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REVIEW

Nuclear receptors as therapeutic targets in cholestatic liver diseases

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Cholestasis results in intrahepatic accumulation of cytotoxic bile acids, which cause liver damage ultimately leading to biliary fibrosis and cirrhosis. Cholestatic liver injury is counteracted by a variety of adaptive hepatoprotective mechanisms including alterations in bile acid transport, synthesis and detoxification. The underlying molecular mechanisms are mediated mainly at a transcriptional level via a complex network involving nuclear receptors including the farnesoid X receptor, pregnane X receptor, vitamin D receptor and constitutive androstane receptor, which target overlapping, although not identical, sets of genes. Because the intrinsic adaptive response to bile acids cannot fully prevent liver injury in cholestasis, therapeutic targeting of these receptors via specific and potent agonists may further enhance the hepatic defence against toxic bile acids. Activation of these receptors results in repression of bile acid synthesis, induction of phases I and II bile acid hydroxylation and conjugation and stimulation of alternative bile acid export while limiting hepatocellular bile acid import. Furthermore, the use of nuclear receptor ligands may not only influence bile acid transport and metabolism but may also directly target hepatic fibrogenesis and inflammation. Many drugs already used to treat cholestasis and its complications such as pruritus (e.g. ursodeoxycholic acid, rifampicin, fibrates) may act via activation of nuclear receptors. More specific and potent nuclear receptor ligands are currently being developed. This article will review the current knowledge on nuclear receptors and their potential role in the treatment of cholestatic liver diseases.
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Keywords: nuclear receptors; cholestasis; bile acids; bilirubin; statins; fibrates; glitazones; primary biliary cirrhosis; primary sclerosing cholangitis; obstructive cholestasis; fibrosis

Abbreviations: ASBT/Asbt (*SLC10A2/Slc10a2*), apical sodium-dependent bile acid transporter; BSEP/Bsep (*ABCB11/Abcb11*), bile salt export pump; CA, cholic acid; CAR (NR1I3), constitutive androstane receptor; CDCA, chenodeoxycholic acid; CYP, cytochrome P450 enzyme; CYP27A1/Cyp27a1, sterol 27-hydroxylase; CYP7A1/Cyp7a1, cholesterol 7alpha-hydroxylase; CYP8B1/Cyp8b1, sterol 12alpha hydroxylase; DCA, deoxycholic acid; FTF (NR5A2), fetoprotein transcription factor; FXR (NR1H4), farnesoid X receptor; GR (NR3C1), glucocorticoid receptor; HNF1α, (*TCF1*); hepatocyte nuclear factor 1 alpha, HNF4α; (NR2A1), hepatocyte nuclear factor 4 alpha; LCA, lithocholic acid; LRH1 (NR5A2), liver receptor homologue; Mdr2 (*Abcb4*), multidrug resistance gene 2; MDR3 (ABCB4), human homologue to rodent Mdr2; MRP/Mrp (*ABCC/Abcc*), multidrug resistance-associated protein; NCoR, nuclear receptor corepressor; NR, nuclear receptor; NTCP/Ntcp (*SLC10A1/Slc10a1*), Na^+/taurocholate cotransporter; OATP/Oatp (*SLCO/Slco*), organic anion transporting peptide; OSTα/OSTβ/Ostα/Ostβ, organic solute transporter alpha/beta; PFIC, progressive familial intrahepatic cholestasis; PGC1, proliferator-activated receptor-gamma coactivator-1; PPARα (NR1C1), peroxisome proliferator-activated receptor alpha; PXR (NR1I2), pregnane X receptor; RARα (NR1B1), retinoic acid receptor alpha; RXRα (NR2B1), retinoid X receptor alpha; SHP (NR0B2), short heterodimer partner; SMRT, silent mediator of retinoic acid receptor and thyroid receptor; SULT2A1, dehydroepiandrosterone sulfotransferase; UDCA, ursodeoxycholic acid; UGT, UDP-glucuronosyl transferase; VDR (NR1I1), vitamin D receptor

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Please note that human genes and their products are capitalized, whereas rodent genes and their products are written in lower case. Transporter genes are set *in italic* whereas gene products are set in normal font.
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Introduction

Cholestasis is defined as a disturbance of bile secretion that can result from a functional defect in bile formation at the level of hepatocytes or from impaired bile secretion and flow at the bile duct level (Trauner *et al.*, 1998; Trauner and Boyer, 2003). Cholestasis results in the retention of substances normally secreted into bile. Retention of these cholephiles

(particularly bile acids that are cytotoxic at high concentrations) can lead to chronic liver disease with development of biliary fibrosis, cirrhosis and ultimately end-stage liver disease requiring liver transplantation. So far, ursodeoxycholic acid (UDCA) is one of the few widely used drugs in the treatment of chronic cholestatic disorders such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). However, the effectiveness of UDCA in PBC has been questioned by several meta-analyses, and UDCA delays disease progression only when initiated at early stages (Paumgartner and Pusl, 2008). UDCA improves liver tests in patients with PSC, but its effects on survival remain also unclear (Cullen and Chapman, 2006). Therefore future research is needed to develop novel and more effective drugs for cholestatic diseases. Nuclear receptors (NRs) are promising therapeutic targets for cholestatic liver diseases.

Principal therapeutic targets in cholestasis

Because cholestasis can be defined from a pathophysiological perspective as a reduction in bile secretion leading to retention of substances normally secreted into bile, at a first glance, restoration of bile flow might be considered a primary therapeutic target (Fig. 1). However, it has to be kept in mind that a mere increase of bile flow in obstructive cholestasis without resolution of the cause may worsen the disease course due to increase of biliary pressure leading to rupture of cholangioles and to the development of bile infarcts. This has been observed in bile duct-ligated mice and in a mouse model of sclerosing cholangitis after administration of UDCA at choleretic doses (Fickert *et al*., 2002). Many relevant human cholestatic disorders, which are primarily not considered to be obstructive, may have obstructive components to various extents. Potential examples include PSC, secondary sclerosing cholangitis and late stage PBC with pronounced ductopenia.

Which mechanisms should be targeted by novel therapeutic strategies? A major aim of therapy may be to reduce accumulation of toxic biliary constituents such as bile acids. This can be achieved by reducing basolateral hepatic uptake and by increasing hepatocellular excretion of these compounds (Fig. 1). As mentioned above, canalicular secretion should be kept low in order to prevent an increase in biliary pressure in obstructive forms of cholestasis. Stimulating alternative basolateral secretion of water soluble compounds into sinusoidal blood is expected to enhance renal elimination of accumulating substances in cholestasis (Fig. 1). Increasing water solubility and reducing toxicity can be achieved by phase I and phase II detoxification reactions (Fig. 1). In addition, repression of bile acid synthesis will also reduce bile acid accumulation (Fig. 1). Another strategy is to reduce toxicity of bile by increasing the biliary phospholipid content. This may be relevant under conditions with stagnant or low bile flow and increased exposure of cholangiocytes to toxic biliary constituents. Because long-term cholestasis leads to the development of biliary cirrhosis, direct inhibition of fibrosis may also be an attractive strategy (Fig. 1).

Bile acid homeostasis is regulated to large extent at a transcriptional level via NRs that play a key role in the regula-

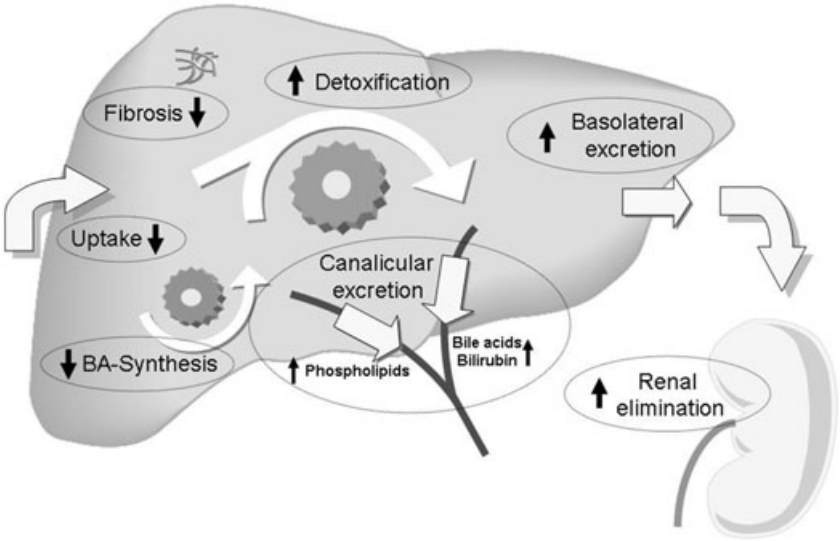

Figure 1 Targets for nuclear receptor ligands in cholestasis. Therapeutic approaches in cholestasis should primarily aim at limiting accumulation of toxic biliary constituents (especially bile acids, BA). This can be achieved by reducing basolateral hepatic uptake and by increasing orthograde canalicular and retrograde (alternative) basolateral secretion. Increasing canalicular secretion will also lead to an increase in bile flow, which might be beneficial in some (early and primarily canalicular) forms of cholestasis. In obstructive cholestasis, however, an increase in bile flow will lead to an increase in biliary pressure with subsequent rupture of cholangioles and liver injury. Stimulating alternative basolateral secretion of water soluble compounds into sinusoidal blood is followed by increased renal elimination of these substances. Increasing water solubility and reducing toxicity can be achieved by phase I and phase II detoxification reactions that also facilitate renal elimination due to reduced albumin binding. In addition, repression of bile acid synthesis will also reduce bile acid accumulation. Other therapeutic strategies in cholestasis include reduction of the toxicity of bile by increasing the biliary phospholipids content and direct inhibition of fibrosis. All these protective mechanisms are regulated to a large extent at a transcriptional level by nuclear receptors. Therapeutic administration of nuclear receptor ligands can activate these defence pathways. Adapted from SJ Karpen, *Hepatology* 2005 (Karpen, 2005).

tion of hepatobiliary transport systems, bile acid synthesis and of enzymes involved in bile acid detoxification (Karpen, 2002; Eloranta and Kullak-Ublick, 2005; Zollner *et al.*, 2006b). NRs comprise a family of transcription factors that regulate gene expression in a ligand-dependent manner. All NRs share several structural domains that are essential for receptor function (Kumar *et al.*, 2004). The carboxy-terminal region includes the ligand-binding domain, dimerization interface and a ligand-dependent activation function (Chawla *et al.*, 2001). Upon ligand binding, NRs undergo a conformational change that coordinately dissociates corepressors and facilitates recruitment of coactivator proteins to enable transcriptional activation (McKenna *et al.*, 1999). The NR ligand-binding domain is connected to the DNA-binding domain by a short flexible linker and mediates ligand-dependent transactivation functions (Glass and Rosenfeld, 2000). The DNA-binding domain is highly conserved and contains two α helices and two zinc fingers that are involved in the specificity of response-element recognition and in receptor dimerization (Staudinger, 2008). Most NRs bind to their DNA response elements in a sequence-specific manner as dimers, functioning either as homodimers or as heterodimers with the retinoid X receptor (RXR) (Mangelsdorf and Evans, 1995). The N-terminal region is highly variable but always contains a region called activation function 1 with many phosphorylation sites (Staudinger, 2008). Increasing knowledge on the three-dimensional structure of NRs (e.g. through crystallization studies) has facilitated the design of small molecules specifically targeting their ligand-binding domain (Pellicciari *et al.*, 2005; Westin *et al.*, 2005).

The precise regulation of transcription by NRs requires the recruitment of intermediary factors characterized as coregulators. These factors modulate transcriptional initiation at regulated promoters by modifying chromatin structures and assembling transcriptional initiation complexes. These coregulators can have positive and negative actions on target gene expression. Coregulator proteins modulate the transcription of NR target genes by participating in chromatin remodelling or interacting with general transcription machinery to affect the formation of the preinitiation complex (Perissi and Rosenfeld, 2005). Coactivators such as peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) promote NR-transcriptional activation in the presence of NR ligands while corepressors such as nuclear receptor corepressor (NCoR) and silent mediator of retinoic acid receptor and thyroid receptor (SMRT) mediate NR-dependent transcriptional silencing in absence of ligands (Nishihara *et al.*, 2004).

Because NRs are the central regulators of bile acid synthesis, transport and detoxification and also play a role in modulating fibrosis, specific targeting of NRs represents an innovative approach for the treatment of cholestasis. Extensive research in the field of cholestasis has provided a detailed understanding of the molecular mechanisms involved in bile formation, bile acid homeostasis and NR function under physiological and pathological conditions over the last decade. This knowledge is required for the development of such NR-targeted therapies and will therefore be briefly reviewed.

Molecular mechanisms of bile formation and cholestasis

Bile acids are synthesized from cholesterol by a complex pathway consisting of a cascade of 16 reactions (Chiang, 1998). The cholesterol 7α-hydroxylase (CYP7A1) initiates the first, rate limiting step in bile formation in the classical bile acid synthesis pathway finally producing cholic acid (CA) and chenodeoxycholic acid (CDCA) in equal amounts (Myant and Mitropoulos, 1977). Sterol 12alpha hydroxylase (CYP8B1) controls the ratio of CA to CDCA in this pathway. The alternative pathway is initiated by sterol 27-hydroxylase (CYP27A1) leading to the production of CDCA (Pikuleva *et al.*, 1998). The driving force for hepatocellular bile formation is the active transport of bile acids from sinusoidal blood into the canaliculus. Specific transport proteins are localized to the basolateral (sinusoidal) and canalicular (apical) membrane of hepatocytes and cholangiocytes (Fig. 2) and have recently been reviewed elsewhere (Trauner and Boyer, 2003; Pellicoro and Faber, 2007). Defects in transporter systems can cause rare forms of inherited cholestatic syndromes leading to cholestasis already in childhood (Oude Elferink *et al.*, 2006). Transporter defects may be also incomplete not causing any phenotype under physiologic concentrations but may become evident when the patient is challenged with a cholestatic agent (e.g. drugs, sex-hormones, cytokines released by inflammation) (Oude Elferink *et al.*, 2006). However, in most cholestatic disorders transporter alterations may rather be the consequence than the cause of cholestasis and largely represent an attempt to adapt to accumulating biliary constituents in cholestasis and protect hepatocytes from intracellular accumulation of toxic bile acids. A complex machinery of coordinated mechanisms is activated by bile acids to counteract cholestatic liver injury (Zollner *et al.*, 2006b). Such adaptive mechanisms include repression of basolateral bile acid uptake and bile acid synthesis, induction of bile acid detoxification systems (i.e. phase I bile acid hydroxylation and phase II conjugation with sulphate or glucuronidate) and recruitment of alternative export pumps for cholephiles at the basolateral membrane. Adaptive mechanisms in response to cholestasis are not only restricted to the hepatocytes but also observed in kidney, intestine and bile duct epithelium (Zollner and Trauner, 2006).

Limiting hepatic bile acid uptake and bile acid synthesis during cholestasis are considered as protective mechanisms to reduce hepatocellular bile acid overload. Expression of the main basolateral bile acid uptake systems, the Na^+/taurocholate cotransporter (NTCP/Ntcp) and the organic anion transporter OATP1B1/*SCLO1B1* (formerly known as OATP-C or OATP2) is reduced in human cholestatic liver diseases and in rodent models of cholestasis and bile acid overload (for review see Zollner and Trauner, 2008). CYP7A1 is repressed by bile acids and by other bile acid-independent mechanisms (Chiang, 2004), and CYP7A1 is down-regulated in late stage PBC (Zollner *et al.*, 2007).

Bile acid hydroxylation (phase I) and conjugation (phase II) renders bile acid more hydrophilic, less toxic and more amenable for urinary excretion as a result of reduced albumin binding. Phase I detoxification (hydroxylation) is mediated by CYP3A4 (and by its rodent homologue Cyp3a11) (Araya

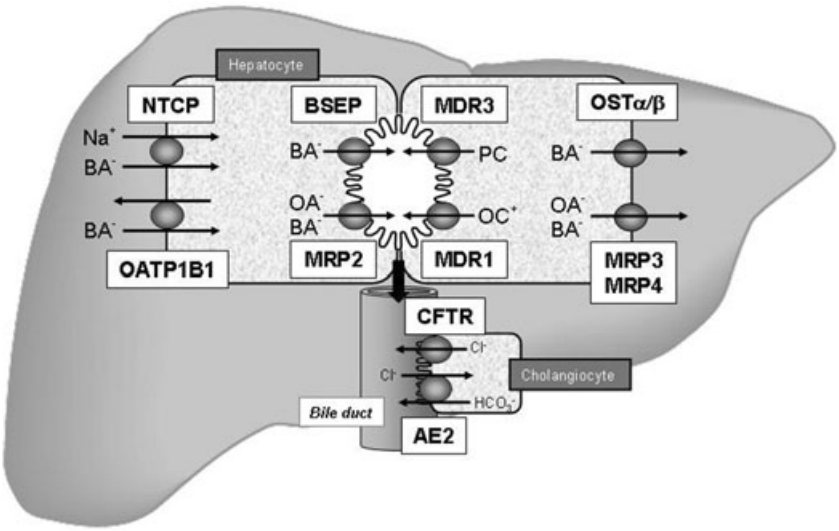

Figure 2 Hepatobiliary transport systems in the liver. Bile acids (BA^-) are taken up by the Na^+/taurocholate cotransporter (NTCP) and organic anion transporting protein2 (OATP2) at the basolateral membrane of hepatocytes. Monovalent BA^- are excreted into bile by the canalicular bile salt export pump (BSEP), divalent BAs and anionic anions (OA^-) are exported by the canalicular conjugate export pump (MRP2). The phospholipid export pump (MDR3) mediates excretion of phosphatidylcholine (PC), which forms mixed micelles together with BA^- and cholesterol in bile. Cationic drugs (OC^+) are excreted by the multidrug export pump (MDR1). At the basolateral membrane of hepatocytes, MRP3, MRP4 and the heteromeric organic solute transporter OSTα/β provide an alternative excretion route for BA^- and other OA^- into the systemic circulation. BA^- secreted into bile can be reabsorbed by cholangiocytes via apical Na^+-dependent bile salt transporter (ASBT) and effluxed by Ostα/β and Mrp3 (not shown). Cholangiocytes also express a chloride channel that is the cystic fibrosis transmembrane regulator (CFTR) that drives bicarbonate secrtion via a chloride/anion exchanger (AE2). Adapted from Zollner and Trauner, *Wien Med Wochenschr* 2006 (Zollner and Trauner, 2006).

and Wikvall, 1999; Handschin and Meyer, 2003; Bodin *et al.*, 2005). Cyp3a11 levels are increased in rodent models of obstructive cholestasis and in bile acid-challenged mice leading to increased urinary excretion of (poly-)hydroxylated bile acids (Schuetz *et al.*, 2001; Staudinger *et al.*, 2001b; Xie *et al.*, 2001; Makishima *et al.*, 2002; Stedman *et al.*, 2004; Marschall *et al.*, 2006; Zollner *et al.*, 2006a). Phase II conjugation reactions of bile acids with sulphate or glucuronidate are catalysed by dehydroepiandrosterone-sulfotransferase (SULT2A1) and by the UDP-glucuronosyltransferases UGT2B4 and UGT2B7 respectively (Falany, 1997; Weinshilboum *et al.*, 1997; Gall *et al.*, 1999; King *et al.*, 2000). The appearance of hydroxylated, sulphated and glucuronidated bile acids in urine of patients with cholestatic diseases indicates that these detoxification pathways are activated in human cholestatic diseases (Makino *et al.*, 1975; Berge Henegouwen *et al.*, 1976; Frohling and Stiehl, 1976; Alme *et al.*, 1977; Bremmelgaard and Sjovall, 1979; 1980; Thomassen, 1979; Alme and Sjovall, 1980; Shoda *et al.*, 1990).

Hepatocellular bile acid efflux via the basolateral membrane may become an important alternative spill-over route for accumulating bile acids during cholestasis. This alternative (or retrograde) basolateral bile acid export is mediated by the multidrug resistance-associated proteins MRP3, MRP4 and the heteromeric organic solute transporter OSTα/OSTβ (Dawson *et al.*, 2005). These export systems are normally expressed at very low levels at the basolateral membrane but are dramatically up-regulated after bile acid feeding and in experimental cholestasis in rodents as well as in human cholestatic liver diseases (Hirohashi *et al.*, 1998; Ogawa *et al.*, 2000; Donner and Keppler, 2001; Fickert *et al.*, 2001; Schuetz *et al.*, 2001; Shoda *et al.*, 2001; Soroka *et al.*, 2001; Tanaka *et al.*, 2002; Zollner *et al.*, 2003a,b; 2006c; 2007; Keitel *et al.*, 2005). The substrate specificity of MRP3, MRP4 and OSTα/OSTβ includes phase II conjugation products suggesting an interplay between detoxification and subsequent basolateral export systems. Bile acids reaching the systemic circulation are filtered at the glomerulus from plasma into urine thus establishing an alternative way for their excretion (Wilson *et al.*, 1981).

The data derived from animal models of cholestasis and human cholestatic diseases indicate that adaptive mechanisms aimed at counteracting liver injury are intrinsically activated in cholestasis. However, these protective mechanisms do not suffice to completely avoid liver damage. Thus additional targeting these pathways via key regulatory NRs appears as an innovate approach (Fig. 3). This will be the focus of this review.

Farnesoid X receptor [FXR (*NR1H4*)] and short heterodimer partner [SHP (*NR0B2*)]

FXR is a major intracellular bile acid receptor that regulates the expression of a wide variety of genes involved in bile acid synthesis, metabolism and transport (Fig. 3, Table 1). FXR binds to its response element generally as a heterodimer with retinoid X receptor RXR (NR2B1). The preferred DNA-binding motifs are inverted repeat elements separated by one nucleotide [inverse repeat (IR)-1] (Laffitte *et al.*, 2000). Physiologic ligands for FXR are CDCA, deoxycholic acid (DCA), lithocholic acid (LCA) and CA (Parks *et al.*, 1999). In addition to bile acid homeostasis, FXR also regulates triglyceride, cholesterol and glucose metabolism (Lee *et al.*, 2006a). Moreover, FXR has been shown to modulate liver regeneration, carcinogenesis, inflammation, bacterial overgrowth in the intestine

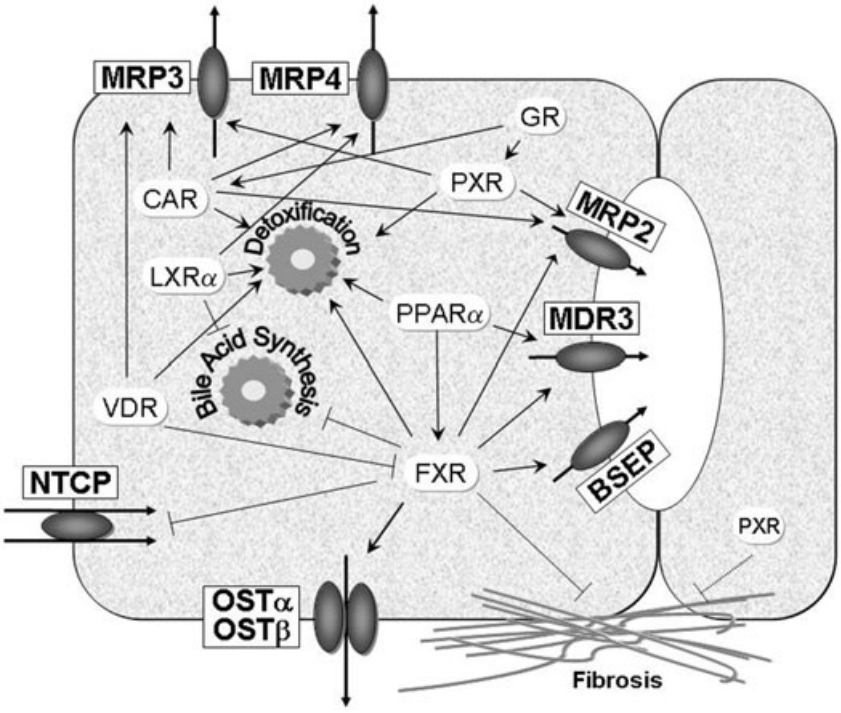

Figure 3 Nuclear receptors as therapeutic targets in cholestasis. Nuclear receptors regulate a large number of target genes mediating transport, synthesis and detoxification of biliary constituents including bile acids. This figure summarizes the anti-cholestatic properties of nuclear receptors that can be targeted therapeutically by synthetic and natural ligands. As indicated in the figure, nuclear receptors regulate overlapping sets of target genes. This may be of relevance, when expression of one nuclear receptor is low due to disease state (as observed in cholestasis). In addition, nuclear receptors may directly regulate fibrogenesis Arrows indicate stimulatory effects, the other lines indicate suppressive effects on target genes. BSEP, bile salt export pump; CAR, constitutive androgen receptor; FXR, farnesoid X receptor; GR, glucocorticoid receptor; LXR, liver X receptor; MRP, multidrug resistance-associated protein; NTCP, Na^+/taurocholate cotransporter; OST, organic solute transporter; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; VDR, vitamin D receptor.

and hepatitis C virus replication making it a very attractive target for hepatobiliary and gastrointestinal disorders (Huang *et al.*, 2006; Inagaki *et al.*, 2006; Kim *et al.*, 2007c; Yang *et al.*, 2007; Scholtes *et al.*, 2008).

Bile acid synthesis

FXR plays a central role in regulation of bile acid synthesis. Bile acid-activated FXR represses *CYP7A1* gene transcription by induction of the nuclear repressor SHP. SHP was suggested to negatively interact with fetoprotein transcription factor (FTF/*NR5A1*, also known as liver receptor homologue, LRH-1), which binds to the bile acid response element (BARE) located within the proximal *CYP7A1* promoter region together with hepatocyte nuclear factor (HNF) 4α (Stroup *et al.*, 1997; Chiang *et al.*, 2000; Goodwin *et al.*, 2000; Lu *et al.*, 2000). A similar mechanism has been proposed for regulation of *Cyp8b1* (Castillo-Olivares and Gil, 2000; Goodwin *et al.*, 2000; Zhang and Chiang, 2001). However, recent studies indicate that LRH-1 is not involved in feedback regulation of either *Cyp7a1* or *Cyp8b1* because LRH-1 deficiency in hepatocytes has no significant effects on FXR-mediated repression of these genes (Lee *et al.*, 2008). An additional gut-liver signalling pathway in the regulation of bile acid homeostasis is mediated via fibroblast growth factor Fgf15 (the rodent homologue of human FGF19). Bile acids induce intestinal Fgf15 expression in an FXR-dependent fasion in mice. Fgf15 signals from intestine to the liver to repress Cyp7a1 through a mechanism involving Fgf receptor 4 (FgfR4) and a c-Jun N-terminal kinase (JNK)-dependent pathway (Holt *et al.*, 2003; Inagaki *et al.*, 2005). The role of this intestinal FXR/Fgf-15 pathway has recently been confirmed in intestine-specific FXR knockout mice (Kim *et al.*, 2007b). In this study, the FXR agonist GW4064 repressed *Cyp7a1* only in intestine- but not in liver-specific FXR-deficient animals. In contrast, *Cyp8b1* repression by the synthetic FXR ligand GW4064 was more dependent on the presence of FXR in liver than in intestine indicating mechanistic differences in feedback repression of *Cyp7a1* and *Cyp8b1* (Kim *et al.*, 2007b). In addition to FXR/SHP, multiple redundant pathways regulate expression of bile acid synthesis enzymes. Bile acids can also directly block recruitment of the transcriptional coactivator PGC-1, which is bound to HNF4α thereby abolishing the activating effects of HNF4α (De Fabiani *et al.*, 2003). A detailed review of these redundant pathways is given elsewhere (Chiang, 2004; Eloranta and Kullak-Ublick, 2005; Zollner *et al.*, 2006b).

Bile acid uptake

FXR negatively regulates the main bile acid uptake system *Ntcp* via SHP. Bile acid-activated FXR induces expression of SHP, which in turn interferes with RXRα : RARα (retinoic acid receptorα) mediated activation of the rat *Ntcp* promoter (Denson *et al.*, 2001). In addition, SHP reduces *Ntcp* expres-

Table 1. Nuclear receptors as therapeutic targets in cholestasis

NR	*Ligands*	*Therapeutic*	*Target Genes*	*Therapeutic Effects*
FXR (*NR1H4*)	CDCA, DCA, CA, synthetic: GW4064, 6-ECDCA	Synthesis:	Indirect repressive effects via SHP	Repression of bile acid synthesis indirectly via SHP
		Detoxification:	*CYP3A4, SULT2A1, UGT2B4, UGT2B7*	Induction of bile acid detoxification
		Transport:	*MRP2/Mrp2, OATP1B3, OSTα/β, SHP, BSEP/ Bsep, I-BABP; MDR3/ Mdr2* indirect repressive effects via SHP	Induction of canalicular and alternative basolateral bile acid excretion Increase in bile flow Increase in bililary phospholipid content Repression of bile acid uptake (effect may be restricted to rodents)
		Fibrosis:	*SHP, PPARγ*	Indirect antifibrotic activity via SHP and PPARγ.
SHP (*NR0B2*)	No ligand, FXR target	Synthesis:	*CYP7A1/Cyp7a1, CYP8B1/Cyp8b1, CYP27A1*	Repression of bile acid synthesis
		Transport:	*Ntcp, ASBT/Asbt*	Repression of hepatic and intestinal bile acid uptake
		Fibrosis:	*α1(I)collagen*	Induction of quiescent HSC phenotype, increase in HSC apoptosis
PXR (*NR1/2*)	Rifampicin in humans, phenobarbital, dexamethasone, statins, St. John's wort, clotrimazole pregnenolone-16α-carbonitrile (PCN) in rodents	Synthesis:	*CYP7A1*	Indirect repression (via interaction with HNF4α) of CYP7A1 *in vitro*
		Detoxification:	*CYP3A4, SULT2A1/ Sult2a1, UGT1A1*	Induction of phase I and II bile acid and bilirubin detoxification systems
		Transport:	*MRP2/Mrp2, MRP3, Oatp1a4, MDR1*	Induction of orthograde canalicular and alternative basolateral bile acid excretion
		Fibrosis:		Antifibrotic activities, inhibition of HSC transdifferentiation
CAR (*NR1/3*)*	Yin Chin, CITCO, bilirubin, Phenobarbital, 1,4-bis-[2-(3,5-ichlorpyidyloxy)] benzene (TCPOBOP), dimethoxycoumarin, dimethylesculetin	Detoxification:	*CYP3A4, Sult2a1, UGT1A1*	Induction of bile acid and bilirubin detoxification systems
		Transport:	*MRP2/Mrp2, Mrp3, MRP4/Mrp4*	Induction of orthograde canalicular and alternative basolateral bile acid excretion Minor increase in bile flow
VDR (*NR1/1*)	1α,25-dihydroxy-vitamin, D_3, LCA	Detoxification:	*CYP3A4/Cyp3a11, SULT2A1/Sult2a1*	Induction of bile acid detoxification systems
		Transport:	*Mrp3, ASBT*	Induction of alternative basolateral bile acid excretion and induction of of biliary and intestinal bile acid reabsorption
		NRs:	*FXR*	Inhibition of FXR transactivation
HNF4α (*NR2A1*)	Fatty acyl-coenzyme A thioesters	Synthesis:	*Cyp7a1, CYP8B1/Cyp8b1, CYP27A1*	Reduced HNF4α binding or expression down-regulates bile acid synthesis and detoxification pathways
		Detoxification:	*CYP3A4, Cyp3, SULT2A1*	
		Transport:	*ASBT, OATP1B1, Oatp1a1, Oatp1b2, Oatp1a4, Ntcp*	Required for constitutive expression of various basolateral uptake systems
		NRs, Transcription factors	*PXR, CAR, HNF1α*	Indirect regulation of PXR, CAR and HNF1α target genes
LRH-1 (*NR5A2*)	Phospholipids	Synthesis:	*CYP7A1, CYP8B1/Cyp8b1*	Required for basal expression of bile acid
		Transport	*MRP3, Asbt, Ntcp, Bsep, Mdr2*	synthesis enzymes, transporters and NRs
		NRs:	*FXR, SHP*	
PPARα (*NR1C1*)	Fatty acids, fibrates, statins, eicasaonoids, leukotriens, NSAIDs, WY-14643	Synthesis:	*CYP7A1*	Repression of bile acid synthesis
		Detoxification:	*SULT2A1, UGT2B4, UGT1A3*	Induction of bile acid detoxification systems
		Transporti:	*Mdr2, ASBT*	Protection of bile duct epithelium via increased phospholipid secretion and increased bile acid reabsorption
		NRs:	*FXR*	Indirect induction of FXR targets such as *UGT2B4*
PPARγ (*NR1C3*)	thiazolidinediones (glitazones)	NRs:	*SHP*	Effects on SHP targets?
		Fibrosis:	*α1(I)collagen*	Inhibits HSC activation
		General		Attenuation of the inflammatory response, senzitation to insulin

Table 1. *Cont.*

NR	*Ligands*	*Therapeutic*	*Target Genes*	*Therapeutic Effects*
LXRα (NR1H3)	Oxysterols, fatty acids, 6α-hydroxylated bile acids, TO1317	Synthesis:	*Cyp7a1/CYP7A1*	Induction of rodent Cyp7a1, repression of human CYP7A1
		Detoxification:	*Sult2a9, UGT1A3*	Induction of bile acid sulfation and glucuronidation
		Transport:	*Mrp4*	Induction of alternative basolateral bile acid excretion
GR (*NR3C1*)	Glucocorticoids, potentially UDCA	Transport:	*ASBT, NTCP*, potentially *AE2*, *Bsep*, Mrp2	Contribution of effects on transporters to anti-inflammatory properties in treatement of inflammatory cholestasis ("steroid whitewash")
		NRs:	*CAR, PXR, RXRα*	Potentiates action of PXR and CAR

*Please note that CAR can be activated either indirectly or directly by ligand binding (e.g. TCPOBOP (Goodwin *et al.* 2004).

sion via a complex pathway involving repression of HNF4α and HNF1α, the latter being an essential *Ntcp* transactivator (Karpen *et al.*, 1996; Lee *et al.*, 2000; Jung and Kullak-Ublick, 2003). While the role of SHP in *NTCP/Ntcp* regulation has been questioned (Wang *et al.*, 2002; Jung *et al.*, 2004b), data obtained in CA-fed and bile duct-ligated FXR knockout mice indicate an important role for FXR in *Ntcp* regulation by bile acids (Zollner *et al.*, 2005). In contrast, the human *NTCP* promoter does not contain the rat RXRα : RARα and HNF4α response elements (Jung *et al.*, 2004b). Reduced NTCP expression in various human cholestatic diseases (Shoda *et al.*, 2001; Zollner *et al.*, 2001; 2003b) might be explained by suppression of glucocorticoid receptor (GR)-mediated activation of human *NTCP* by FXR/SHP (Eloranta *et al.*, 2006). Additional FXR/SHP independent pathways in bile acid-mediated *Ntcp* regulation also play an important role (Li *et al.*, 2002; Wang *et al.*, 2002; Zollner *et al.*, 2006b).

Bile acid detoxification

In addition to the pregnane X receptor (PXR) and the constitutive androgen receptor (CAR) as the central regulators of phases I and II enzymes, FXR is also involved in controlling bile acid detoxification pathways. FXR positively regulates human *CYP3A4* (Gnerre *et al.*, 2004), while it is not required for up-regulation of mouse orthologue *Cyp3a11* (Schuetz *et al.*, 2001; Marschall *et al.*, 2006; Zollner *et al.*, 2006a). On the contrary, FXR-deficient bile duct-ligated mice even have higher levels of Cyp3a11 and increased bile acid hydroxylation rates indicating species differences (Marschall *et al.*, 2006) and that other NRs such as PXR may take over when FXR is absent. FXR also positively regulates *SULT2A1* by binding to an IR-0 response element in its gene promoter (Song *et al.*, 2001). Bile acids can induce human *UGT2B4* via activation of FXR (Barbier *et al.*, 2003b). Of note, *UGT2B4* is the only gene described so far to be activated by FXR through binding of an FXR monomer to a single hexameric DNA motif without its common heterodimeric partner RXR.

Bile acid efflux

While most of FXR's repressive effects are indirect and largely mediated by the activation of SHP, bile salt export pump (*BSEP*) is directly transactivated by FXR. (Ananthanarayanan *et al.*, 2001; Gerloff *et al.*, 2002; Plass *et al.*, 2002). Bile acids increase BSEP expression in primary human hepatocytes or HepG2 cells with the same rank order of potency that activates FXR (Schuetz *et al.*, 2001). In addition, MRP2 is also induced by FXR ligands (Kast *et al.*, 2002). The human phospholipid export pump *MDR3* contains an FXR response element in its gene promoter, and expression is stimulated by the natural and synthetic FXR ligands CDCA and GW4046 respectively (Huang *et al.*, 2003a). Murine Mdr2 (the rodent homologue to human MDR3) expression is lower in FXR-deficient mice, and Mdr2 induction by GW4064 is abolished in these animals (Moschetta *et al.*, 2004). Thus, bile acids not only induce their own efflux into bile by increasing BSEP expression but also stimulate phospholipid secretion, which is needed to maintain the bile acid/lipid ratio in bile to prevent bile duct injury by non-micellar bound bile acids. When orthograde biliary bile acid output is reduced, retrograde bile acid secretion represents an alternative elimination route to reduce accumulation of toxic bile acids within hepatocytes. Bile acid-activated FXR transactivates the basolateral efflux system Ostα/Ostβ. Two functional FXR-binding motifs were identified in the human *OSTα* gene, and one in the *OSTβ* gene indicating a role of FXR in modulation of alternative bile acid secretory pathways (Landrier *et al.*, 2006; Lee *et al.*, 2006b).

FXR and fibrosis

FXR also plays a role in regulation of hepatic stellate cells (HSCs), which are the major source for extracellular matrix deposition in the liver. Activation of FXR by the synthetic CDCA derivate 6-ethyl chenodeoxycholic acid (6-ECDCA), reduces liver fibrosis in a rat model of bile duct obstruction and reduces human and rat HSC transdifferentiation (Fiorucci *et al.*, 2004; 2005b,c). The antifibrotic properties of FXR are mediated via SHP and the peroxisome proliferator-activated receptor (PPAR)γ. SHP reduces α1(I) collagen mRNA by interfering with activator protein 1 (AP1), promotes the development of a quiescent HSC phenotype and increases apoptosis of HSCs (Fiorucci *et al.*, 2004; 2005c). FXR induces PPARγ expression, which also leads to down-regulation of α1(I) collagen mRNA expression and to counter-regulation of HSC activation in rodent models of fibrosis (including obstructive cholestasis) (Fiorucci *et al.*, 2005b). However, these data have to be validated in further studies.

Table 2 Nuclear receptor ligands currently tested in clinical trials for cholestasis

Drug	*Nuclear receptor target*	*Cholestatic disorder*	*ClinicalTrials. gov weblink*
INT-747 (6-ECDCA)	FXR	PBC/monotherapy	http://clinicaltrials.gov/ct2/show/NCT00570765
INT-747 (6-ECDCA)	FXR	PBC/combination therapy with UDCA	http://clinicaltrials.gov/ct2/show/NCT00550862
Fenofibrate	PPARα	PBC	http://clinicaltrials.gov/ct2/show/NCT00575042

6-ECDCA, 6-ethyl chenodeoxycholic acid; FXR, farnesoid X receptor; PBC, primary biliary cirrhosis; PPAR, peroxisome proliferator-activated receptor.

Therapeutic targeting of FXR

Taken together, FXR is critically involved in the regulation of bile acid transport, synthesis and metabolism, of biliary phospholipid secretion and may also play a role in modulation of HSC activity (Table 1). This makes FXR a highly interesting target for drug therapy. Further data suggesting that stimulation of FXR would be an ideal therapeutic approach in cholestasis include: (i) low FXR expression and activity in cholestasis (especially in inflammatory cholestasis) (Kim *et al.*, 2003); (ii) increased liver injury in FXR-deficient mice upon bile acid challenge (Sinal *et al.*, 2000; Zollner *et al.*, 2003a); and (iii) the association of low expression of FXR and FXR target genes (i.e. BSEP) with various human cholestatic disorders (i.e. progressive intrahepatic cholestasis, intrahepatic cholestasis of pregnancy) (Strautnieks *et al.*, 1998; Chen *et al.*, 2004; Van Mil *et al.*, 2007).

Indeed, beneficial effects of pharmacologic FXR activation have been observed in oestrogen-induced cholestasis in rodents. Administration of the synthetic FXR ligands 6-ECDCA and GW4064 to oestrogen-treated rats restored bile flow and reduced serum markers of cholestasis. This was attributed to repression of basolateral bile acid uptake and bile acid synthesis and to induction of canalicular transporters (Fiorucci *et al.*, 2005a). Reduced transporter function may be causative in oestrogen-induced cholestasis (Kullak-Ublick *et al.*, 2000), and stimulation of reduced transport function may indeed be beneficial. This may be true for other forms of cholestasis where transporter defects are suspected to contribute to cholestasis (e.g. hereditary cholestatic diseases such as progressive familial intrahepatic cholestasis, sepsis-associated cholestasis and intrahepatic cholestasis of pregnancy). Most clinically relevant cholestatic disorders, however, are the consequence of bile duct obstruction (e.g. large bile duct obstruction by stones or tumours, small bile duct obstruction as observed in PSC) or bile duct loss (i.e. vanishing bile duct syndromes like late stage PBC). In these diseases, alterations of transporter expression and function are not causing cholestasis but rather are secondary events as a consequence of bile acid retention (Zollner and Trauner, 2006). Especially in obstructive cholestasis, stimulation of biliary bile flow may be detrimental. Stimulation of bile flow even with the hydrophilic bile acid UDCA in a mouse model of sclerosing cholangitis and in bile duct ligated mice increased liver injury, aggravated bile infarcts and induced hepatocyte necroses (Fickert *et al.*, 2002). Increased liver injury is caused by increased biliary pressure due to UDCA's choleretic activity leading to rupture of cholangioles (Fickert *et al.*, 2002). Moreover, the central role for FXR in worsening cholestatic injury in obstructive cholestasis was confirmed in bile duct-ligated and UDCA-fed FXR-deficient mice, which are protected from cholestasis and lack the development of bile infarcts (Wagner *et al.*, 2003; Stedman *et al.*, 2006). Serum bile acid levels in bile duct-ligated mice lacking FXR were even lower, and urinary bile acid output was increased, indicating enhanced adaptation to cholestasis in FXR-deficient mice (Marschall *et al.*, 2006). Some of UDCA's effects can be attributed to activation of FXR because UDCA is a weak FXR ligand (Lew *et al.*, 2004). UDCA may also exert FXR antagonistic properties by changing the bile acid pool composition and reducing the relative amounts of stronger FXR ligands like CDCA and CA. However, most of UDCA's negative effects in biliary obstruction are probably due to inducing choleresis and not to activation or inactivation of FXR.

Taken together, FXR agonists should be used with caution in human cholestasis with an obstructive component or with bile duct loss (e.g PSC, late stage PBC). Whether these substances are of benefit when initiated early in the course of vanishing bile duct syndromes, is currently tested in ongoing clinical studies. As such, a clinical phase II study is addressing the effects of 6-ECDCA in PBC patients, but results are still pending (Table 2). Moreover, future studies will have to differentiate between potential direct hepatic versus indirect intestinal (e.g. FGF-mediated) therapeutic effects of FXR agonists.

PXR (*NR1I2*) and CAR (*NR1I3*)

PXR and CAR are master regulators of phases I and II detoxification and regulate numerous hepatic genes in response to a large group of xenobiotics and endobiotics (Fig. 3, Table 1). These two receptors share some common ligands and regulate an overlapping set of target genes. CAR and PXR form heterodimers with RXR. PXR and CAR reside in the cytoplasm and are translocated to the nucleus after ligand binding (Goodwin and Moore, 2004; Squires *et al.*, 2004; Moreau *et al.*, 2008). PXR is activated by a broad range of xenobiotics but also by LCA (Staudinger *et al.*, 2001b; Xie *et al.*, 2001; Tirona and Kim, 2005). CAR is activated by xenobiotics but has also been shown to be activated by bilirubin, and a role of CAR for sensing bile acids has been suggested (Guo *et al.*, 2003; Huang *et al.*, 2003b; Xie *et al.*, 2004; Tirona and Kim, 2005).

Bile acid detoxification

Phase I hydroxylation of endobiotic and xenobiotic is largely mediated by CYP3A4. Both PXR and CAR are key regulators of CYP3A4 expression in hepatocytes and ligands for these receptors including xenobiotics, drugs but also bile acids can induce CYP3A4 expression (Bertilsson *et al.*, 1998; Lehmann

et al., 1998; Staudinger *et al.*, 2001a,b; Xie *et al.*, 2001; Goodwin *et al.*, 2002a,b). The importance of PXR in defence to toxic bile acids is underlined in LCA-fed PXR-deficient mice. While Cyp3a11 is induced after LCA feeding in wild type mice, Cyp3a11 induction is absent in PXR knockout mice leading to increased liver injury (Staudinger *et al.*, 2001b; Xie *et al.*, 2001). The human CAR response elements also mediates transactivation of CYP3A4 by human PXR, suggesting that interplay between these receptors is likely to be an important determinant of CYP3A4 expression (Goodwin *et al.*, 2002b).

Phase II bile acid sulphation via SULT2A1/Sult2a1 is regulated by numerous NRs including PXR, CAR, FXR and vitamin D receptor (VDR) (Runge-Morris *et al.*, 1999; Song *et al.*, 2001; Sonoda *et al.*, 2002; Assem *et al.*, 2004; Echchgadda *et al.*, 2004; Saini *et al.*, 2004; Echchgadda *et al.*, 2007). PXR, CAR and FXR bind to the same IR-0 element within the rodent *Sult2a1* gene promoter (Runge-Morris *et al.*, 1999; Song *et al.*, 2001; Sonoda *et al.*, 2002; Assem *et al.*, 2004; Saini *et al.*, 2004). CAR appears to be the central regulator of bile acid sulphation because CAR transgenic mice are resistant against LCA toxicity due to increased LCA sulphation (Saini *et al.*, 2004). Furthermore, CAR is required to up-regulate basolateral Mrp4, which is able to transport steroid sulphate conjugates (Assem *et al.*, 2004). Thus, CAR coordinates an integrated pathway mediating bile acid sulphation and subsequent basolateral export. Besides hydroxylation and sulphation, PXR and CAR also control glutathione S-transferases and UGTs (Falkner *et al.*, 2001; Huang *et al.*, 2003b; Xie *et al.*, 2003; Gong *et al.*, 2006).

Bile acid synthesis

In vitro studies suggested that PXR also inhibits human *CYP7A1* gene transcription by reducing interaction of peroxisome PGC-1α with HNF4α leading to inhibition of human *CYP7A1* gene transcription (Li and Chiang, 2005). However, *in vivo* administration of the PXR ligand rifampicin did not significantly reduce *CYP7A1* expression or bile acid synthesis in humans (Lutjohann *et al.*, 2004; Marschall *et al.*, 2005) questioning the physiologic significance of PXR-mediated repression.

Bile acid transport

Both CAR and PXR not only coordinate detoxification enzymes but also regulate transport of products of phases I and II detoxification. CAR and PXR share the same response element in rat *Mrp2* promoter together with FXR (Kast *et al.*, 2002), and ligands for these receptors induce MRP2/Mrp2 expression (Courtois *et al.*, 1999; 2002; Cherrington *et al.*, 2002; Kast *et al.*, 2002; Guo *et al.*, 2003; Marschall *et al.*, 2005; Teng and Piquette-Miller, 2005; Wagner *et al.*, 2005). Both NRs also positively regulate basolateral MRP3/Mrp3 expression while only CAR but not PXR ligands induce MRP4/Mrp4 (Cherrington *et al.*, 2002; Guo *et al.*, 2003; Teng *et al.*, 2003; Assem *et al.*, 2004; Zhang *et al.*, 2004a; Maher *et al.*, 2005; Wagner *et al.*, 2005). Taken together, both PXR and CAR play a central role in regulating the elimination of phases I and II detoxification products from hepatocytes.

Increased bile acid toxicity in PXR and CAR knockout models

The importance of both PXR and CAR in the defence against cholestasis is underlined by multiple studies. Mice lacking PXR develop increased liver injury after LCA feeding due to absent Cyp3a11 induction (Staudinger *et al.*, 2001b; Xie *et al.*, 2001). In contrast, a recent paper reported reduced liver injury in mice lacking PXR fed CA (Teng and Piquette-Miller, 2007). The authors attributed these controversial results to higher basal expression levels of Cyp3a11, Ostα/Ostβ, Mrp2 and Mrp3 in PXR knockout animals. These data are also in line with increased bilirubin clearance due to increased expression of bilirubin-detoxifying enzymes and transporters in PXR knockout mice (Saini *et al.*, 2005). The increased expression of CAR target genes in these studies may be explained by de-repression of the constitutive activity of CAR in the absence PXR (Saini *et al.*, 2005). CAR knockout and CAR/PXR double knockout animals showed similar sensitivity to bilirubin challenge as wild-type mice (Saini *et al.*, 2005). In addition, the combined loss of PXR and CAR results in increased sensitivity to LCA-induced liver injury when compared with loss of PXR or CAR alone (Uppal *et al.*, 2005). Similar findings were observed in FXR/PXR double knockout mice displaying more severe toxicity in response to CA feeding than mice lacking FXR or PXR alone (Guo *et al.*, 2003). These data indicate, that PXR, CAR but also FXR protect against hepatic bile acid-induced toxicity in a complementary manner regulating redundant but distinct defence pathways.

PXR and fibrosis

In addition to FXR, PXR also seems to play a role in modulating liver fibrosis. The PXR ligand PCN inhibited HSC transdifferentiation to a pro-fibrogenic phenotype in a non-cholestatic model of liver fibrosis in rats (Marek *et al.*, 2005). In addition, rifampicin inhibited the expression of various fibrosis- and proliferation-related genes in human HSCs and reduced HSC proliferation and transdifferentiation in a PXR-dependent manner (Haughton *et al.*, 2006). One explanation of PXR's inhibitory effects on liver fibrosis could be PXR-dependent inhibition of NF-κB leading to reduced inflammation (Axon *et al.*, 2008). However, the exact molecular mechanisms remain to be determined. Whether these findings also can be extended to therapy of fibrosis and cirrhosis caused by long-lasting cholestasis is currently unknown.

Therapeutic targeting of PXR and CAR

Because of their central role in bile acid detoxification and transport, PXR and CAR represent attractive targets for drug therapy of cholestasis (Table 1). Ligands for both receptors have already been used to treat cholestasis even long before their mode of action has been explored. Rifampicin is a ligand for PXR and is effectively used to treat pruritus of cholestasis but also ameliorates elevated liver function tests (Bachs *et al.*, 1989; Cancado *et al.*, 1998; Yerushalmi *et al.*, 1999). The CAR agonist phenobarbital not only improves pruritus but also reduces serum bile acid concentrations in cholestasis (Stiehl *et al.*, 1972; Bloomer and Boyer, 1975; Bachs *et al.*, 1989). However, both drugs can cause significant side effects ranging

from fatigue and somnolence (phenobarbital) to hepatoxicity and liver failure (rifampicin) (Bachs *et al.*, 1992; Prince *et al.*, 2002). NR ligands have also been used in traditional Chinese medicine for centuries. For example, Yin Zhin Huang and a number of other herbal decoctions containing Yin Chin have been used in Asia to prevent and treat neonatal jaundice. Yin Chin has been identified as a CAR ligand and accelerates bilirubin clearance *in vivo* (Huang *et al.*, 2004). The underlying molecular mechanisms of the beneficial effects of these PXR and CAR ligands have been elucidated in various animal models over the last years. In a rodent model, activation of PXR counteracted LCA-induced liver toxicity by induction of Cyp3a11, Sult2a1 and 3′-phosphoadenosine 5′-phosphosulfate synthase 2 (PAPSS2), an enzyme that generates the sulphate donor for the sulphation reaction (Staudinger *et al.*, 2001b; Xie *et al.*, 2001; Saini *et al.*, 2004). PXR activation also induced bilirubin detoxification and clearance via induction of its glucuronidation and export (Kast *et al.*, 2002; Chen *et al.*, 2003a; Ellis *et al.*, 2006). Administration of PXR ligands reduced liver injury, bilirubin and bile acid levels in CA-fed mice via induction of Cyp3a11 and Mrp3 (Teng and Piquette-Miller, 2007). In obstructive cholestasis in mice, administration of PXR and CAR ligands reduced serum parameters of cholestasis (i.e. bilirubin and serum bile acid levels) by induction of phases I and II detoxification and transport systems (Wagner *et al.*, 2005). However, increased transaminases in these animals indicate potential hepatotoxic side effects of the used substances at least under conditions when bile flow is completely blocked (Wagner *et al.*, 2005). Stimulation of PXR and CAR may be therapeutically superior to activation of FXR in obstructive cholestasis because this does not increase bile flow. However, these substances should be used with care because of potential hepatotoxicty when biliary elimination is hampered and the risk of promoting hepatic cancerogenesis via continuous stimulation of CAR (Yamamoto *et al.*, 2004; Huang *et al.*, 2005). Novel compounds targeting PXR and CAR with fewer side effects are needed for the treatment of cholestasis. Whether herbals from traditional Chinese medicine in analogy to Yin Chin may contribute to the armentarium of CAR and PXR agonists needs further exploration.

VDR (*NR1I1*)

The VDR is a member of the superfamily of steroid hormone receptors and regulates calcium homeostasis, cell proliferation and differentiation, and exerts immunomodulatory as well as antimicrobial functions (Campbell and Adorini, 2006). VDR binds to and mediates the calcemic effects of calcitriol (1α,25-dihydroxyvitamin D3) after forming an heterodimer with RXR. 1α,25-dihydroxyvitamin D3 negatively regulates its own synthesis by repressing the 25-hydroxyvitamin D_3 1α-hydroxylase (*CYP27B1)* (Turunen *et al.*, 2007).

VDR has been demonstrated to be an intestinal receptor for LCA (Makishima *et al.*, 2002). Activation of VDR by vitamin D or LCA *in vitro* induces expression of CYP3A4, which can detoxify LCA via phase I hydroxylation (Makishima *et al.*, 2002). Expression of VDR however is high in intestine but low in liver (McCarthy *et al.*, 2005), where it is restricted to Kupffer cells, endothelial cells, biliary epithelial cells and HSCs (Gascon-Barre *et al.*, 2003). Despite low hepatic VDR expression, LCA, vitamin D and a synthetic VDR ligand were able to stimulate Cyp3a11 expression in mouse liver (Makishima *et al.*, 2002). These effects were also present in mice lacking PXR indicating a VDR-mediated stimulation of *Cyp3a11*. *SULT2A1/Sult2a1* is another target for VDR (Echchgadda *et al.*, 2004). Vitamin D stimulates SULT2A1/Sult2a1 expression in HepG2 cells *in vitro* as well as *in vivo* in mice (Chatterjee *et al.*, 2005), indicating that even low VDR expression in hepatocytes may be sufficient to up-regulate Sult2a1. *Mrp3* also harbours a VDR response element in its promoter region and is transactivated upon calcitriol and LCA treatment (McCarthy *et al.*, 2005). However, Mrp3 induction was only present in colon but not in liver (McCarthy *et al.*, 2005). The ileal bile acid uptake system apical sodium-dependent bile acid transporter (*ASBT*) is another target of VDR, and calcitriol increases *ASBT* mRNA and promoter activity (Chen *et al.*, 2006). Moreover, VDR seems to play an indirect role in bile acid homeostasis because VDR negatively interacts with FXR and calcitriol inhibits FXR transactivation *in vitro* (Honjo *et al.*, 2006).

Thus VDR is an important regulator of bile acid transport and metabolism in the intestine due to its high expression in enterocytes but also plays an important role in hepatic phases I and II detoxification reactions (Fig. 3, Table 1). The use of vitamin D or synthetic VDR agonist represents an attractive therapeutic option to treat cholestatic liver diseases and should be investigated in future studies. However, the rather complex role of VDR in regulation of bile acid uptake in intestine and regulation of bile acid metabolism in liver as well as its negative effects on FXR makes the outcome of such studies rather unpredictable.

Peroxisome proliferator-activated receptors (PPARs)

PPARs are ligand-activated NRs that heterodimerize with RXR and bind to DR-1 response elements upon activation (Willson *et al.*, 2000; Brown and Plutzky, 2007). PPARα, β, γ are dietary lipid sensors, which control lipid homeostasis and cellular differentiation from adipocytes. As such, almost all occurring natural fatty acids and eicosanoids are natural ligands for PPARs. PPARα (*NR1C1*) is highly expressed in heart, liver, kidney and brown fat, tissues with a high rate of β-oxidation of fatty acids, while PPARγ (*NR1C3*) is mainly expressed in white adipose tissue (Brown and Plutzky, 2007). PPARs regulate the expression of various genes crucial for lipid, glucose, bile acid and drug metabolism (Kota *et al.*, 2005; Nakata *et al.*, 2006).

PPARα (NR1C1)

PPARα is involved in the regulation of bile acid metabolism indicated by the presence of PPAR response elements in the *SULT2A1* and *UGT2B4* gene promoters. These genes are activated by lipid-lowering fibrates, which are PPARα activators (Willson *et al.*, 2000; Barbier *et al.*, 2003a; Fang *et al.*, 2005).

In addition, there is crosstalk between the PPARα and FXR transcriptional pathways because *PPARα* is an FXR target gene harbouring an FXR response element in its gene promoter (Pineda *et al.*, 2003). For example, *UGT2B4* expression can be directly induced via activation of PPARα and indirectly via FXR-dependent induction of PPARα, which then activates *UGT2B4* transcription. Treatment of human hepatocytes with fibrates as classic PPARα ligands induced expression and activity of UGT1A3, which is responsible for the glucuronidation of CDCA (Trottier *et al.*, 2006). PPARα also negatively affects bile acid synthesis by repressing *CYP7A1* by reducing HNF4α binding to the DR-1 response element in the *CYP7A1* promoter (Marrapodi and Chiang, 2000; Patel *et al.*, 2000; Post *et al.*, 2001; Rudling *et al.*, 2002; Roglans *et al.*, 2004).

PPARα is not only involved in regulation of bile acid synthesis and detoxification but also modulates biliary phospholipid secretion. Phospholipids protect the bile duct epithelium from detergent bile acids by formation of mixed micelles. Fibrates and other PPARα activators directly induce expression of Mdr2 in the canalicular membrane thereby inducing biliary phospholipid output (Chianale *et al.*, 1996; Miranda *et al.*, 1997; Kok *et al.*, 2003; Shoda *et al.*, 2004). PPARα induces ASBT/Asbt expression in liver (cholangiocytes) and intestine (Jung *et al.*, 2002) resulting in increased bile acid absorption from the intestine and bile ducts. Reabsorption of bile acids from obstructed bile ducts might minimize cholangiocyte damage.

The effects of PPARα on biliary phospholipid secretion, bile acid metabolism and synthesis make stimulation of PPARα an interesting therapeutic approach in the treatment of cholestasis (Table 1). Especially increased phospholipid secretion into bile may reduce the aggressiveness of bile thus protecting cholangiocytes. Fenofibrate administration to bile duct-ligated rats moderately reduced serum markers of cholestasis and histological parameters liver injury. However, these effects were only moderate (Cindoruk *et al.*, 2007). Clinical trials by using fibrates showed beneficial effects on biochemical parameters and in part also on histological findings in patients with PBC (Kurihara *et al.*, 2000; Nakai *et al.*, 2000; Ohmoto *et al.*, 2001; Kurihara *et al.*, 2002; Yano *et al.*, 2002; Kanda *et al.*, 2003). However, these studies were pilot studies including only a small number of patients and where not randomized controlled trials. Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase ('statins') are PPARα activators and stimulate phospholipid secretion by induction of Mdr2 (Carrella *et al.*, 1999; Hooiveld *et al.*, 1999; Landrier *et al.*, 2004) Statins have also been tested in the treatment of PBC. While initial mostly anecdotal reports suggested improvement of cholestasis under statin treatment (Kurihara *et al.*, 1993; Kamisako and Adachi, 1995; Ritzel *et al.*, 2002), a recent dose finding study was unable to demonstrate improvement of cholestasis in PBC patients with an incomplete prior response to UDCA (Stojakovic *et al.*, 2007). Whether long-term application of PPARα ligands improves cholestasis and disease outcome in a larger cohort of patients remains to be demonstrated. Currently, a phase II study is under way investigating the effects of fenofibrate in PBC patients with incomplete response to UDCA (Table 2). However, results of this study will not be available before 2010.

PPARγ (NR1C3)

PPARγ is therapeutically targeted by thiazolidinediones (glitazones) and is a key regulator of adipogenesis and insulin sensitivity. The transcriptional coactivator PGC-1α orchestrates PPARγ effects thus playing a critical role in the regulation of mitochondrial functional capacity and cellular energy metabolism. Of note, PGC-1α also targets many other NRs including PPARα, PPARβ, thyroid hormone receptor, retinoid receptors, GR, oestrogen receptor, FXR, PXR, HNF4α, liver X receptor (LXR) and the oestrogen-related receptors (Finck and Kelly, 2006). While PPARγ induces expression of the cholesterol transporters ABCA1 and ABCG1 and ABCG2 (a protective pump against toxic agents) (Takeda *et al.*, 2000; Szatmari *et al.*, 2006), a direct role for PPARγ in the regulation of bile acid homeostasis has not yet been reported. A PPARγ/RXRα heterodimer has been shown to bind to a PPAR response element in the *SHP* promoter and rosiglitazone increased SHP expression in primary rat hepatocytes (Kim *et al.*, 2007a). Whether PPARγ ligands affect bile acid homeostasis via this pathway *in vivo* remains to be determined. However, PPARγ agonists might be of use in inflammatory cholestasis. Pretreatment of lipopolysaccharide (LPS)-injected mice with rosiglitazone attenuated repression of Ntcp, Bsep and Cyp3a11 without affecting cytokine levels (Ghose *et al.*, 2007). These anti-cholestatic effects were attributed to prevention of the nuclear export of RXRα caused by LPS (Ghose *et al.*, 2004; Ghose *et al.*, 2007). In addition, PPARγ represses transcriptional activation of inflammatory response genes in mouse macrophages by a complex mechanism involving SUMOylation of the PPARγ ligand-binding domain. This prevents recruitment of the ubiquitination/19S proteosome machinery that normally mediates the signal-dependent removal of corepressor complexes required for gene activation. As a result, NCoR complexes are not cleared from the promoter and proinflammatory target genes are maintained in a repressed state (Pascual *et al.*, 2005). Moreover, PPARγ agonists inhibit HSC activation and counteract liver fibrosis in models of cholestasis (Dubuquoy *et al.*, 2002; Galli *et al.*, 2002; Fiorucci *et al.*, 2005b). The crosstalk between FXR and PPARγ is described above.

Safety issues are an important concern when using glitazones. Troglitazone was the first glitazone on the market but was withdrawn later because of hepatotoxic side effects (Lee, 2003). Second generation glitazones like rosiglitazone and pioglitazone are rarely associated with liver injury; however, the manufacturers do not recommend the use of these substances in patients with liver disease. These drugs show beneficial effects in non-alcoholic fatty liver disease and do not seem to lead to liver injury in patients with fatty liver and increased baseline liver function tests (Belfort *et al.*, 2006; Caldwell *et al.*, 2006). However, the use of these drugs in cholestasis could have hepatotoxic side effects. Glitazones have been demonstrated to inhibit Na^+- and ATP-dependent bile acid transport in a dose dependent manner (Funk *et al.*, 2001; Snow and Moseley, 2007). This was evident for rosiglitazone, ciglitazone and troglitazone indicating a class effect (Snow and Moseley, 2007). Beneficial effects of troglitazone observed in a rat model of obstructive cholestasis on cholangiocellular proliferation and fibrosis have not yet been extended to other models (Marra *et al.*, 2005). Taken together,

glitazones should be tested with great care in patients with cholestasis.

LXRα (NR1H3) and LXRβ (NR1H2)

The LXR subfamily consists of LXRα and LXRβ. LXRα is mainly expressed in liver, adipose tissue, intestine, kidney and macrophages whereas LXRβ is ubiquitously expressed (Lu *et al.*, 2001). LXRα and LXRβ are activated by naturally occurring oxysterols, certain unsaturated fatty acids and 6α-hydroxylated bile acids (Song *et al.*, 2000; Lu *et al.*, 2001; Ou *et al.*, 2001). Both LXRs heterodimerize with RXR and preferentially bind to DR-4 DNA response elements.

LXRα acts as a cholesterol sensor and regulates cholesterol and lipid homeostasis. In cholesterol-enriched diet-fed rodents, the expression of *Cyp7a1* is induced via LXRα, which is activated by oxysterol metabolites of cholesterol (Janowski *et al.*, 1996; Lehmann *et al.*, 1997). In contrast to rodent *Cyp7a1*, human *CYP7A1* is repressed upon activation of LXRα (Chiang *et al.*, 2001; Goodwin *et al.*, 2003), which was attributed to induction of LXRα-activated SHP (Goodwin *et al.*, 2003). LXRα also regulates various genes involved in lipid metabolism including ABCA1, ABCG1, ABCG4, ABCG5, and ABCG8, apolipoprotein E, cholesterol ester transport protein, lipoprotein lipase, fatty acid synthase, and the sterol-regulatory element-binding protein 1 (SREBP-1), a key transcription factor for regulation of hepatic lipogenesis (Tall *et al.*, 2000; Edwards *et al.*, 2002; Laffitte and Tontonoz, 2002; Repa *et al.*, 2002). In addition, LXRα modulates immune and inflammatory responses in macrophages (Zelcer and Tontonoz, 2006). LXR activation inhibits LPS-mediated induction of various proinflammatory cytokines (Joseph *et al.*, 2003). This mechanism has been linked to SUMOylation-dependent pathways. Ligand-dependent conjugation of SUMO2/3 to LXR prevents the signal-dependent removal of the corepressor NCoR from proinflammatory genes leading to transrepression of inflammation (Ghisletti *et al.*, 2007).

A role for LXRα in reducing cholestatic liver injury has recently been demonstrated. In this study, LXRα-transgenic mice and mice treated with a synthetic LXRα agonist were resistant to liver damage induced by LCA feeding and bile duct ligation (Uppal *et al.*, 2007). Moreover, LXR knockout animals displayed severe liver injury after bile duct ligation. The beneficial effects of LXRα stimulation were attributed to increased expression of sulfotransferase Sult2a9 and Mrp4 leading to increased urinary bile acid elimination (Uppal *et al.*, 2007). Interestingly, the findings regarding protection by Sult2a9 induction via LXR were restricted to female mice, and the protective effects of LXR were absent in male mice exposed to cholestatic injury. The underlying mechanism still remains unresolved but may be linked to sex hormone-dependent regulation of detoxifying enzymes (Uppal *et al.*, 2007). In addition, the bile acid-glucuronidating enzyme *UGT1A3* has also been identified as a LXRα target with a LXR response element in its gene promoter (Verreault *et al.*, 2006). Thus, LXRα is not only an attractive target for intervention in metabolic disorders but also for the treatment of cholestasis.

GR (*NR3C1*)

The GR is ubiquitously expressed in the body and regulates numerous functions including repression of transcriptional responses to inflammatory signals. The natural ligands for GR are glucocorticoids but also UDCA was reported to activate GR (Tanaka and Makino, 1992; Miura *et al.*, 2001). GR transactivates human *NTCP* (Eloranta *et al.*, 2006) and *ASBT* (Jung *et al.*, 2004a). GR also appears to modulate anion exchanger AE2 expression (Alvaro *et al.*, 2002; Arenas *et al.*, 2008). Interestingly, the combination of UDCA and the GR ligand dexamethasone but not UDCA or dexamethasone alone increased AE2 expression and function via interaction of HNF1 and GR on the *AE2* alternate promoter (Arenas *et al.*, 2008). These findings might explain the beneficial effects of the combination of glucocorticoids and UDCA in patients with PBC, because AE2 expression is reduced in PBC (Prieto *et al.*, 1993). A role for glucocorticoids has been suggested for the regulation of Bsep in rat hepatocytes *in vitro* (Warskulat *et al.*, 1999) but this has been questioned by other studies (Gerloff *et al.*, 2002; Cheng *et al.*, 2007). A GR response element has so far not been identified in the *Bsep* promoter. Rodent Mrp2 is stimulated by dexamethasone (Courtois *et al.*, 1999; Kubitz *et al.*, 1999; Turncliff *et al.*, 2004), while human MRP2 is unaffected after dexamethasone treatment (Pulaski *et al.*, 2005; Nishimura *et al.*, 2006). One has to be aware that effects of glucocorticoids may not only be direct effects of GR on target genes but may also be modulated indirectly by other NRs. As such, CAR has been identified as a primary GR response gene with a glucocorticoid responsive element in its promoter region. In addition, glucocorticoids increase the levels of PXR and RXRα mRNA and protein (Pascussi *et al.*, 2000; 2003). In addition to its transcriptional induction, dexamethasone also increases translocation of CAR protein into the nucleus (Pascussi *et al.*, 2000). Taken together, GR modulates and potentiates action of PXR and CAR target genes, but also directly regulates expression of various genes involved in bile acid transport and detoxification.

Glucocorticoids have been used in the treatment of various cholestatic disorders. Beneficial effects of glucocorticoids (i.e. prednisone, budesonide) on serum parameters of cholestasis and liver histology have been noted especially when added to the standard treatment with UDCA (Mitchison *et al.*, 1992; Leuschner *et al.*, 1996; 1999; Rautiainen *et al.*, 2005). Whether these effects are only the consequence of the anti-inflammatory properties or whether they can in part be attributed to modulation of bile acid transport and metabolism remains elusive.

LRH-1 (*NR5A1*)

LRH-1 is expressed in tissues derived from endoderm, including intestine, liver and exocrine pancreas, as well as in the ovary. In these tissues, LRH-1 plays a predominant role in development, reverse cholesterol transport, steroidogenesis and bile acid homeostasis (Fayard *et al.*, 2004). For a long time, LRH-1 was considered to be an orphan NR, but recently, phospholipids were shown to bind human LRH-1 (Krylova *et al.*, 2005; Ortlund *et al.*, 2005; Wang *et al.*, 2005).

LRH-1-binding sites have been identified in the promoters of *CYP7A1* and *CYP8B1* (Nitta *et al.*, 1999; Castillo-Olivares and Gil, 2000). LRH-1 has also been implicated in repression of these enzymes via a FXR/SHP-dependent mechanisms (Goodwin *et al.*, 2000; Lu *et al.*, 2000). However, this was questioned by a recent study demonstrating preserved FXR-mediated repression of Cyp7a1 and Cyp8b1 in liver-specific LRH-1 knockout mice (Lee *et al.*, 2008). The repressive effects of FXR on *Cyp7a1* in this study were attributed to SHP-mediated repression of HNF4α, which also binds to the same response element in the *Cyp7a1* promoter (Lee *et al.*, 2000; De Fabiani *et al.*, 2001). While basal expression of Cyp7a1 was unaffected in LRH-1 deficient mice, Cyp8b1 expression was markedly reduced (Mataki *et al.*, 2007; Lee *et al.*, 2008). Bile acid pool composition in these animals changed drastically towards a more hydrophilic pool with low levels of CA but higher levels of muricholic acid and UDCA, while levels of CDCA and DCA acid remained unchanged (Mataki *et al.*, 2007). Loss of LRH-1 had also dramatic effects on expression of hepatobiliary transport systems and NRs. Expression of Ntcp, Bsep, Mrp3, Mrp2, Mdr2, FXR and SHP was markedly reduced in these animals (Mataki *et al.*, 2007; Lee *et al.*, 2008). Some of these findings can be attributed to direct LRH-1-mediated target gene activation as described for MRP3/Mrp3 (Inokuchi *et al.*, 2001; Bohan *et al.*, 2003), Bsep (Song *et al.*, 2008), Asbt (Chen *et al.*, 2003b), Ostα/Ostβ (Frankenberg *et al.*, 2006), FXR and SHP (Oiwa *et al.*, 2007). Decreased expression of FXR may also contribute to low expression of its target genes (i.e. Bsep, Mdr2, Mrp2 and SHP) (Goodwin *et al.*, 2000; Lu *et al.*, 2000; Ananthanarayanan *et al.*, 2001; Gerloff *et al.*, 2002; Plass *et al.*, 2002; Moschetta *et al.*, 2004). Two recent studies in LRH-1-deficient mice indicate a central role for this receptor in the regulation of bile acid homeostasis (Mataki *et al.*, 2007; Lee *et al.*, 2008). Whether LRH-1 knockout mice are more susceptible (due to reduced transporter expression) or on the contrary even protected from cholestatic injury (due to a more hydrophilic bile acid pool) remains to be investigated. Therapeutic targeting of LRH-1 has not been tested so far but would be expected to lead to deregulation of a large number of genes involved in bile acid metabolism, and the subsequent effects are hardly predictable.

HNF4α (*NR2A1*)

HNF4α is a highly conserved member of the NR superfamily and is expressed at highest levels in liver, intestine, kidney and pancreas (Miquerol *et al.*, 1994; Sladek, 1994). HNF4α has an essential role in development, oncogenesis and maintenance of organ function (Odom *et al.*, 2004). HNF4α functions as a homodimer and can activate gene transcription in the absence of exogenous ligands (Sladek *et al.*, 1990; Ladias *et al.*, 1992). Fatty acids may be ligands for HNF4α (Dhe-Paganon *et al.*, 2002; Wisely *et al.*, 2002), and fatty acyl-coenzyme A (CoA) thioesters may modulate HNF4α-binding activity (Hertz *et al.*, 1998), suggesting an important role in the control of metabolic status. Furthermore, mutations in the HNF4α gene cause maturity onset diabetes of the young (MODY1), a rare form of non-insulin-dependent diabetes mellitus inherited in an autosomal dominant pattern and characterized by defective secretion of insulin (Yamagata *et al.*, 1996; Dhe-Paganon *et al.*, 2002; Wisely *et al.*, 2002). A number of CYP genes including *CYP3A4/Cyp3* harbour putative HNF4α-binding sites in their promoter and enhancer sequences and HNF4α positively regulates their gene expression (Huss and Kasper, 1998; Ogino *et al.*, 1999; Tirona *et al.*, 2003; Matsumura *et al.*, 2004). Furthermore, HNF4α regulates the basal and CAR-/PXR-induced expression of human *SULT2A1* (Echchgadda *et al.*, 2007). HNF4α is also an important regulator of bile acid synthesis. *CYP7A1*, *CYP27A1* and *CYP8B1* harbour HNF4α–binding sites in their gene promoters (Zhang and Chiang, 2001; Yang *et al.*, 2002; Chen and Chiang, 2003). HNF4α may be the key target of FXR-activated SHP leading to suppression of Cyp7a1 and Cyp8b1 expression (Mataki *et al.*, 2007; Lee *et al.*, 2008).

In addition to direct target gene regulation, HNF4α may also indirectly act via activation of other NRs. An HNF4α-binding site was characterized in the *PXR* promoter thereby regulating responses to xenobiotics through activation of the *PXR* gene during fetal liver development (Li *et al.*, 2000; Kamiya *et al.*, 2003). The human *CAR* promoter is regulated by HNF4α (Ding *et al.*, 2006), and expression of CAR is reduced in mice lacking HNF4α (Tirona *et al.*, 2003). Moreover, FXR and PPARα gene transcription is activated by HNF4α (Lu *et al.*, 2000; Pineda Torra *et al.*, 2002; Zhang *et al.*, 2004b). HNF4α is also a critical regulator of the liver-enriched transcription factor HNF1α (Tian and Schibler, 1991; Miura and Tanaka, 1993; Wang *et al.*, 2001; Jung and Kullak-Ublick, 2003), which itself plays a key role in the regulation of bile acid transport and metabolism (Shih *et al.*, 2001; Arrese and Karpen, 2002).

Taken together, these data indicate that HNF4α is a major regulator of genes involved in the control of bile acid homeostasis. HNF4α controls target genes either directly or indirectly via interactions with other NRs and transcription factors. Targeting HNF4α in cholestasis has not yet been tested, but many side effects are to be expected due to its central role in regulation of organ development function and the metabolism.

Conclusions and outlook

NRs are critically involved in regulation of bile formation and bile acid homeostasis under physiological and pathological conditions. Various compounds accumulate as a result of bile secretory failure in cholestasis and induce a complex machinery of defence pathways involving bile acid detoxification, synthesis and transport via activation of NRs. However, these intrinsic adaptive mechanisms do not suffice to overcome cholestatic liver injury damage. Therefore, additional stimulation of these defence pathways represents an attractive target of drug therapy (Fig. 3). Some NR activators (e.g. phenobarbital, rifampicin) are already successfully used in the treatment of cholestasis on an empiric basis and have been introduced as therapeutics long before their mode of action was identified. However, these drugs are sometimes associated with substantial side effects including severe liver injury. In addition, NR ligands stimulating bile flow may cause hepatotoxicity when bile duct obstruction or substantial bile duct loss is present. The increasing knowledge on the pathomechanisms of cholestasis and the action of NRs in health and

disease made the use NR ligands possible while keeping their potential side effects minimal. Clinical trials have already investigated or currently investigate the effects of various NR ligands (e.g. FXR, PPARα and GR ligands) in human cholestatic disorders. Some clinical results have been disappointing and have not fulfilled the expectations that were raised based on animal experimental findings. However, initiation of such studies would not haven been possible without the increasing knowledge on NR function in cholestasis derived from *in vitro* experiments, animal models of cholestasis as well as from human liver disease. Further basic research in the field will probably identify other, more potent substances with a lower rate of side effects, which hopefully can be applied to human cholestatic diseases.

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Conflicts of interest

None.

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