2010, 52(4):1465-76. https://doi.org/10.1002/hep.23846.

[82] Manning BD, Toker A: AKT/PKB signaling: Navigating the network. Cell 2017, 169(3):381-405. https://doi.org/10.1016/j.cell.2017.04.001.

[83] Webster CR, Anwer MS; Role of the PI3K/PKB signaling pathway in cAMP-mediated translocation of rat liver Ntcp. Am J Physiol 1999, 277(6):G1165-72. https://doi.org/10.1152/ajpgi.1999.277.6.G1165.

[84] Wu T, Zhang Q, Li J, Chen H, Wu J, Song H: Up-regulation of BSEP and MRP2 by Calculus Bovis administration in 17alpha-ethynylestradiol-induced cholestasis: Involvement of PI3K/Akt signaling pathway. J Ethnopharmacol 2016, 190:22-32. https://doi.org/10.1016/j.jep.2016.05.056.

[85] Beuers U, Denk GU, Soroka CJ, Wimmer R, Rust C, Paumgartner G, et al.: Taurolithocholic acid exerts cholestatic effects via phosphatidylinositol 3-kinase-dependent mechanisms in perfused rat livers and rat hepatocyte couplets. J Biol Chem 2003, 278(20):17810-8. https://doi.org/10.1074/jbc.M209898200.

[86] Boaglio AC, Zucchetti AE, Sanchez PE, Pellegrino JM, Ochoa JE, Mottino AD, et al.: Phosphoinositide 3-kinase/protein kinase B signaling pathway is involved in estradiol 17beta-D-glucuronide-induced cholestasis: Complementarity with classical protein kinase C. Hepatology 2010, 52(4):1465-76. https://doi.org/10.1002/hep.23846.

[87] Wang T, Liu C, Jia L: The roles of PKCs in regulating autophagy. J Cancer Res Clin Oncol 2018, 144(12):2303-11. https://doi.org/10.1007/s00432-018-2731-4.

[88] Mcconkey M, Gillin H, Webster CR, Anwer MS: Cross-talk between protein kinases Czeta and B in cyclic AMP-mediated sodium taurocholate co-transporting polypeptide translocation in hepatocytes. J Biol Chem 2004, 279(20):20882-8. https://doi.org/10.1074/jbc.M309988200.

[89] Park SW, Schonhoff CM, Webster CR, Anwer MS: Protein kinase Cdelta differentially regulates cAMP-dependent translocation of NTCP and MRP2 to the plasma membrane. Am J Physiol Gastrointest Liver Physiol 2012, 303(5):G657-65. https://doi.org/10.1152/ajpgi.00529.2011.

[90] Schonhoff CM, Gillin H, Webster CR, Anwer MS: Protein kinase Cdelta mediates cyclic adenosine monophosphate-stimulated translocation of sodium taurocholate cotransporting polypeptide and multidrug resistant associated protein 2 in rat hepatocytes. Hepatology 2008, 47(4):1309-16. https://doi.org/10.1002/hep.22162.

[91] Schonhoff CM, Yamazaki A, Hohenester S, Webster CR, Bouscarel B, Anwer MS: PKC{epsilon}-dependent and -independent effects of taurolithocholate on PI3K/PKB pathway and taurocholate uptake in HuH-NTCP cell line. Am J Physiol Gastrointest Liver Physiol 2009, 297(6):G1259-67. https://doi.org/10.1152/ajpgi.00177.2009.

[92] Muhlfeld S, Domanova O, Berlage T, Stross C, Helmer A, Keitel V, et al.: Short-term feedback regulation of bile salt uptake by bile salts in rodent liver. Hepatology 2012, 56(6):2387-97. https://doi.org/10.1002/hep.25955.

[93] Sekine S, Ito K, Horie T: Oxidative stress and Mrp2 internalization. Free Radic Biol Med 2006, 40(12):2166-74. https://doi.org/10.1016/j.freeradbiomed.2006.02.015.

[94] Anwer MS: Role of protein kinase C isoforms in bile formation and cholestasis. Hepatology 2014, 60(3):1090-7. https://doi.org/10.1002/hep.27088.

[95] Grune S, Engelking LR, Anwer MS: Role of intracellular calcium and protein kinases in the activation of hepatic Na+/taurocholate cotransport by cyclic AMP. J Biol Chem 1993, 268(24):17734-41.

[96] Chandra P, Zhang P, Brouwer KL: Short-term regulation of multidrug resistance-associated protein 3 in rat and human hepatocytes. Am J Physiol Gastrointest Liver Physiol 2005, 288(6):G1252-8. https://doi.org/10.1152/ajpgi.00362.2004.

[97] Guo GL, Klaassen CD: Protein kinase C suppresses rat organic anion transporting polypeptide 1- and 2-mediated uptake. J Pharmacol Exp Ther 2001, 299(2):551-7.

[98] Barosso IR, Zucchetti AE, Boaglio AC, Larocca MC, Taborda DR, Luquita MG, et al.: Sequential activation of classic PKC and estrogen receptor alpha is involved in estradiol 17ss-D-glucuronide-induced cholestasis. Plos One 2012, 7(11):e50711. https://doi.org/10.1371/journal.pone.0050711.

[99] Muhlfeld S, Domanova O, Berlage T, Stross C, Helmer A, Keitel V, et al.: Short-term feedback regulation of bile salt uptake by bile salts in rodent liver. Hepatology 2012, 56(6):2387-97. https://doi.org/10.1002/hep.25955.

[100] Kubitz R, Saha N, Kuhlkamp T, Dutta S, Vom DS, Wettstein M, et al.: Ca2+-dependent protein kinase C isoforms induce cholestasis in rat liver. J Biol Chem 2004, 279(11):10323-30.

DOI: 10.55415/deep-2022-0052.v2

https://doi.org/10.1074/jbc.M306242200.
[101] Wimmer R, Hohenester S, Pusl T, Denk GU, Rust C, Beuers U: Tauroursodeoxycholic acid exerts anticholestatic effects by a cooperative cPKC alpha-/PKA-dependent mechanism in rat liver. Gut 2008, 57(10):1448-54. https://doi.org/10.1136/gut.2007.140871.
[102] Park SW, Schonhoff CM, Webster CR, Anwer MS: Protein kinase Cdelta differentially regulates cAMP-dependent translocation of NTCP and MRP2 to the plasma membrane. Am J Physiol Gastrointest Liver Physiol 2012, 303(5):G657-65. https://doi.org/10.1152/ajpgi.00529.2011.
[103] Gliki G, Wheeler-Jones C, Zachary I: Vascular endothelial growth factor induces protein kinase C (PKC)-dependent Akt/PKB activation and phosphatidylinositol 3'-kinase-mediates PKC delta phosphorylation: Role of PKC in angiogenesis. Cell Biol Int 2002, 26(9):751-9. https://doi.org/10.1016/s1065-6995(02)90926-1.
[104] Reyland ME: Protein kinase C isoforms: Multi-functional regulators of cell life and death. Front Biosci (Landmark Ed) 2009, 14:2386-99. https://doi.org/10.2741/3385.
[105] Webster CR, Johnston AN, Anwer MS: Protein kinase Cdelta protects against bile acid apoptosis by suppressing proapoptotic JNK and BIM pathways in human and rat hepatocytes. Am J Physiol Gastrointest Liver Physiol 2014, 307(12):G1207-15. https://doi.org/10.1152/ajpgi.00165.2014.
[106] Schonhoff CM, Webster CR, Anwer MS: Taurolithocholate-induced MRP2 retrieval involves MARCKS phosphorylation by protein kinase C in HUH-NTCP Cells. Hepatology 2013, 58(1):284-92. https://doi.org/10.1002/hep.26333.
[107] Beuers U, Bilzer M, Chittattu A, Kullak-Ublick GA, Keppler D, Paumgartner G, et al.: Tauroursodeoxycholic acid inserts the apical conjugate export pump, Mrp2, into canalicular membranes and stimulates organic anion secretion by protein kinase C-dependent mechanisms in cholestatic rat liver. Hepatology 2001, 33(5):1206-16. https://doi.org/10.1053/jhep.2001.24034.
[108] Scholmerich J, Baumgartner U, Miyai K, Gerok W: Tauroursodeoxycholate prevents taurolithocholate-induced cholestasis and toxicity in rat liver. J Hepatol 1990, 10(3):280-3. https://doi.org/10.1016/0168-8278(90)90133-c.
[109] Sekine S, Ito K, Horie T: Oxidative stress and Mrp2 internalization. Free Radic Biol Med 2006, 40(12):2166-74. https://doi.org/10.1016/j.freeradbiomed.2006.02.015.
[110] Murray JW, Thosani AJ, Wang P, Wolkoff AW: Heterogeneous accumulation of fluorescent bile acids in primary rat hepatocytes does not correlate with their homogenous expression of ntcp. Am J Physiol Gastrointest Liver Physiol 2011, 301(1):G60-8. https://doi.org/10.1152/ajpgi.00035.2011.
[111] Stross C, Keitel V, Winands E, Haussinger D, Kubitz R: Expression and localization of atypical PKC isoforms in liver parenchymal cells. Biol Chem 2009, 390(3):235-44. https://doi.org/10.1515/BC.2009.031.

DOI: 10.55415/deep-2022-0052.v2

Table 1 Main Nuclear Receptors Involved in Transporter Regulation

NR	Name	Ligands
FXR (NR1H4)	Farnesoid X-activated receptor	Bile acids (CDCA, DCA, LCA, CA); possibly UDCA (weak ligand) synthetic: GW4064, 6α-ethyl-CDCA, fexaramines
PXR (NR1I2)	Pregnane X receptor	Bile acids, rifampicin in humans, phenobarbital, dexamethasone, statins, St. John's wort, clotrimazole pregnenolone-16a-carbonitrile (PCN)
CAR (NR1I3)	Constitutive androstane receptor	Bilirubin, phenobarbital, TCPOBOP, dimethoxycoumarin, xenobiotics, Yin Chin, CITCO in humans
VDR (NR1I1)	Vitamin D receptor	Vitamin D, LCA
LRH-1 (NR5A2)	Liver receptor homolog-1	Phospholipids
PPARα (NR1C1)	Peroxisome proliferator-activated receptor α	Fatty acids, fibrates, statins, eicasaonoids, leukotriens, NSAIDs, WY-14643

Figure Legends

Figure 1 The role of transporters in enterohepatic circulation of BAs. BAs are synthesized from cholesterol. Subsequently, BAs are secreted into bile in canalicular by membrane transporters. Most of BAs are reabsorbed into the portal vein by transporters on cholangiocytes and enterocytes. Then in the sinusoids of the liver, BAs are taken up by NTCP and OATPs to be recycled back to the liver.

Figure 2 The roles of transporters in IC. IC results in intrahepatic accumulation of BAs, leading to toxic hepatocellular bile acid burden. In addition, the uptake of BAs is restricted due to downregulation of NTCP and OATPs. Export of BAs is mediate by basolateral transporters, such as BSEP, MRP2, MRP3, MRP4 and OSTα/β. Decreased expression of these transporters results in decreased bile acid excretion, further increasing BAs accumulation in the liver to trigger IC.

Figure 3 Proposed model for the regulation of NTCP and MRP2 by PKC isoforms. Activation of

DOI: 10.55415/deep-2022-0052.v2

nPKCδ and aPKCζ by cAMP leads to translocations of NTCP and MRP2 to the PM. Activation of nPKCδ by GCDCA facilitates MRP2 translocation to the PM. Activation of cPKCa by PMA and TCDCA induces retrieval of NTCP from the PM. Activation of cPKCa and nPKCε have been implicated in MRP2 retrieval from the PM by $E_2$17G-, EA- and TLCA-induced cholestasis, respectively.