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Review Article

Molecular mechanisms of transporter regulation and their impairment in intrahepatic cholestasis

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ABSTRACT

Intrahepatic cholestasis (IC) is a liver disease caused by disorders in bile formation and excretion, owing to structural and functional abnormalities in hepatocytes and/or bile capillaries. IC is commonly caused by hepatitis virus, alcohol consumption, drug-induced liver damage, autoimmune liver disease and heredity. In the absence of effective treatment, IC can progress to liver fibrosis, cirrhosis and ultimately liver failure. However, the mechanisms underlying IC remain poorly understood. IC is believed to be closely associated with changes in the transcription, function and localization of hepatocellular transport proteins. To better understand the molecular mechanisms of transport proteins in IC, herein, we review the roles of these transport proteins and discuss their underlying regulatory mechanisms in IC. Our aim is to provide a reference for understanding IC pathogenesis and developing effective drug therapies.

Keywords: intrahepatic cholestasis, transport proteins, localization, regulation

1. 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, mainly including primary biliary cirrhosis (PBC), primary sclerosing cholangitis and intrahepatic cholestasis of pregnancy (ICP), can be caused by inflammatory disorders, drugs, heredity and the environment [2, 3]. Any functional perturbation in the bile secretory process may lead to IC, which is associated with intracellular accumulation of toxic bile constituents and consecutive cholestatic liver cell damage [4]. The main pathogenic mechanisms of IC may include deregulation of bile secretion, impaired cell-membrane fluidity, inflammatory responses, and changes in hepatocyte tight junctions and transporters [5].

Many transporters expressed in hepatocytes and cholangiocytes are involved in bile formation and excretion. The secretion of bile is a hepatocellular transport processes occurring mainly across the canalicular membranes of hepatocytes. Perturbations in the function, expression and/or localization of transporters lead to the intracellular accumulation of toxic bile acids (BAs), thus promoting cholestatic liver injury [6]. Alterations in hepatobiliary transporter function are important risk factors for susceptibility to IC development [7]. Mutations in transporter genes can cause hereditary cholestatic liver disease [8]. Mutations in multidrug resistance 3 (MDR3) and bile salt export pump (BSEP) can cause an array of cholestatic syndromes, including progressive and benign forms of familial IC and ICP [9, 10], have been well established to cause inherited cholestatic syndromes [11, 12]. Furthermore, genetically determined functional changes in hepatobiliary transport systems have been demonstrated to cause acquired cholestatic syndromes, such as ICP and drug-induced cholestasis [7]. The transcription and expression of transporters are regulated by complex networks. Transporters are regulated by multiple nuclear receptors (NRs) at the transcriptional level [13]. After their transcription and translation, transporters can also be regulated by various protein kinases (PKs) such as protein kinase B (Akt) and protein kinase C family members (PKCs) [14-16]. The first-line treatment for IC is ursodeoxycholic (UDCA). However, approximately 40% of patients have inadequate responses [17]. Therefore, herein, we review the molecular mechanisms of transporter dysregulation under IC, to provide a reference

for understanding its pathogenesis and developing effective drug therapies.

2. 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, the bile composition undergoes further modification in the canaliculus, through reabsorption and secretion processes maintained by apical and basolateral transport systems in cholangiocytes. **Figure 1** shows a scheme of the hepatocellular and bile duct transport proteins involved in the uptake and efflux of bile compounds (e.g., BAs).

Bile, comprising mainly BAs, cholesterol, bilirubin, bile pigment, phosphatidylcholine, water and inorganic salts, plays crucial roles in the digestion and absorption of lipids and lipid-soluble drugs. Bile production begins at the canaliculus in hepatocytes, and bile is modified downstream by cholangiocytes [18, 19]. Bile formation is a fundamental physiological process comprising the active transport of BAs and other solutes across the canalicular membrane [20]. BAs, 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. Most primary BAs, such as cholic acid and deoxycholic acid, are metabolized immediately into taurine- and glycine-binding BAs. They are transported into the canaliculus by hepatic transporters, then mixed with other components to form bile [22].

BAs in bile enter the intestine through the contraction of the gallbladder. Under the action of the intestinal microbiota, cholic acid and deoxycholic acid are transformed into UDCA and lithocholic acid (LCA), respectively, and all taurine- and glycine-binding BAs are deamidated in the terminal ileum and colon. In the entire process of enterohepatic circulation, 90% of BAs are reabsorbed by intestinal epithelium, and the rest is discharged from the human body through the feces [23]. In the small intestine, some free BAs and glycine-binding BAs can be reabsorbed passively, but most are actively absorbed in the terminal ileum by the apical sodium-dependent BA transporter (ASBT, SLC10A2) and

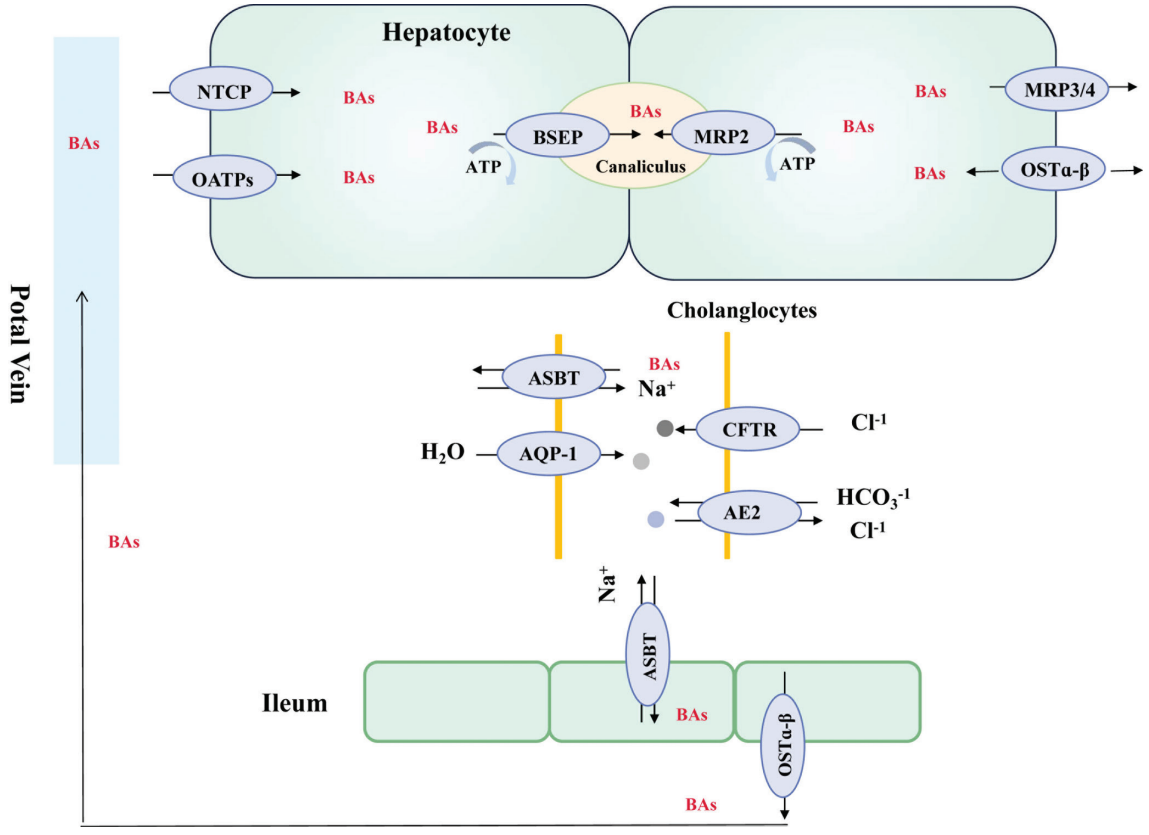

Figure 1 | The roles of transporters in enterohepatic circulation of BAs.
BAs are synthesized from cholesterol. Subsequently, BAs are secreted into bile in the canaliculus by membrane transporters. Most BAs are reabsorbed into the portal vein by transporters on cholangiocytes and enterocytes. In the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

organic solute transporter α/β (OSTα/β, SLC51a/b) on the basement membrane [24]. Most BAs in the hepatic portal vein, together with bilirubin and a variety of other organic anions, are reabsorbed from the blood into the liver through uptake transporters, and the rest are eliminated from the kidneys through the blood circulation. BAs can generally be eliminated after 20 enterohepatic circulations. Therefore, BA uptake disorders, obstructed BA efflux and bile duct injury in the liver can all lead to cholestasis [25].

2.1 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 membranes of hepatocyte, mainly including ATP-binding cassette (ABC) and solute carrier family (SLC) transporters. ABC transporters efflux substrates, whereas SLC transporters mediate the uptake of substrates into cells. These transporters play important roles in bile formation and the biliary excretion of xenobiotics. In bile flow obstruction, cholestasis has been attributed to the expression and functional abnormalities of various transporters [26]. The transporters in cholangiocytes take up electrolytes and water into the blood, thus forming a "biliary-liver" cycle, and excrete them into the bile duct, thereby further promoting bile flow (**Figure 1**). Therefore, transporters play important roles in the secretion and excretion of bile in cholestasis.

2.1.1 SLC transporters. SLC transporters include Na^+-taurocholate co-transport polypeptide (NTCP, SLC10A1), organic anion transporter polypeptides (OATPs, SLCO), OSTα/β and ASBT. On the basolateral membranes of hepatocytes, BAs are taken up primarily by NTCP and OATPs. Furthermore, NTCP also transports steroidal hormones and a variety of drugs. Repression and translocation of NTCP contribute to the etiopathogenesis of IC [27]. OATPs take up other cholephilic compounds, including glucuronidated bilirubin, exogenous organic anions, leukotrienes, estrogen conjugates (e.g., estrone-3-sulfate and 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, whereas small organic cations are taken up by organic cation transporter 1 (OCT1, SLC22A1) [31].

2.1.2 ABC transporters. In hepatocytes, bile excretion occurs mainly at the canalicular membrane, predominantly via ABC transporters. ABC transporters are a superfamily of membrane proteins that mediate diverse ATP-driven transport processes; main members include BSEP (ABCB11), multidrug resistance protein 2 (MRP2, ABCC2), P-glycoprotein (P-gp/MDR1, ABCB1), breast cancer drug resistance protein (BCRP, ABCG2) and MDR3 (ABCB4) [32, 33]. BSEP and MRP2, two main transporters on the canalicular membrane, excrete BAs into the bile duct. Monoanionic bile salts are excreted mainly into the canalicular pole by BSEP [34]. In contrast, canalicular efflux of divalent, sulfated or glucuronidated bile salts, glutathione or glucuronidated bilirubin is mediated by MRP2 [33]. In addition, phosphatidylcholine, cholesterol and other compounds are excreted into the canaliculus through ATP-binding cassette subfamily B member 4 (MDR2, ABCB4), ATP-binding cassette subfamily G member 5/8 (ABCG5/G8), ATP-binding cassette subfamily B member 1 (MDR1, ABCB1) and MRP2 [35].

Cooperation among transporters is critical to maintaining bile homeostasis (**Figure 2**). In early stages of acute and chronic cholestasis, the NTCP-BSEP axis is blocked, thus leading to accumulation of BAs in hepatocytes and spontaneously activating the OATP-MRP2 axis, thereby accelerating the excretion of BAs. It maintains bile homeostasis, delays rapid increases in intracellular BA concentrations, and alleviates hepatocyte structural and functional damage [21].

2.2 Transporters in intrahepatic cholangiocytes

During bile flow, formation and excretion, the intrahepatic bile duct secretes the electrolytes Cl^{-1} and HCO_3^{-1} into the bile via several transporters or channels expressed on cholangiocyte membranes, thus synergistically regulating the fluidity and pH of bile in the bile duct [36]. These transporters 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, and alterations in bile composition and flow [37]. Impairment of cholangiocyte transporters and aquaporin leads to "toxic" bile, owing to both a lack of the "HCO_3^{-1}" and increased intraluminal levels of damaging Bas [38]. CTFR transports intracellular Cl^{-1} outside the plasma membrane [39]. Subsequently, Cl^{-1} on the plasma membrane secondarily drives the Cl^{-1}-HCO_3^{-1} transporter AE-2, which actively secretes HCO_3^{-1} into the bile [40], whereas AQP1 transports water molecules into the bile [41]. Genetic abnormalities in CFTR result in attenuation of bile hydration, accumulation of toxic BAs, cholangiocyte damage and cholestasis, and ultimately progression to cystic fibrosis [42]. Abnormal function and expression of AE-2 are also associated with PBC [43].

3. TRANSCRIPTIONAL REGULATION OF HEPATIC TRANSPORTERS BY NRS

NRs, a family of 48 members, play important roles in BA homeostasis, lipid metabolism, and mechanisms involved in fibrosis and inflammation. Several of the adaptive changes in cholestasis are mediated by NRs, because biliary compounds retained during cholestasis (e.g., BAs, bilirubin, oxysterols, hormones and drugs) act as NR ligands and coordinately affect target-gene expression [44, 45]. NRs, mainly farnesoid X receptor (FXR, NR1H4), pregnane X receptor (PXR, NR1I2), constitutive androgen

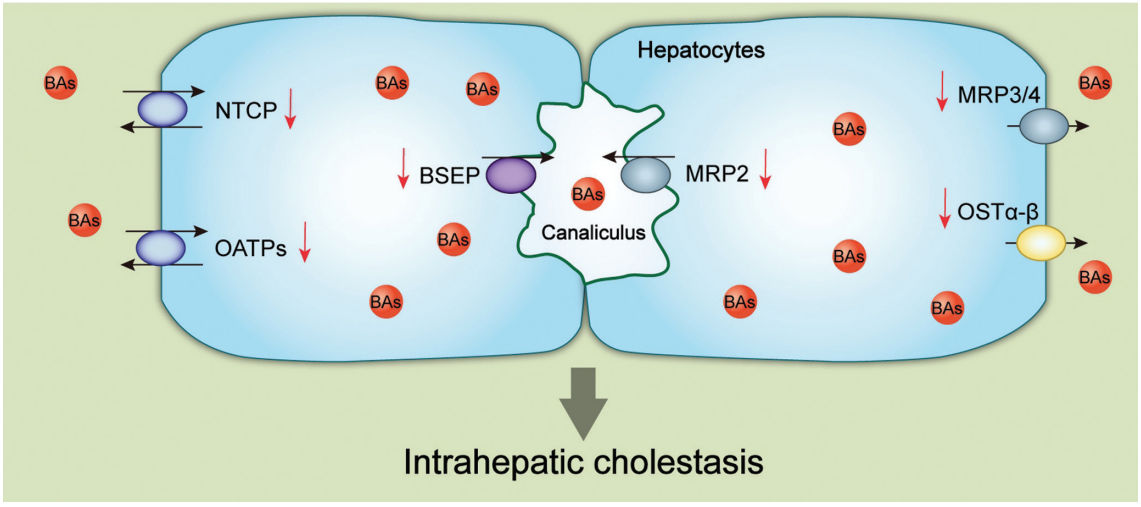

Figure 2 | The roles of transporters in IC.
IC results in intrahepatic accumulation of BAs, thus leading to a toxic hepatocellular bile acid burden. In addition, the uptake of BAs is restricted, owing to downregulation of NTCP and OATPs. Export of BAs is mediated by basolateral transporters, such as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

receptor (CAR, NR1I3), liver X receptor α (LXRa, NR1H3) and vitamin D receptor (VDR, NR1I1), are involved in the maintenance of BA homeostasis in IC [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 [46]. Changes in transporter regulation comprise a complex interaction network of several ligand-activated NRs as well as liver-enriched hepatocyte nuclear factors.

3.1 FXR

FXR is a major NR regulating the expression of transporters and maintaining BA homeostasis during pathogenesis of cholestasis [47]. In early stages of chronic cholestasis, FXR is rapidly activated, thus forming FXR-RXR (retinoid X receptor, NR2B1) heterodimers. Subsequently, the dimers bind inverted repeat 1 (IR-1) elements in the target-gene promoter and significantly up-regulate the expression of BSEP and MRP2, thereby accelerating BA excretion. FXR also induces the expression of a nuclear orphan receptor small heterodimer partner (SHP), and consequently inhibits the functions of other NRs, such as liver X receptor (NR1H3) and hepatocyte nuclear factor 4α (HNF4α), and ultimately suppresses the expression of CYP7A1/CYP8B1 and NTCP in hepatocytes, thereby decreasing BA synthesis and uptake, and indirectly accelerating BA clearance [48]. Similarly to NTCP, OATP1B1 and ASBT are negatively regulated by FXR through the interaction of

Table 1 | Main nuclear receptors involved in transporter regulation.

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

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SHP with HNF4 [49]. In addition, FXR directly promotes cellular bile clearance via directly inducing canalicular BSEP and MRP2 [50, 51].

In another regulatory pathway inhibiting BA synthesis, intestinal FXR induces the expression of an intestinal hormone-like peptide, fibroblast growth factor 15/19 (murine FGF15 or 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 pathway and consequently inhibits CYP7A1 activity and decreases BA synthesis [52]. Thus, the FXR-FGF19 pathway, through a typical negative feedback regulation mechanism, plays a critical role in the pathogenesis of cholestatic diseases [53]. FXR may therefore be a promising therapeutic target for novel drug development in IC.

3.2 PXR and CAR

Recent studies have revealed that, beyond FXR, PXR and CAR are key NRs regulating many adaptive responses in IC. They coordinate protective hepatic responses to toxic stimuli, induced by endogenous compounds (BAs or bilirubin) and xenobiotics [54, 55]. As sensors of toxic byproducts, they are central in the detoxification pathways involving phase I/II detoxification and transporters [54, 56, 57]. Levels of PXR and CAR are diminished in IC [58], whereas PXR polymorphisms are associated with greater susceptibility to ICP [59]. MRP3/4 expression is upregulated via PXR and CAR, thereby alleviating cholestatic liver injury [60, 61]. In addition, FXR induces BA export and metabolism via transcriptional activation of PXR [62]. Targeting these NRs may provide therapeutic benefits for patients with cholestasis in the future.

3.3 VDR

VDR, expressed in the intestines, kidney and liver, is also activated by BAs. Recent reports have shown that VDR regulates BA transporters, and its polymorphic variants may affect individual susceptibility and quality of life in patients with IC, such as PBC or ICP [63, 64]. However, the direct effects of VDR polymorphisms on the pathogenesis of IC are unclear. Loss of VDR exacerbates cholestatic liver injury through the disruption of biliary epithelial cell junctions in mice [65]. VDR increases ASBT mRNA expression and promoter activity [66]. Moreover, VDR appears to play an indirect role in BA homeostasis. Furthermore, vitamin D intake has been suggested to relieve biliary fibrosis in ABCB-knockout mice and ameliorate cholestatic disease [67]. Therefore, VDR may serve as a therapeutic target in cholestatic diseases.

3.4 LRH-1

LRH-1, a transcription factor in bile salt synthesis, is expressed mainly in the liver, intestines, exocrine pancreas and reproductive tissue [68]. It binds DNA in its monomeric form and regulates other NRs and the transcription of genes involved in the biosynthesis and transport of BAs, including CYP7A1 [69], BSEP, MRP2, ASBT, NTCP, MRP3 and MDR2 [70-73]. LRH-1 induces the expression of CYP7A1, BSEP [71] and ASBT [73]. In addition, deletion of LRH-1 significantly decreases the expression of FXP and SHP as well as multiple transporters (i.e., NTCP, BSEP, MRP3, MRP2 and MDR2) [70]. However, the effect of LRH-1 remains to be fully elucidated in IC.

3.5 PPARα

PPARα, a ligand-activated nuclear receptor, plays a central role in maintaining cholesterol, lipid and BA homeostasis by regulating genes involved in BA synthesis and transport. PPARα primarily down-regulates BA synthesis through inhibition of BA-synthesizing enzymes (i.e., CYP7A1 and CYP27A1) [74]. In addition, PPARα induces biliary phospholipid output by activating canalicular MDR3 [75]. PPARα activators directly induce canalicular MDR2, thereby inducing biliary phospholipid output [76]. In addition, ASBT expression in cholangiocytes and the intestines is induced by PPARα [77], thus increasing BA absorption from the intestines and bile ducts. Bezafibrate, a dual PPAR and PXR agonist, increases the expression of NTCP, MDR1, MDR3 and MRP2, thus protecting against cholestatic liver injury [78]. Agonists of PPARα are promising therapeutic approaches in IC.

4. LOCALIZATION REGULATION OF HEPATIC TRANSPORTERS

Accurate expression and localization of transporters on the plasma membrane require interactions among various proteins between the membrane and cytoskeleton—a complex process regulated by PKs. 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, regulate the localization of hepatic bile transporters after transcription, and the activation of phosphoinositide-3-kinase (PI3K)/Akt signaling causes sustained internalization of MRP2 and BSEP, thus eventually leading to cholestasis [79, 80]. Via second messengers, PKs initiate signaling cascades, regulate the phosphorylation and dephosphorylation of the hepatobiliary transport system and corresponding crosslinked proteins or scaffolding proteins, alter the membrane localization of transporters and rapidly adjust bile composition, thus subsequently promoting cholestasis and/or exerting choleretic effects.

4.1 PKB (Akt)

The Ser/Thr kinase PKB (Akt) has been widely studied as a cell growth factor regulating the functions of multiple downstream anti-apoptotic proteins [81]. Akt is considered the characteristic target protein and terminal effector of PI3K [82, 83]. Beuers et al. [84] have found that in a taurolithocholic acid (TLCA)-induced cholestasis model, wortmannin, a PI3K-specific inhibitor, decreases Akt activity and attenuates cholestasis, thus suggesting a causal link between these events. Furthermore, in an $E_2$17G- and TLCA-induced cholestasis

model, the anti-cholestatic effect of an Akt inhibitor (Calbiochem 124005) is similar to that of a PI3K inhibitor (LY294002), and identical anti-cholestatic outcomes have been achieved by combining wortmannin with Calbiochem 124005 or a conventional PKC (cPKC) inhibitor (G6976) [80]. In addition, the activation of PI3K and Akt contributes to sustaining the internalization of transporters and the consequent impairment of their activity. Thus, the PI3K/Akt pathway is largely responsible for cholestasis.

4.2 PKC

PKCs are a group of PKs mediating the function of targeted proteins through phosphorylation of serine and threonine amino acid residues. Ten subtypes of PKC have been found in mammalian tissues, and can be divided into three groups: cPKC, including α, βI, βII and γ subtypes; novel PKC (nPKC), including δ, ε, η and θ subtypes; and atypical PKC (aPKC), including ι (also known as λ in mice) and ζ subtypes [85]. The activation of several PKC subtypes in the liver, such as aPKCζ and nPKCδ, depends on PI3K [86-88]. However, the activation of cPKCs does not rely on PI3K [80, 89, 90], and oxidative stress can mediate the activation of cPKCs and nPKCs [91]. In the past, the activation of PKCs has been found to induce cholestasis [16], inhibit cAMP-induced intake of taurocholate and decrease MRP3- and OATP-mediated transport of organic solutes [92-94]. Different PKCs are activated by various compounds and subsequently exert pro-cholestatic, anti-cholestatic and choleretic effects. Accordingly, different compounds appear to function differently by affecting various subtypes of PKCs and corresponding signaling pathways (**Figure 3**).

4.2.1 The roles of PKC subtypes in IC. Both cPKCα and nPKCδ participate in the pathogenesis of cholestasis. In an $E_2$17G-induced cholestasis model, cPKCα participates in pathogenesis by activating downstream estrogen receptor (ERα) signaling [95]. cPKCα mediates NTCP internalization induced by phorbol myristate acetate and taurochenodeoxycholate acid [90, 96]. Taurodeoxycholic acid exerts post-translational anticholestatic effects through a cooperative cPKCα-/PKA-dependent mechanism in an experimental model of TLCA-induced cholestasis [97]. A recent study has indicated that nPKCδ is activated by cAMP, and is involved in cAMP-mediated NTCP and MRP2 translocation in hepatocytes [87]. In contrast to those of cPKCα, the effects of nPKCδ are associated with its phosphorylation sites, which are activated by various signals. Activation of nPKCδ may lead to cholestatic effects via Tyr phosphorylation, whereas its activation may lead to anti-cholestatic effects via Thr phosphorylation [98, 99]. In agreement with this hypothesis, the activation of nPKCδ by cAMP and GCDCA is associated with Thr and not Tyr phosphorylation in rat hepatocytes [88, 100]. Nevertheless, the effects and underlying mechanism of differential phosphorylation of nPKCδ in IC still require validation.

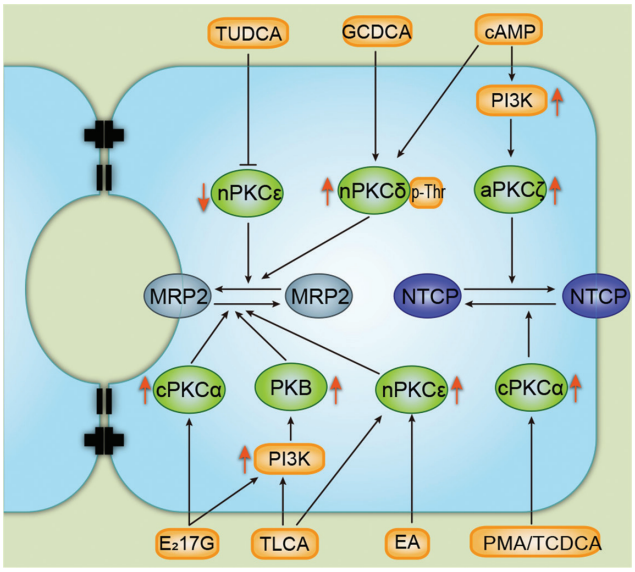

Figure 3 | Proposed model for the regulation of NTCP and MRP2 by PKC isoforms.
Activation of nPKCδ and aPKCζ by cAMP leads to translocation of NTCP and MRP2 to the plasma membrane. Activation of nPKCδ by GCDCA facilitates MRP2 translocation to the plasma membrane. Activation of cPKCa by phorbol myristate acetate and taurochenodeoxycholate acid induces retrieval of NTCP from the plasma membrane. Activation of cPKCa and nPKCε has been implicated in MRP2 retrieval from the plasma membrane by cholestasis induced by $E_2$17G, ethacrynic acid and TLCA.

nPKCε and aPKCζ are two important PKC subtypes. In primary hepatocytes, TLCA activates nPKCε and induces MRP2 endocytosis. Knockdown of nPKCε reverses TLCA-induced internalization of MRP2 [101]. In addition, cAMP and taurodeoxycholic acid reverse TLCA-induced cholestasis and MRP2 retrieval by inhibiting nPKCε [84, 102, 103]. MRP2 retrieval induced by ethacrynic acid is also mediated via nPKCε in rats [91]. In contrast to nPKCε, cAMP promotes the delivery and localization of NTCP toward the basement membrane via the PI3K/aPKCζ pathway [104]. Because aPKCζ, BSEP and MRP2 are all expressed on the membranes of hepatocytes [105], aPKCζ may also be involved in the canalicular localization of the two transporters, a possibility that should be further studied.

5. CONCLUSION

Transporters participate in the transmembrane transport of bile, and their disorders play important roles in the pathogenesis of cholestasis. In past decades, the essential roles of hepatic transporters in the pathogenesis of cholestasis have been gradually revealed. However, studies on the molecular mechanism underlying cholestasis have been limited mainly to transcriptional, expression and functional abnormalities in individual or several transporters, thus yielding limited conclusions. Notably,

abnormal transporter localization can also cause cholestasis. The localization of hepatocyte transporters has been studied, but a comprehensive understanding is lacking. In addition, few studies have assessed the location and function of cholangiocyte transporters. Overall, the pathogenesis of cholestasis induced by abnormal bile transport is a complex network comprising multiple transporters that synergistically secrete and excrete bile from the liver. Furthermore, given the compensatory protective mechanisms in the human body, transporters exert distinctly different effects on acute and chronic cholestasis, thus enabling the underlying molecular mechanisms to be unraveled.

NRs, as transcription factors, regulate transporter genes required for hepatobiliary transport, as well as phase I and II metabolic enzymes involved in processing their substrates. Impaired NR signaling may affect the expression of transporters, and genetic variants of NR-encoding genes are associated with IC susceptibility and progression. In addition, altered localization of transporters participates 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 [91]. In contrast, cPKCα and nPKCδ may be involved in choleretic, cholestatic and anticholestatic effects by inserting, retrieving and inhibiting retrieval of transporters, respectively. Thus, we reviewed the molecular mechanisms through which transporters are regulated through various proteins such as NRs and PKCs in cholestasis, to provide a reference for understanding IC pathogenesis and developing effective drug therapies. Nevertheless, considerable in-depth studies remain necessary to comprehensively clarify the network of regulatory mechanisms of cholestasis-associated transporters.

ABBREVIATIONS

BA, bile acid; UDCA, ursodeoxycholic acid; LCA, lithocholic 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, farnesoid 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; PXR, pregnane X receptor; PPARα, peroxisome proliferator-activated receptor α; SHP, small heterodimer partner; 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; VDR, Vitamin D receptor; LRH-1, Liver receptorhomolog-1; CDCA, Chenodeoxycholic acid; DCA, Deoxycholic acid; CA, Cholic acid; TCDCA, Taurochenodeoxycholic Acid; GCDCA, Glycochenodeoxycholic Acid; E217G, Estradiol-17beta-glucuronide.

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CONFLICTS OF INTERESTS

The authors have no conflicts of interest associated with this publication.

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