**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.


**Keywords:** nuclear receptors; cholestasis; bile acids; bilirubin; statins; fibrates; glitazones; primary biliary cirrhosis; primary sclerosing cholangitis; obstructive cholestasis; fibrosis

**Abbreviations:** ASBT/Asbt (*SLC10A2/SIc10a2*), 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; MRP1/Mrp (*ABCC/Abcc3*), multidrug resistance-associated protein; NCoR, nuclear receptor corepressor; NR, nuclear receptor; NTCP/Ntcp (*SLC10A1/SIc10a1*), Na+/taurocholate cotransporter; OATP/Oatp (*SLCO/Sico*), organic anion transporting peptide; OSTα/OSTβ (OSTα/Ostβ or OSTβ/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

**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-

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**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 S J 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 coordinate 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 (Pelliciari 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 hydroylase (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...
et al. (1991) have shown that 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), while divalent BAs and anionic anions (OAs) 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 OST\(\beta\)/OST\(\alpha\) provide an alternative excretion route for BA\(^-\) and other OAs into the systemic circulation. BA\(^-\) secreted into bile can be reabsorbed by cholangiocytes via apical Na\(^+\)-dependent bile salt transporter (ASBT) and effluxed by Ost\(\alpha\)/OST\(\beta\) and Mrp3 (not shown). Cholangiocytes also express a chloride channel that is the cystic fibrosis transmembrane regulator (CFTR) that drives bicarbonate secretion via a chloride/anion exchanger (AE2). Adapted from Zollner and Trauner, *Wien Med Wochenschr* 2006 (Zollner and Trauner, 2006).

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), while divalent BAs and anionic anions (OAs) 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 OST\(\beta\)/OST\(\alpha\) provide an alternative excretion route for BA\(^-\) and other OAs into the systemic circulation. BA\(^-\) secreted into bile can be reabsorbed by cholangiocytes via apical Na\(^+\)-dependent bile salt transporter (ASBT) and effluxed by Ost\(\alpha\)/OST\(\beta\) and Mrp3 (not shown). Cholangiocytes also express a chloride channel that is the cystic fibrosis transmembrane regulator (CFTR) that drives bicarbonate secretion 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 glucuronate 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 OST\(\alpha\)/OST\(\beta\) (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 OST\(\alpha\)/OST\(\beta\) 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...
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 fashion 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 receptor) 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

<table>
<thead>
<tr>
<th>NR</th>
<th>Ligands</th>
<th>Therapeutic</th>
<th>Target Genes</th>
<th>Therapeutic Effects</th>
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<tbody>
<tr>
<td><strong>FXR (NR1H4)</strong></td>
<td>CDCA, DCA, CA, synthetic: GW4064, 6-ECDCA</td>
<td>Synthesis: Indirect repressive effects via SHP</td>
<td></td>
<td>Repression of bile acid synthesis indirectly via SHP</td>
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<td></td>
<td></td>
<td>Detoxification: CYP3A4, SULT2A1, UGT1B84, UGT2B7</td>
<td></td>
<td>Induction of bile acid detoxification</td>
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<td></td>
<td></td>
<td>Transport: MRP2/Mrp2, OATP1B3, OSTAβ1, SHP, BSEP, Bsep, I-BABP, MDR3/Mdr2</td>
<td></td>
<td>Induction of canalicular and alternative basolateral bile acid excretion</td>
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<td></td>
<td></td>
<td>Fibrosis: SHP, PPARγ</td>
<td></td>
<td>Increase in bile flow</td>
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<td></td>
<td></td>
<td></td>
<td>Fibrosis: SHP, PPARγ</td>
<td>Increase in biliary phospholipid content</td>
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<td></td>
<td></td>
<td></td>
<td>Fibrosis: SHP, PPARγ</td>
<td>Repression of bile acid uptake (effect may be restricted to rodents)</td>
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<tr>
<td><strong>SHP (NR0B2)</strong></td>
<td>No ligand, FXR target</td>
<td>Synthesis: CYP7A1, CYP8B1/Cyp8b1, CYP27A1</td>
<td></td>
<td>Repression of bile acid synthesis</td>
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<tr>
<td></td>
<td></td>
<td>Transport: Ntcp, ASBT/Asbt</td>
<td></td>
<td>Repression of hepatic and intestinal bile acid uptake</td>
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<td></td>
<td></td>
<td>Fibrosis: α1(I)collagen</td>
<td></td>
<td>Induction of quiescent HSC phenotype, increase in HSC apoptosis</td>
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<td><strong>PXR (NR1/2)</strong></td>
<td>Rifampicin in humans, phenobarbital, dexamethasone, statins, St. John’s wort, clotrimazole pregnenolone-16α-carbonitrile (PCN) in rodents</td>
<td>Synthesis: CYP7A1</td>
<td></td>
<td>Induction of phase I and II bile acid and bilirubin detoxification systems</td>
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<td></td>
<td></td>
<td>Detoxification: CYP3A4, SULT2A1/ Sult2A1, UGT1A1</td>
<td></td>
<td>Induction of orthograde canalicular and alternative basolateral bile acid excretion</td>
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<td></td>
<td></td>
<td>Transport: MRP2/Mrp2, MRP3, Oatp1α4, MDR1</td>
<td></td>
<td>Minor increase in bile flow</td>
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<tr>
<td><strong>CAR (NR1/3)</strong></td>
<td>Yin Chin, CITCO, bilirubin, Phenobarbital, 1,4-bis-[2-(3,5-ichlorpyidyloxy)] benzene (TCPOBOP), dimethoxycoumarin, dimethylesculetin</td>
<td>Synthesis: CYP7A1</td>
<td></td>
<td>Antifibrotic activities, inhibition of HSC transdifferentiation</td>
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<td></td>
<td></td>
<td>Detoxification: CYP3A4, SULT2A1/ Sult2A1, UGT1A1</td>
<td></td>
<td>Deregulation of bile acid synthesis and fibrosis pathways</td>
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<td></td>
<td></td>
<td>Transport: MRP2/Mrp2, Mrp3, MRP4/Mrp4</td>
<td></td>
<td>Required for constitutive expression of various basolateral uptake systems</td>
</tr>
<tr>
<td><strong>VDR (NR1/1)</strong></td>
<td>1α,25-dihydroxy-vitamin, D₃, LCA</td>
<td>Detoxification: CYP3A4/Cyp3a11, SULT2A1/Sult2A1</td>
<td></td>
<td>Induction of bile acid detoxification systems</td>
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<tr>
<td></td>
<td></td>
<td>Transport: MRP3, ASBT</td>
<td></td>
<td>Induction of alternative basolateral bile acid excretion and induction of of biliary and intestinal bile acid reabsorption</td>
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<tr>
<td><strong>HNF4α (NR2A1)</strong></td>
<td>Fatty acyl-coenzyme A thioesters</td>
<td>NRs: FXR</td>
<td>Synthesis: Cyp7a1, CYP8B1/Cyp8b1, CYP27A1</td>
<td>Reduced HNF4α binding or expression down-regulates bile acid synthesis and detoxification pathways</td>
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<td></td>
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<td>Detoxification: CYP3A4, Cyp3, SULT2A1/ Sult2A1, UGT1A1</td>
<td></td>
<td>Required for constitutive expression of various basolateral uptake systems</td>
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<td>Transport: ASBT, OATP1B1, Oatp1α1, Oatp1β2, Oatp1α4, Ntcp</td>
<td></td>
<td>Indirect regulation of PXR, CAR and HNF1α target genes</td>
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<tr>
<td><strong>LRH-1 (NR5A2)</strong></td>
<td>Phospholipids</td>
<td>NRs, Transcription factors: PXR, CAR, HNF1α</td>
<td>Synthesis: CYP7A1, CYP8B1/Cyp8b1, CYP27A1</td>
<td>Required for basal expression of bile acid synthesis enzymes, transporters and NRs</td>
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<tr>
<td></td>
<td></td>
<td>Transport: MRP3, Asbt, Ntcp, Bsep, Mdr2</td>
<td></td>
<td>Repression of bile acid synthesis</td>
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<tr>
<td><strong>PPARα (NR1C1)</strong></td>
<td>Fatty acids, fibrates, statins, eicosanoids, leukotriens, NSAIDs, WI-14643</td>
<td>NRs: FXR, SHP</td>
<td>Synthesis: CYP7A1</td>
<td>Repression of bile acid synthesis</td>
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<tr>
<td></td>
<td></td>
<td>Detoxification: SULT2A1, UGT2B4, UGT1A3</td>
<td></td>
<td>Induction of bile acid detoxification systems</td>
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<tr>
<td></td>
<td></td>
<td>Transport: Mdr2, ASBT</td>
<td></td>
<td>Protection of bile duct epithelium via increased phospholipid secretion and increased bile acid reabsorption</td>
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<tr>
<td><strong>PPARγ (NR1C3)</strong></td>
<td>Thiazolidinediones (glitazones)</td>
<td>NRs: FXR</td>
<td>Synthesis: SHP, α1(I)collagen</td>
<td>Indirect induction of FXR targets such as UGT2B4</td>
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<td></td>
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<td>Fibrosis: α1(I)collagen</td>
<td></td>
<td>Effects on SHP targets?</td>
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</table>

**Toxicology**

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 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 (FXR) 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 (Landrieu 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-ECDDA), 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 (PPARγ). 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.

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Table 1. Cont.

<table>
<thead>
<tr>
<th>NR</th>
<th>Ligands</th>
<th>Therapeutic</th>
<th>Target Genes</th>
<th>Therapeutic Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>LXRα(NR1H3)</td>
<td>Oxysterols, fatty acids, 6α-hydroxylated bile acids, TO1317</td>
<td>Synthesis: Cyp7a1/CYP7A1 Detoxification: Sul2a9, UGT1A3 Transport: Mrp4</td>
<td></td>
<td>Induction of rodent Cyp7a1, repression of human CYP7A1 Induction of bile acid sulfation and glucuronidation Induction of alternative basolateral bile acid excretion Contribution of effects on transporters to anti-inflammatory properties in treatment of inflammatory cholestasis (&quot;steroid whitewash&quot;) Potentiates action of PXR and CAR</td>
</tr>
<tr>
<td>GR(NR3CI)</td>
<td>Glucocorticoids, potentially UDCA</td>
<td>Transport: ASBT, NTCP, potentially AE2, Bsep, Mrp2</td>
<td></td>
<td><em>Please note that CAR can be activated either indirectly or directly by ligand binding (e.g. TCPOBOP (Goodwin et al. 2004).</em></td>
</tr>
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</table>

*NRs: CAR, PXR, RXRα*
and UDCA-fed FXR-deficient mice, which are protected from over, the central role for FXR in worsening cholestatic injury increased biliary pressure due to UDCA’s choleretic activity et al.

causative in oestrogen-induced cholestasis (Kullak-Ublick aggravated bile infarcts and induced hepatocyte necroses and cholestasis but rather are secondary events as a consequence of bile transporters like late stage PBC). In these diseases, alterations of 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-ECDA 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 canaliculare 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 cholelithiasis 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-ECDA 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.

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Nuclear receptor target</th>
<th>Cholestatic disorder</th>
<th>ClinicalTrials.gov weblink</th>
</tr>
</thead>
<tbody>
<tr>
<td>INT-747 (6-ECDA)</td>
<td>FXR</td>
<td>PBC/monotherapy</td>
<td><a href="http://clinicaltrials.gov/ct2/show/NCT00570765">http://clinicaltrials.gov/ct2/show/NCT00570765</a></td>
</tr>
<tr>
<td>INT-747 (6-ECDA)</td>
<td>FXR</td>
<td>PBC/combo combination therapy with UDCA</td>
<td><a href="http://clinicaltrials.gov/ct2/show/NCT00550862">http://clinicaltrials.gov/ct2/show/NCT00550862</a></td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>FXR</td>
<td>PBC</td>
<td><a href="http://clinicaltrials.gov/ct2/show/NCT00575042">http://clinicaltrials.gov/ct2/show/NCT00575042</a></td>
</tr>
</tbody>
</table>

6-ECDA, 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).

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-kB 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 hepatotoxicity 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 Chin 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, Sul2a1 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 hepatotoxicity 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 herbs from traditional Chinese medicine in analogy to Yin Chin may contribute to the armamentarium 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 D3 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 (Echchegadda 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 (NR1C2) 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 Mrd2 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 Mrd2 (Carrela et al., 1999; Hooiveld et al., 1999; Landrier et al., 2004). Statins have also been tested in the treatment of PBC. While initially 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)-selected 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 proteasome 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, cigitazone and troglitazone indicating a class effect (Snow and Moseley, 2007). Beneficial effects of troglitazone observed in a rat model of obstructive cholestasis on cholangiocyte 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), OstO/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 SHF 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 have 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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