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Emerging functions of the nuclear receptor LRH-1 in liver physiology and pathology

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ABSTRACT

Nuclear receptors play pleiotropic roles in cell differentiation, development, proliferation, and metabolic processes to govern liver physiology and pathology. The nuclear receptor, liver receptor homolog-1 (LRH-1, NR5A2), originally identified in the liver as a regulator of bile acid and cholesterol homeostasis, was recently recognized to coordinate a multitude of other hepatic metabolic processes, including glucose and lipid processing, methyl group sensing, and cellular stress responses. In this review, we summarize the physiological and pathophysiological functions of LRH-1 in the liver, as well as the molecular mechanisms underlying these processes. This review also focuses on the recent advances highlighting LRH-1 as an attractive target for liver-associated diseases, such as non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC).

1. Introduction

Nuclear receptors comprise one of the largest families of transcription factors that play critical roles in numerous biological processes, including development, proliferation, and metabolism [1,2]. Many nuclear receptors are ligand-dependent transcription factors that are activated by steroid hormones, such as estrogen, androgen and progesterone, and various other lipid-soluble molecules, including

Abbreviations: LRH-1, liver receptor homolog 1; NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; HNF4, hepatocyte nuclear factor 4; DLPC, dilauroyl phosphatidyl choline; PC, phosphatidyl choline; IBD, inflammatory bowel disease; SF-1, steroidogenic factor 1; Ftz-F1, fushi tarazu factor 1; DBD, DNA binding domain; LBD, ligand binding domain; AF-2, activation function 2; PI, phosphatidyl inositol; PG, phosphatidyl glycerol; PE, phosphatidyl ethanolamine; SHP, short heterodimer partner; DAX1, dosage-sensitive sex-reversal adrenal hypoplasia critical region on chromosome X, gene 1; PROX1, prospero homeobox 1; NCOR1 and NCOR2, nuclear receptor co-repressors; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; SRCs, steroid receptor coactivators; MBF-1, multiprotein bridging factor 1; FOXA1, forkhead box protein A1; PTM, posttranslational modification; ERK, extracellular signal-regulated kinase; PKA, protein kinase A; SUMO, small ubiquitin-like modifier; CUL4, cullin 4; DDB2, DNA damage binding-protein 2; VLDL, very low-density lipoprotein; ER, endoplasmic reticulum; UPR, unfolded protein response; ALD, alcoholic liver diseases; Cyp7a1, cholesterol 7α hydroxylase; Cyp8b1, sterol 12α hydroxylase; FXR, farnesoid X receptor; LXR, liver X receptor; LOF, loss-of-function; BSEP, bile salt export pump; RCT, reverse cholesterol transport; HDL, high-density lipoprotein; ApoA1, apolipoprotein A1; CETP, cholesteryl ester transfer protein; SR-BI, scavenger receptor class B type I; NPC1L1, Niemann-Pick C1-like 1; PPARα, peroxisome proliferator-activated receptor α; SREBP2, sterol regulatory element-binding protein 2; GCK, glucokinase; G6P, glucose-6-phosphate; ChREBP, carbohydrate response element binding protein; Cyp17a1, steroid 17α-monooxygenase; DHEA, dehydroepiandrosterone; Fasn/FAS, fatty acid synthase; Mttp/MTP, microsomal triglyceride transfer protein; GOF, gain-of-function; OSBPL3, oxysterol binding protein-like 3; MRG15, MORF4-related gene on chromosome 15; SAM, S-adenosylmethionine; MAT1A, methionine adenosyltransferase 1A; NASH, non-alcoholic steatohepatitis; GNMT, glycine *N*-methyltransferase; CDP, cytidine diphosphate; PEMT, phosphatidyl ethanolamine *N*-methyl transferase; MCD, methionine- and choline-deficient diet; Mdr2, multidrug-resistance protein 2; APR, acute phase response; APPs, acute phase proteins; IL, interleukin; Il-1ra, IL-1 receptor antagonist; Plk3, polo-like kinase 3; GSH, glutathione; GSSG, glutathione disulfide; NADPH, nicotinamide adenine dinucleotide phosphate; GLS2, glutaminase 2; ME1, cytosolic malic enzyme; T2D, type 2 diabetes; AFP, α-fetoprotein; FTF, α-fetoprotein transcription factor; HBV, hepatitis B virus; ENII, Enhancer II; Ccnd1, cyclin D1; Ccne1, cyclin E1; TALENs, transcription activator-like effector nucleases; Dlk1, delta-like non-canonical Notch ligand 1; Dio3, iodothyronine deiodinase type III; α-KG, α-ketoglutarate; GPT2, glutamate pyruvate transaminase 2; GOT1, glutamate oxaloacetate transaminase 1; mTORC1, mammalian target of rapamycin complex 1; DEN, diethylnitrosamine; NTD, N terminal domain; G-1-P, glucose-1-phosphate; R-5-P, ribose 5-phosphate; Gln, glutamine; Glu, glutamate; OAA, oxaloacetate; Asp, aspartate; SAH, S-adenosylhomocysteine; Cpt1α, carnitine palmitoyltransferase 1α; C/EBPβ, CCAAT/enhancer-binding protein β; TCF4, transcription factor 4.

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thyroid hormone, vitamin D, and retinoic acid. With the advancement of technology and science, several orphan nuclear receptors were found to be activated by endogenous metabolites at concentrations fluctuating within the micromolar range, distinguishing them from the classical steroid hormone receptors, whose activation only requires picomolar concentrations of hormones. The identification of fatty acids and various cholesterol derivatives as natural ligands for these "metabolic nuclear receptors" has opened an entirely new field of research with tremendous potential for therapeutic interventions. For several nuclear receptors, however, the ligand-dependent regulations have only begun to be unraveled. This is the case for hepatocyte nuclear factor 4 (HNF4; or NR2A1), originally classified as a nuclear receptor with constitutive transactivation activity, but later proposed to be modulated by linoleic acid, an essential dietary polyunsaturated fatty acid [3]. Another example is liver receptor homolog 1 (LRH-1, also known as NR5A2). Because the endogenous ligands of LRH-1 remained elusive for many years, it was initially designated as a constitutively active nuclear receptor. Later on, crystallographic studies revealed that several phospholipid species can efficiently bind to and further enhance the activity of human, but not mouse LRH-1 [4–9]. However, both human and mouse LRH-1 respond to dilauroyl phosphatidyl choline (DLPC), a phospholipid composed of phosphatidyl choline (PC) and two medium-chain lauric acid moieties [10].

Like many of the nuclear receptors, LRH-1 has diverse functions in development, differentiation, and metabolism. LRH-1 is expressed from the initial phases of embryonic development with key functions in embryonic stem cells [11,12]. The embryonic lethal phenotype observed in homozygous *Lrh-1* knockout mice furthermore supports its essential role throughout development [13]. In adult mammals, LRH-1 is strongly expressed in the pancreas, ovary, brain, intestine, and liver, with very low levels in the heart and lungs [14]. In the pancreas, it is essential for the production and secretion of pancreatic digestive fluid [15,16]. In the ovary, LRH-1 regulates steroidogenesis, ovulation, and mouse gestation [17–19]. Moreover, its action in the hypothalamus also affects ovarian follicle maturation and fertility [20]. In the intestine, LRH-1 coordinates cell renewal and local immune function, and can modulate the development and progression of common intestinal pathologies, such as colorectal cancer and inflammatory bowel disease (IBD) [21–24]. Furthermore, LRH-1 is also linked to the development of several other cancers, including pancreatic, breast, gastric, lung, prostate, and liver cancers (reviewed in [25]). The tumorigenic effects of LRH-1 are due to enhanced proliferation, cell cycle progression of cancer cells, metabolic reprogramming, regulation of apoptotic signaling, as well as promotion of migration and invasion.

While these findings highlight distinct functions of LRH-1 in development and differentiation, they also underscore its diverse actions in a tissue-specific context. Since its initial discovery in hepatocytes, the role of LRH-1 has been extensively studied in the liver. In this review, we discuss the molecular mechanisms by which the activity of LRH-1 is fine-tuned. Moreover, we focus on the physiological and pathophysiological functions of LRH-1 in the liver and highlight the recent advances that position LRH-1 as a putative target for liver-associated diseases, such as non-alcoholic fatty liver disease (NAFLD) and hepatocellular carcinoma (HCC).

2. Structure and regulation of LRH-1

2.1. LRH-1 structure and potential ligands

LRH-1 belongs to the NR5A subfamily of nuclear receptors and shares a high degree of sequence identity with the other member of this family, steroidogenic factor-1 (SF-1, also known as NR5A1). LRH-1 was first identified in Drosophila and named fushi tarazu factor 1 (Ftz–F1). Since then, its orthologs have been identified in many species, including mouse, rat, Xenopus, zebrafish, frog, chicken, horse, and human [14]. As with other nuclear receptors, LRH-1 has a highly conserved modular architecture that involves a DNA binding domain (DBD), a flexible hinge linker domain, and a ligand binding domain (LBD) (Fig. 1). At the C terminal of the DBD domain, a subtype-specific helix, termed Ftz-F1 motif, dictates the specificity of DNA binding. Contrary to the classic model of dimeric nuclear receptors, NR5A receptors bind to DNA as a monomer to response elements containing characteristic Ftz-F1 consensus binding site YCAAGGYCR (Y = pyrimidine, R = purine). The LBD of LRH-1 consists of 12 conserved α-helices, and contains the ligand-binding pocket and a conserved activation function-2 (AF-2) domain that mediates interaction with co-regulators. Moreover, the integrated structural model of human full-length LRH-1 predicts that the Ftz-F1 helix directly interacts with the second helix on the LBD to form an inter-domain communication, which contributes to NR5A receptor subclass function [26].

Several studies have provided detailed crystallographic information on the 3D structure of the LBD and on its ability to bind different phospholipid species, such as PC, phosphatidyl inositol (PI), phosphatidyl glycerol (PG), phosphatidyl ethanolamine (PE), and DLPC [4–10]. Notably, mutations within the human LRH-1 LBD significantly reduce binding to specific phospholipids, thereby diminishing its transcriptional potential [8]. However, mouse LRH-1 was found to possess a large but unoccupied hydrophobic pocket, and to display an active conformation, even in the absence of ligand. Mutations in mouse LRH-1 LBD fail to reduce its transcriptional activity [7], suggesting that ligands might be dispensable for mouse LRH-1. This divergence is likely due to variations in the amino acid sequence of human and mouse LRH-1, which differentially modulate the sensitivity to phospholipid ligand binding [6]. However, the phospholipid, DLPC, appears to activate both human and mouse LRH-1 [10] and to confer molecular and physiological responses in rodent models. In mice, DLPC treatment increases bile acid levels, reduces serum glucose, and hepatic triglycerides by modulating the expression of LRH-1 target genes [10] (see Sections 4.1, 4.3, and 6). At the molecular level, DLPC binding is a dynamic process altering the stability of human LRH-1 and its ability to interact with co-regulators [9], further substantiating its potential to modulate LRH-1 mediated biological processes.

Several synthetic small molecule agonists of LRH-1 have been reported and explored for their ability to modulate (patho)-physiological processes [27–31]. The LRH-1 agonist BL001, for instance, was shown to attenuate the development of diabetes in mice by blunting apoptosis of beta cells and maintaining the release of anti-inflammatory factors, which appears necessary for islet regeneration [30]. Similarly, the first nanomolar-range LRH-1 agonist induces intestinal epithelial steroidogenesis in an organoid model of IBD [31]. Based on structural analyses, a series of synthetic small molecules have been identified and characterized as antagonists of human LRH-1 from a library of commercially available compounds *via* molecular docking [32]. Experimental evaluation shows that these antagonists inhibit the transcriptional activity of LRH-1 and reduce the expression of LRH-1 target genes, resulting in the inhibition of LRH-1-mediated cancer cell proliferation.

2.2. Co-regulator recruitment

The transcriptional activity of LRH-1 is to a major extent determined by the recruitment of transcriptional co-regulators, a diverse family of proteins that act as chromatin remodeling factors (or which recruit such factors) to control promoter accessibility. Co-regulator interactions can both positively (co-activators) or negatively (co-repressors) impact LRH-1 activity, and occur at the level of the AF-2 domain *via* a helical LXXLL motif (Fig. 1). For instance, the atypical nuclear receptor, short heterodimer partner (SHP; NR0B2), as one of the well-defined co-repressors of LRH-1 [33], blunts LRH-1 transcriptional activity by binding the C-terminal AF-2 on the LBD [8,34]. Importantly, LRH-1 is critically required for SHP expression [35], which in turn inhibits its own expression by reducing the transcriptional activity of LRH-1 in a negative feedback loop [36,37]. Other proteins such as dosage-sensitive sex-

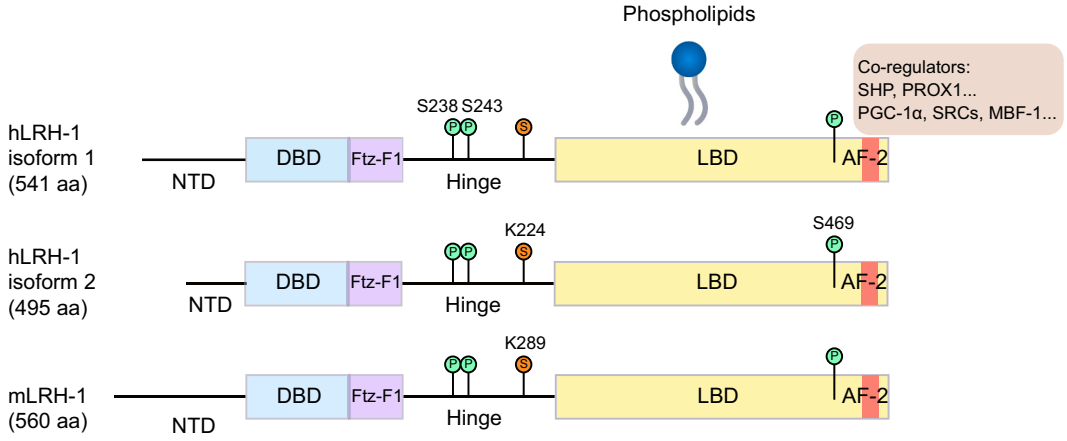

Fig. 1. LRH-1 structure and regulation. Canonical structure of human LRH-1 (hLRH-1) and mouse LRH-1 (mLRH-1). Isoform 1 is the largest isoform, while isoform 2 is the most abundant. Phosphorylation (P) and SUMOylation (S) sites are identified on different isoforms: S238 and S243 on isoform 1 of hLRH-1; S469 on isoform 2 of hLRH-1; K224 on isoform 2 of hLRH-1; K289 on mLRH-1. All these sites are conserved on these three forms. Phospholipids bind to the ligand binding pocket formed by LBD, such as PI, PG, PC and DLPC. Among these, DLPC is a well-studied LRH-1 agonist with reported functional relevance in rodent models. Co-regulators (including co-activators and co-repressors) interact with LRH-1 *via* the AF-2 domain.

reversal adrenal hypoplasia critical region on chromosome X, gene 1 (DAX1; NR0B1), prospero homeobox 1 (PROX1), and nuclear receptor co-repressors (NCOR1 and NCOR2) bind and repress LRH-1 in a tissue-dependent fashion (reviewed in [38]). For example, SHP is mainly expressed in the pancreas, liver, and heart, while its putative paralog, DAX1, is abundant in embryonic stem cells, ovary, and testis. PROX1, on the other hand, is primarily expressed in the heart, liver, and hippocampus. In addition to co-repressors, well-described co-activators of LRH-1 include peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), steroid receptor coactivators (SRC1, SRC2, and SRC3), multiprotein bridging factor-1 (MBF-1), and β-catenin (reviewed in [38]). These co-activators act as strong LRH-1 regulators by binding to its AF-2 and potentiating its transcriptional activity.

Nuclear receptors, including the glucocorticoid receptor [39], have been demonstrated to act as pioneer factors in differentiating tissues where they modify chromatin accessibility to other transcriptional constituents and thus, act as a transcription factor that shapes the epigenome during cell differentiation [40]. There are several lines of evidence that would support a similar function for LRH-1. Chromatin immunoprecipitation analyses demonstrate that LRH-1 cooperates with forkhead box protein A1 (FOXA1), to enhance the expression of cell cycle genes [41]. Knockdown of LRH-1 altered FOXA1 binding and induced the epigenetic depletion of histone deacetylase 2 from the regulatory region of cell cycle proteins [41]. In addition, SHP, the well-known co-repressor of LRH-1, can also recruit the class III histone deacetylase SIRT1 to deacetylate histone H3 and H4 on LRH-1 target genes and inhibit their transcription [42]. Furthermore, SHP interacts with G9a, an H3-K9 methyltransferase essential in maintaining the H3-K9 methylation silencing mark in euchromatic DNA regions [43–45]. The notion that LRH-1 may act as a pioneer transcription factor to shape the epigenome is an interesting discovery that merits further investigation.

2.3. Posttranslational modification

The transcriptional activity of LRH-1 is also modulated by post-translational modification (PTM) events, including phosphorylation and SUMOylation (Fig. 1). Human LRH-1 can be phosphorylated at serine 238 and serine 243 in the hinge domain by extracellular signal-regulated kinases (ERK) [46]. The phosphorylation is induced by phorbol myristate acetate in HepG2 cells and leads to an increase of LRH-1 activity. Protein kinase A (PKA) also phosphorylates human LRH-1 at serine 469 and induces LRH-1-dependent steroidogenesis by regulating aromatase PII promoter activity in breast cancer cells [47].

SUMOylation is one of the most extensively studied posttranslational modifications of LRH-1. LRH-1 and SF-1 can be modified by E3 SUMO (small ubiquitin-like modifier) ligases on both conserved and distinct lysine residues in different species to modulate their transcriptional activity [12,48–53]. The first SUMOylation site was identified on lysine 224 in the hinge region of human LRH-1 [48]. SUMOylation of this residue causes the protein to be translocated from the chromatin to promyelocytic leukemia protein nuclear bodies, inhibiting its transcriptional activity. A later study demonstrated that lysine 224 is conserved in mouse and acts as a biologically relevant SUMO acceptor site of SUMOylation *in vivo* [52]. When this lysine is mutated to arginine (K289R, an amino acid that cannot be SUMOylated), LRH-1 transcriptional activity is increased, further corroborating previous studies indicating that SUMOylation acts as a negative regulator of LRH-1 [48]. Furthermore, tannic acid, as a non-toxic general SUMOylation inhibitor, inhibits the SUMOylation of LRH-1 and induces the expression of genes that are normally silenced by SUMOylated LRH-1 [54]. In addition to directly modulating the transcriptional activity of LRH-1, SUMOylation affects the recruitment of co-regulators such as PROX1 [52]. Another example is the metabolic co-regulator GPS2, which by acting as a molecular bridge fosters the interaction between SUMOylated LRH-1 and the NCOR/HDACs co-repressor complex [55]. As discussed in the sections below, these interconnections between PTMs and co-regulator recruitment constitute an important way by which LRH-1 coordinates several of its metabolic and immune-regulatory actions in the liver.

Finally, a recent study focused on LRH-1 protein stability reported that LRH-1 is degraded by the ubiquitin-proteasome system [56]. The ubiquitination of LRH-1 is mediated by the cullin 4 (CUL4) E3 ubiquitin ligase complex through the direct interaction with DNA damage binding-protein 2 (DDB2). Overexpression of DDB2 decreases LRH-1 protein levels, whereas knockdown of DDB2 increases LRH-1 protein levels, hence affecting the transcriptional activity of LRH-1. It will be interesting to further investigate the ubiquitination site of LRH-1 and its effect on biological functions.

3. The liver in health and disease

The liver, with its 240 billion cells, is the largest internal organ of the human body. It carries out a wide variety of essential physiological functions, ranging from the production and secretion of livers-specific metabolites, such as bile acids and ketone bodies, to the storage of critical biomolecules like minerals and vitamins. The liver is also the

main site for the synthesis of several blood factors and plays a major role in carbohydrate, protein, and lipid metabolism. Its importance in the processing of nutrients stems from its specialized architecture that facilitates the filtering of the blood flowing from the digestive tract. The presence of a complex and unique blood draining system furthermore allows the liver to secrete a wide range of processed or *de novo*-synthesized biomolecules and lipoprotein particles for distribution in the body. Many of these secreted biomolecules function as fuel molecules for other cells of the organism. The liver thus distributes energy to the whole body by coordinating the systemic supply of nutrients.

Within hepatocytes, the energy derived from fuel molecules after glycolysis or oxidative respiration supports several anabolic processes, including the biosynthesis of fatty acids (*de novo* lipogenesis), very low-density lipoprotein (VLDL) production, and *de novo* synthesis of glucose (gluconeogenesis). The liver is also known for its ability to secrete significant concentrations of transport and bioactive proteins. A significant portion of the energy is thus also consumed for the production and secretion of hepatic proteins. Dysfunctions in this process may trigger endoplasmic reticulum (ER) stress within the hepatocytes and in turn activate the unfolded protein response (UPR), a series of adaptive pathways involved in the resolution of ER stress.

As is the case for other tissues, the liver is a heterogeneous organ made of functionally distinct cell populations. While hepatocytes support much of the liver-specific functions, cholangiocytes and sinusoidal endothelial cells provide structural support by lining the ductal canaliculi and sinusoids respectively. Although hepatocytes itself can produce cytokines as part of the acute phase response (see Section 5.1), the liver is also home to immune cells such as Kupffer cells (liver-resident macrophages) and pit cells (hepatic natural killer cells) that provide immuno-regulatory surveillance against infections or injury. Finally, the liver has a population of vitamin A storing cells called hepatic stellate cells (or Ito cells) that play a key role following liver injury. LRH-1 is moderately to highly expressed in basically all liver cell populations [57], although its role has been mostly studied in hepatocytes. While hepatocytes represent the main cellular population of the liver, they are functionally heterogeneous along the porto-central axis, a phenomenon called liver zonation. Cells located in the periportal zone differ from those in the perivenous zone in key enzymes, receptors, and subcellular structures resulting in different functional capacities for metabolism (reviewed in [58]). While LRH-1 expression itself does not appear to be zonated, some of its key transcriptional interacting partners and co-regulators such as β-catenin and SRC3 are [59], suggesting a potential gradient of LRH-1 activity along the porto-central axis.

Given the complex and heterogeneous architecture of the liver, it is not surprising that some of the most common liver diseases originate from both hepatic and non-hepatic dysfunctions. Liver diseases can have numerous origins such as viral infections, metabolic disorders, poisoning, cancer, or inherited diseases. Based on its key role in coordinating several hepatic metabolic pathways, the function of LRH-1 in diseases stemming from metabolic disorders, such as NAFLD and type 2 diabetes, has been comprehensively studied. Similarly, because of its role in managing fuel availability in the liver, major progress has been made in identifying the contribution of LRH-1 in coordinating liver cancer metabolism. The next sections will hence be devoted to the description of the various functions of LRH-1 in the liver and its important role in NAFLD and HCC.

4. LRH-1 in the control of liver metabolism

LRH-1 is highly expressed in the liver where it regulates several metabolic pathways. In this section, we summarize the hepatic metabolic processes known to be controlled by LRH-1 (Fig. 2), and their interaction with LRH-1 regulatory processes such as ligand binding, co-regulator recruitment, and PTMs.

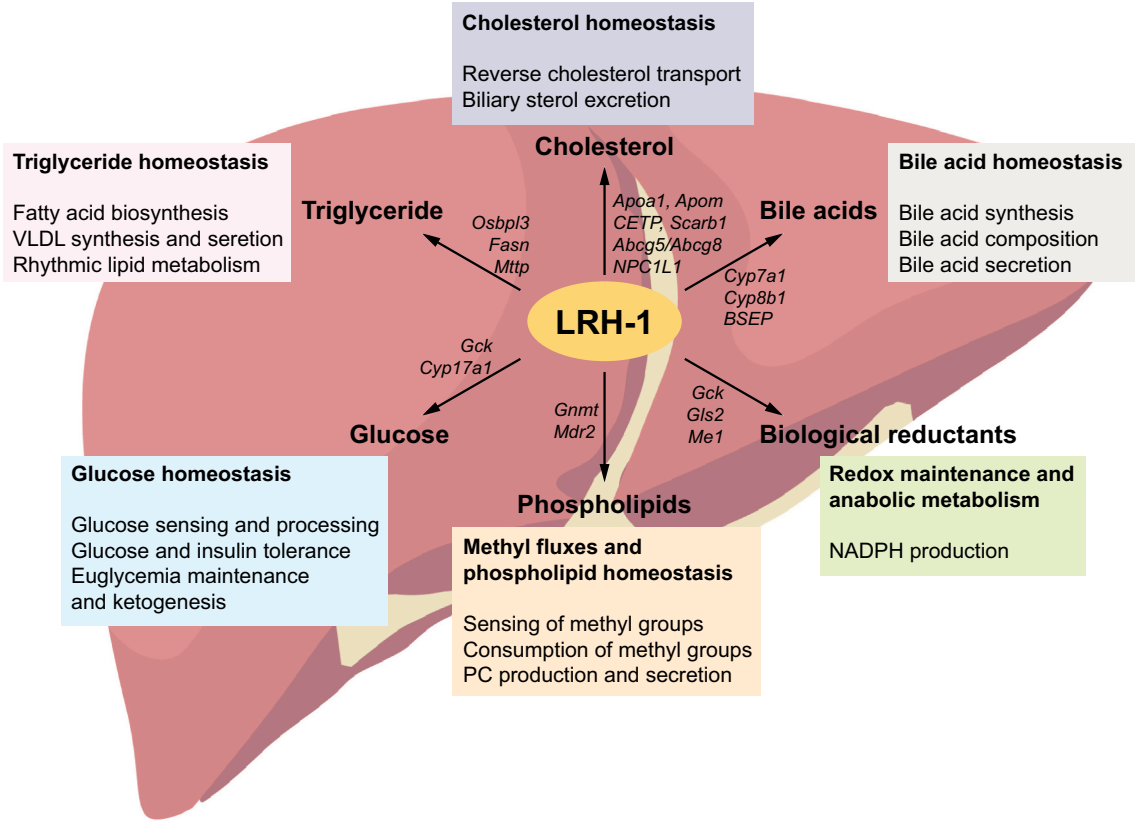

Fig. 2. Overview of diverse functions of LRH-1 in liver metabolism.

4.1. Bile acid homeostasis

One of the critical functions of the liver is to secrete bile. The human liver secretes about 700 to 1200 ml of bile per day to assist the intestinal absorption of lipids. Bile acids are steroid acids that are conjugated with taurine and glycine residues (in humans) or taurine alone (in rodents) *via* liver-specific enzymes before being secreted and stored in the gall-bladder along with phospholipids and cholesterol [60]. They act both as detergents that facilitate solubilization and absorption of dietary fat, and as hormones that alter multiple metabolic pathways in several tissues. After being secreted in the intestine following food ingestion, bile acids are either excreted through the feces or reabsorbed by the intestine before returning to the liver through the enterohepatic circulation, to maintain a constant bile acid pool. The synthesis of bile acids constitutes an important cholesterol consuming pathway in the mammalian liver. The entire bile acid synthesis pathway requires at least 17 different enzymes [60]. The expression of some enzymes in the pathway is tightly regulated by nuclear receptors and other transcription factors. Among all enzymes, the two key ones, cholesterol 7a-hydroxylase (*Cyp7a1*) [61] and sterol 12a-hydroxylase (*Cyp8b1*) [62], are the first identified genes directly regulated by LRH-1. CYP7A1, expressed exclusively in the liver, is the first and rate-limiting enzyme in the classic biosynthetic pathway. CYP8B1 is specifically required for cholic acid synthesis, determining the ratio of cholic acid to chenodeoxycholic acid in humans and thus the relative hydrophobicity of the circulating bile acid pool. Several studies demonstrated that CYP7A1 and CYP8B1 are regulated by SHP through its repression on LRH-1 activity in the context of a well-established feedback regulatory mechanism by which bile acids down-regulate their own synthesis [36,37,63]. When the nuclear bile acid receptor, farnesoid X receptor (FXR), is activated following binding to bile acids, it induces the expression of SHP, which in turn blunts LRH-1 mediated *Cyp7a1* and *Cyp8b1* gene expression, ultimately dampening bile acid synthesis. The LRH-1 agonist, DLPC, induces both bile acid biosynthetic enzymes in mouse liver, albeit with different efficiency, and increases bile acid levels [10]. However, *in vivo* analysis of hepatocyte-specific *Lrh-1* knockout (*Lrh-1*$^{hep-/-}$) mice shows that LRH-1 deficiency almost completely abolishes *Cyp8b1* expression, but only minimally impacts basal *Cyp7a1* expression [64,65]. This could be explained by the fact that *Cyp7a1* is also regulated by HNF-4α [66] and that LRH-1 only acts as a competence factor for liver X receptor (LXR)-mediated transactivation of the *Cyp7a1* promoter [37]. However, LXR only transactivates the promoter of the murine *Cyp7a1* gene and has no effects on the human *CYP7A1*, owing to the lack of a potent LXR binding site in the human *CYP7A1* promoter [37,67]. These results provide an explanation why mice, unlike humans, are better protected against hypercholesterolemia when fed a diet high in cholesterol [67]. Furthermore, while the SHP-LRH-1 axis plays an important role in coordinating bile acid homeostasis, additional mechanisms exist to fine-tune bile acid synthesis [63]. Feedback regulation of hepatic BA production is also coordinated by a gut-driven mechanism, involving FGF15, an enterokine induced and released in the circulation after ileal FXR activation [68]. In this context, it is interesting to note that blunted LRH-1 activity in the gut may also indirectly affect *Cyp7a1* through modulation of intestinal FGF15, further emphasizing the complex enterohepatic regulation of bile acid biosynthesis in the liver [69]. Importantly, LRH-1 loss-of-function (LOF) in hepatocytes, and the consecutive deficiency in *Cyp8b1* expression and cholic acid production, leads to profound changes in the composition and hydrophilicity of the bile acid pool. This remodeling significantly reduces the efficacy of intestinal lipid absorption and bile acid re-uptake, and stimulates the excretion of lipids in feces [64]. In addition to its regulatory control of bile acid synthesis, LRH-1 also transcriptionally activates the promoter of bile salt export pump (BSEP), which is responsible for the canalicular secretion of bile acids, and functions as a modulator in bile acid/FXR-mediated BSEP regulation [70]. Taken together, these data reveal a broad role for LRH-1 in regulating the synthesis and composition, as well as the elimination of bile acids in the liver, which in turn has major consequences on whole body lipid homeostasis.

4.2. Reverse cholesterol transport

Reverse cholesterol transport (RCT), a multi-step process by which the body removes excess cholesterol from peripheral tissues back to the liver, constitutes one of the main mechanisms that protects against atherosclerosis. High-density lipoproteins (HDLs) are the principal vehicles for RCT and collect the extracellular cholesterol secreted from extrahepatic tissues in the plasma through the action of the ATP-binding-cassette transporter A1 (ABCA1) [71]. Apolipoprotein A1 (ApoA1), the major protein component of HDL, acts as an acceptor while the phospholipid component of HDL acts as a sink for the mobilized cholesterol. LRH-1 regulates the expression of *Apoa1*, along with the expression of *Apom*, another component of HDL [72,73]. SHP suppresses the expression of these proteins by inhibiting the transcriptional activity of its binding partner LRH-1. Cholesterol is converted to cholesteryl esters by the enzyme lecithin-cholesterol acyltransferase in HDL. The cholesteryl esters can be transferred to other triglyceride-rich lipoproteins (such as VLDL) with the help of cholesteryl-ester transfer protein (CETP), further facilitating lipid uptake by the liver. Interestingly, hepatic *CETP* gene expression is increased in response to dietary hypercholesterolemia, an effect that is mediated by the activity of LXR and potentiated by LRH-1 [74]. Another crucial regulator of HDL cholesterol flux is the scavenger receptor class B type I (SR-BI), a cell surface receptor that mediates selective uptake of HDL cholesterol [75]. Overexpression of SR-BI in the liver reduces plasma HDL levels and promotes cholesterol excretion, leading to a reduction of atherosclerosis [76,77]. *Lrh-1* heterozygosity (*Lrh-1*$^{+/-}$) furthermore modulates its gene expression [78]. Two ATP-binding-cassette half-transporters *Abcg5* and *Abcg8* have been identified as LRH-1 target genes [79]. ABCG5/ABCG8 modulate the biliary excretion and intestinal reabsorption of cholesterol and other sterols and have been linked with sitosterolemia [80]. Freeman et al. reported that LRH-1 binding to an *Abcg5/Abcg8* intergenic region bi-directionally stimulates the promoter activity of both *Abcg5* and *Abcg8* [79]. Moreover, Niemann-Pick C1-like 1 (NPC1L1), originally identified as an important regulator for cholesterol absorption in the intestine, is also abundant in the human liver, where it promotes the uptake of biliary cholesterol to prevent the extensive loss of cholesterol. *NPC1L1* is regulated by several transcription factors, such as HNF-4α, peroxisome proliferator-activated receptor α (PPARα), and sterol regulatory element-binding protein 2 (SREBP2). LRH-1, which is bound to the response element identified in the *NPC1L1* gene, interacts with SREBP2 to synergistically activate the expression of *NPC1L1* [81].

In line with the studies described above, posttranslational modification of LRH-1 can affect cholesterol homeostasis to a major extent. Reducing the activity of LRH-1 *via* SUMOylation at lysine 289 promotes its interaction with the inhibitor PROX1 and mediates the inhibition of RCT genes, such as *Abcg5*, *Abcg8,* and *Scarb1* (gene name for SR-BI), leading to reduced biliary sterol excretion and RCT. On the contrary, non-SUMOylatable *Lrh-1*^{K289R} mice display increased expression of RCT genes and are less prone to atherosclerosis development [52]. Taken together, these studies suggest that LRH-1 is a master regulator of genes that function to regulate cholesterol transport and maintain homeostasis. This also indicates that LRH-1 could be a therapeutical target to protect against atherosclerosis.

4.3. Glucose homeostasis

LRH-1 has been reported to participate in the regulation of glucose metabolism and insulin resistance. In particular, the LRH-1 agonist DLPC reduces serum glucose and improves glucose and insulin tolerance in both insulin-resistant leptin-receptor-deficient *db/db* mice and high-fat diet-induced obese and insulin-resistant mice [10]. In *Lrh-1*$^{hep-/-}$ mice, hepatic glucose sensing and uptake are significantly dampened

after food intake [82]. Consequently, postprandial glycolysis and glycogen synthesis are substantially blunted in these animals [82]. Mechanistically, glucokinase (GCK), an enzyme that converts glucose into glucose-6-phosphate (G6P), is a direct target of LRH-1. Hepatic ablation of LRH-1 limits the availability of G6P, a key intermediate in glucose homeostasis. Because G6P is an allosteric regulator of glycogen synthase, and a regulator of carbohydrate response element binding protein (ChREBP) nuclear translocation [83], the LRH-1-GCK-G6P axis modulates multiple pathways in liver intermediary metabolism, including glycogen storage, glycolysis, and *de novo* lipogenesis (Figs. 3 and 4). Interestingly, another report focusing on LRH-1 protein stability confirmed the importance of LRH-1 in regulating *GCK* expression [56]. The authors showed that the interaction between DDB2 and LRH-1 prompts LRH-1 protein degradation through the CUL4-DDB1 ubiquitin ligase complex. Furthermore, DDB2 silencing increases the stability of LRH-1, leading to enhanced GCK expression and subsequent glucose uptake and G6P levels in HepG2 cells, further substantiating the link between LRH-1 and hepatic glucose homeostasis.

While these studies implicate a role for LRH-1 in coordinating postprandial liver metabolism, a more recent study indicates that LRH-1 could also be relevant for maintaining euglycemia and for the production of ketone bodies during periods of nutrient deprivation [84]. This process is proposed to involve the same network of nuclear receptors, FXR, SHP, and LRH-1, that control feedback regulation of bile acid biosynthesis through modulation of *Cyp7a1* and *Cyp8b1* (see Section 4.1), but instead conferred by their action on steroid 17α-monooxygenase (*Cyp17a1*), a gene whose enzyme product is involved in the production of dehydroepiandrosterone (DHEA), a putative hormone-ligand for PPARα. While in the fed state, *Cyp17a1* is suppressed by bile acid-mediated repression of LRH-1 activity, bile acid availability declines in the liver during the transition to the fasted state, and derepresses *Cyp17a1*, triggering activation of PPARα signaling in the liver. It will be of interest to further corroborate these novel metabolic functions in LRH-1 LOF mouse models.

4.4. Triglyceride metabolism

The liver acts as a central organ in the control of triglyceride homeostasis. Triglycerides represent the main form of fatty acid storage and transportation. Although a large proportion of fatty acids in the liver have an extrahepatic origin and are taken up from the plasma, about 30% of the fatty acids contained in hepatic fat droplets are produced *de novo* from glucose by the hepatocytes. One of the key genes involved in hepatic *de novo* lipogenesis is fatty acid synthase (*Fasn*), independently regulated at the transcriptional level by hormones, cellular signals, and transcription factors, such as SREBPs, thyroid hormone receptor, and LXR. It is also the first lipogenic gene identified as a direct LRH-1 target [85]. Indeed, LRH-1 appears to be required for the *Fasn* gene to be maximally activated by LXR. Of note, the stimulatory effect of LRH-1 is blocked by SHP, providing a mechanistic explanation for bile acid-mediated repression of lipogenesis. LRH-1 also indirectly coordinates almost every component of the *de novo* lipogenesis pathway by promoting the nuclear translocation and activation of the lipogenic transcription factor ChREBP, which is controlled by LRH-1 through activation of GCK-mediated G6P production [82] (Fig. 4). LRH-1 can thus be considered as a key regulator integrating glucose and lipid homeostasis in the postprandial phase. Moreover, microsomal triglyceride transfer protein (MTP), a key player in hepatic synthesis and secretion of VLDL, is trans-repressed by the SHP-LRH-1 axis, leading to increased expression and activity of MTP, and enhanced VLDL secretion in SHP LOF hepatocytes [86] (Fig. 4).

Increasing evidence indicates that LRH-1 is not acting alone, but rather converges with other transcription factors to regulate hepatic lipid metabolism. A non-biased, genome-wide ChIP-seq analysis revealed that a fraction of genomic sites occupied by LRH-1 are located close to FXR binding sites [87]. Gene Ontology enrichment further indicated that these FXR/LRH-1-regulated genes are involved in lipid metabolism, reinforcing the notion that both LRH-1 and FXR could act together in this biological process in a manner that is reminiscent of the mechanism of action by which LRH-1 and LXR co-operate in the regulation of bile acid biosynthesis [37]. *Lrh-1*^{K289R} mice, an LRH-1 selective gain-of-function (GOF) model, display enhanced *de novo* lipogenesis and accumulation of triglycerides upon refeeding [88]. Mechanistically, removal of repressive SUMO binding sites in LRH-1 leads to a marked induction of oxysterol binding protein-like 3 (OSBPL3), which in turn enhances SREBP-1 processing, culminating in a marked SREBP-1 driven lipogenic response (Fig. 4). Collectively, these findings highlight the complexity by which LRH-1 and other factors coordinate hepatic lipid homeostasis.

LRH-1 is also involved in the circadian regulation of hepatic lipid metabolism through epigenetic remodeling [89]. A study demonstrated that LRH-1 guides *MORF4*-related gene on chromosome 15 (MRG15) to the proximal promoter regions of genes involved in lipid metabolism where it acts as an epigenetic remodeler critical for the rhythmic regulation of hepatic lipid metabolism. At ZT22 (2 h after feeding), when mice are in a situation of nutrient-rich conditions, LRH-1 recruits MRG15 to genomic regions where it alters the histone acetylation status, increases Pol II accessibility, and activates gene transcription and lipid synthesis. However, at ZT10 (in the middle of the light phase) when mice enter the post-absorptive state, attenuated interaction between LRH-1 and MRG15 hinders MRG15 genomic recruitment, and hence, gene expression and lipid synthesis are reduced. These findings attribute a new role to LRH-1 as an integrator of hepatic lipid metabolism and circadian rhythm [89].

4.5. Methyl group and phospholipid homeostasis

The homeostasis of labile methyl groups (choline, betaine, methionine, and folate) involved in one-carbon metabolism is important for maintaining normal liver function. Patients with fatty liver disease or HCC often have reduced levels of methyl groups. In turn, a methionine- and choline-deficient diet (MCD) leads to fatty liver and non-alcoholic steatohepatitis (NASH), highlighting the essential roles of methionine and choline in mitochondrial β-oxidation and VLDL formation [90,91]. Long-term administration of MCD leads to the development of HCC [90,91]. Dietary supplementation with methyl groups improves metabolic function and reverses the negative effects of an obesogenic diet on NAFLD development [92]. The liver must also maintain normal levels of S-adenosylmethionine (SAM), a critical biological methyl donor generated from ATP and methionine *via* a reaction catalyzed by the hepatic enzyme methionine adenosyltransferase 1A (MAT1A). In 2001, Lu et al. speculated that SAM deficiency could be sufficient to induce non-alcoholic steatohepatitis (NASH). This hypothesis was proven to be correct as deletion of *Mat1a* reduces SAM content and leads to spontaneous development of NASH and HCC [93,94]. Of all hepatic methyltransferases, glycine *N*-methyltransferase (GNMT) is particularly important as it acts as a chemical rheostat that maintains constant cellular SAM levels. Accordingly, *Gnmt* deletion in mice induces the accumulation of hepatic SAM and triggers spontaneous NASH and HCC [95], indicating that total transmethylation fluxes need to be tightly regulated. Another important use of labile methyl group is to produce PC species, some of which are endogenous ligands for LRH-1 [8–10]. The production of PC can be achieved *via* two distinct pathways: the cytidine diphosphate (CDP)-choline pathway, which involves the phosphorylation and transfer of choline to phosphatidic acid, and the phosphatidyl ethanolamine *N*-methyl transferase (PEMT) pathway that uses the SAM–dependent transmethylation of PE through PEMT.

The function of LRH-1 in methyl group sensing and metabolism has been studied in *Lrh-1*$^{hep-/-}$ mice. Wagner et al. observed that *Lrh-1*$^{hep-/-}$ mice fed a MCD diet are protected from the development of liver injury. The investigators found that MCD-fed *Lrh-1*$^{hep-/-}$ livers showed higher SAM content and SAM/S-adenosylhomocysteine ratio (an estimation of

the cellular transmethylation capacity) than control mice [96]. The protection is achieved *via* the down-regulation of two LRH-1 target genes, *Gnmt*, and *Mdr2* (multidrug-resistance protein 2), the latter mediating the transport of phospholipids from hepatocytes to bile. Thus, depletion of LRH-1 buffers the content of methyl pool by saving SAM molecules for the PEMT pathway, as well as for other essential transmethylation reactions [96]. These results suggest that LRH-1 controls hepatic labile methyl balance by sensing SAM *via* the PEMT pathway and adjusting their levels by regulating the expression of their molecular rheostat, GNMT. Methyl-pool exhaustion depletes LRH-1 of its natural, endogenous ligands, triggering a response that promotes maintenance of a constant SAM content [96]. In another study, the role of LRH-1 and methyl homeostasis was studied on hepatic mitochondrial function [97]. *Lrh-1* knockout hepatocytes showed reduced mitochondrial number, basal respiration, and ATP production, along with blunted expression of genes involved in mitochondrial biogenesis and β-oxidation (Fig. 4) [97]. Interestingly, the PEMT pathway, but not the CDP-choline pathway, was found to contribute to the production of PC agonists that positively regulates the effect of LRH-1 on mitochondria. Moreover, SAM supplementation was sufficient to transactivate LRH-1 in a PEMT-dependent manner and to promote mitochondrial biogenesis and functions reinforcing the hypothesis that LRH-1 sits at the crossroads between transmethylation reactions and mitochondrial activity (Fig. 4).

5. LRH-1 in the coordination of hepatic cytoprotective mechanisms

LRH-1 plays important roles to protect against various forms of cellular stress in the liver that can be induced by systemic and hepatocyte-specific cues. In this section, we summarize the hepatic cytoprotective pathways known to be regulated by LRH-1, and their specific mechanisms.

5.1. The acute phase response

The liver is the primary site of the acute phase response (APR), which is an orchestrated response to trauma, tissue injury, and infection. This response plays an important role in limiting tissue damage and is characterized by the induction of circulating levels of acute phase proteins (APPs) generated by the liver in response to inflammation, involving various proteins in coagulation, lipid metabolism, and the complement system [98]. Inflammation-associated cytokines, in particular interleukins (ILs), are the major physiological mediators of the APR. Venteclef et al. demonstrated that LRH-1 overexpression dampens IL-1β- and IL-6-mediated haptoglobin, serum amyloid A, and fibrinogen β gene expression in hepatocytes. Conversely, *Lrh-1*$^{+/-}$ mice showed an exacerbated inflammatory response, indicating that LRH-1 is a physiological modulator of the hepatic APR [99]. In addition to the regulation of APPs, the IL-1 receptor antagonist (*Il-1ra*), an inhibitor of IL-1 signaling, was also identified as a target gene of LRH-1. LRH-1 overexpression potentiated cytokine-mediated induction of *IL-1RA* in HepG2 cells, thereby negatively interfering with cytokine-elicited inflammatory response [100]. This regulation was furthermore confirmed in *Lrh-1*$^{+/-}$ mice, which displayed blunted induction of *Il-1ra* after LPS exposure (Fig. 4). Interestingly, modification of LRH-1 activity through SUMOylation also affects the anti-inflammatory pathway and the regulation of the APR [55]. The authors demonstrated that LRH-1-mediated trans-repression is dependent on SUMOylation, in which GPS2 functions as a molecular bridge between SUMOylated LRH-1 and the NCOR/HDAC co-repressor complex. In further support of this finding, *Sumo-1* knockout mice showed an increased hepatic APR, in part explained by the diminished inability of LRH-1 to repress the APR promoters. Taken together, these studies demonstrate the existence of multiple mechanisms contributing to the overall anti-inflammatory properties of LRH-1 in the liver.

5.2. Unfolded protein response

The liver secretes large amounts of transport and bioactive proteins. When physiological conditions or pathological damage disturbs the protein folding process, misfolded proteins accumulate within the ER, causing ER stress. This status, during which the liver's demand to produce functional proteins can no longer be supported by its capacity, is often triggered by chemical compounds or by disease [101]. However, refeeding can also rapidly activate a physiological form of ER stress, which is transient [102]. Chronic unresolved ER stress on the other hand is harmful and contributes to many acute and chronic liver diseases, such as ALD, NAFLD, NASH and hepatic fibrosis [103].

More recently, LRH-1 has been identified as a key player in the resolution of ER stress [104]. Clearance of ER stress is known to be mediated by a set of highly conserved signal transduction cascades that ultimately converge on three transcription factors, ATF4, ATF6, and XBP-1, commonly referred to as the unfolded protein response (UPR). LRH-1-dependent resolution of ER stress has been proposed to constitute a novel pathway, in which ER stress-mediated LRH-1 activation induces the expression of polo-like kinase 3 (*Plk3*), leading to increased phosphorylation and activation of ATF2, which in turn induces the expression of its downstream target genes [104]. Interestingly, *Lrh-1*$^{hep-/-}$, *Plk3* knockout mice and mice with dominant negative *Atf2*, all fail in resolving ER stress upon tunicamycin challenge. Moreover, restoration of *Plk3* expression in *Lrh-1*$^{hep-/-}$ mice rescues ATF2 phosphorylation and ER stress resolution. Therefore, LRH-1 might be a promising therapeutic target for diseases caused by ER stress, including NAFLD.

5.3. Protection against oxidative stress

The liver produces reactive oxygen species derived from oxygen and nitrogen during metabolism, which are known inducers of oxidative stress. Excessive reactive species may lead to oxidative damage and are potentially harmful to cells. To resist oxidative stress and maintain a proper redox status, the liver is well equipped with antioxidant mechanisms. Glutathione (GSH), the most important antioxidant, is produced from the oxidized form of glutathione disulfide (GSSG) in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent glutathione reductase reaction [105]. Thus, maintaining high levels of NADPH is required to buffer oxidative stress. LRH-1 plays an important role in NADPH production as evidenced by the reduced NADPH/NADP^{+} and GSH/GSSG ratios in the livers of *Lrh-1*$^{hep-/-}$ mice [106]. LRH-1 is proposed to facilitate the generation of NADPH by providing substrates for enzymatic reactions that support NADPH synthesis (Figs. 2 and 3). While GCK-dependent G6P production is known to boost NADPH synthesis through the pentose phosphate pathway, glutaminase 2 (GLS2)-mediated breakdown of glutamine constitutes another way of yielding substrates for cytosolic malic enzyme (ME1), another NADPH generating enzyme abundantly expressed in the liver. By controlling the expression of both *Gck* and *Gls2*, LRH-1 coordinates not only the redox state of the liver, but also reductive biosynthesis (Fig. 3), with important implications for liver cancer [106] (see also Section 7).

6. LRH-1 and non-alcoholic fatty liver disease

NAFLD is the most common liver disorder in Western societies and its incidence is strongly associated with obesity, insulin resistance and type 2 diabetes (T2D). NAFLD comprises a spectrum of disorders, of which the earliest stage is characterized by the deposition of lipid droplets within the cytoplasm of the hepatocytes. Hepatic steatosis increases the susceptibility to hepatocyte damage and inflammation, a condition termed NASH, and can ultimately progress to cirrhosis and hepatocellular carcinoma. Liver steatosis develops when there is an imbalance between the supply and the removal of fatty acids. While the removal of fatty acids is mainly governed by VLDL assembly/secretion and mitochondrial fatty acid oxidation, the supply of fatty acids can come

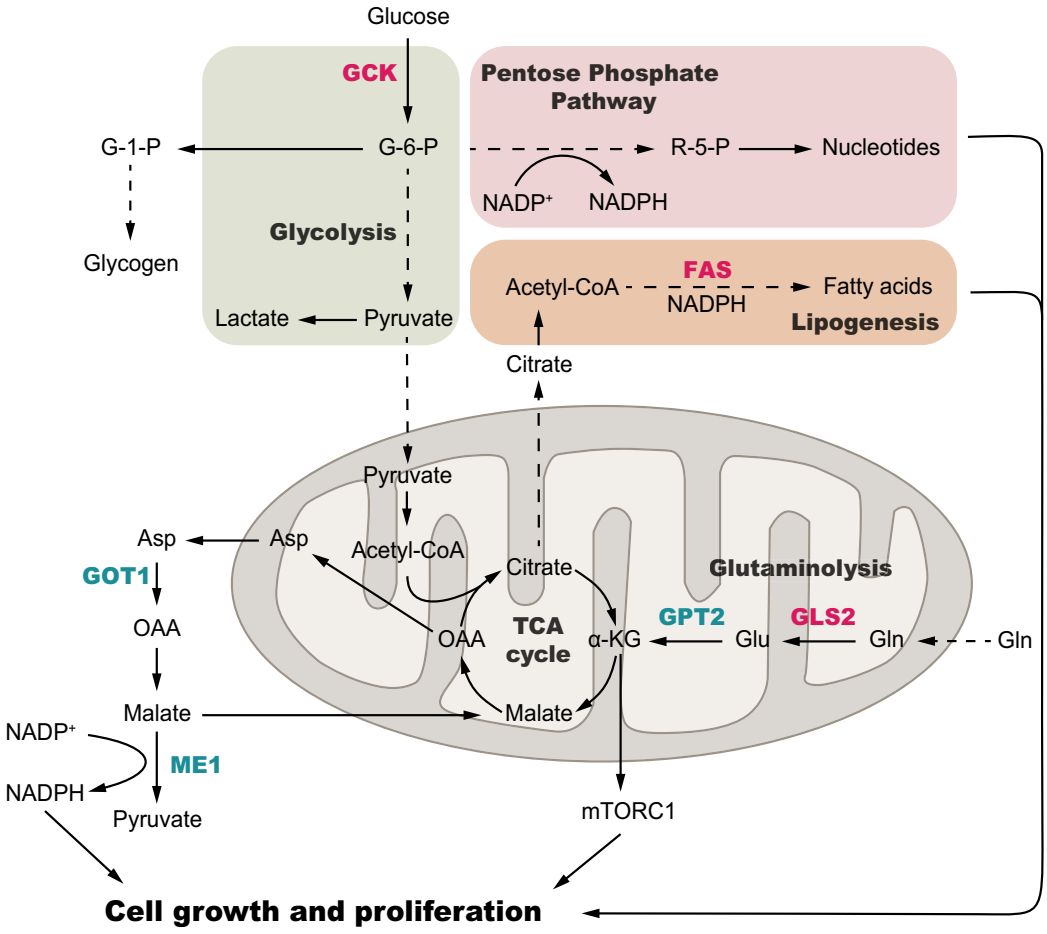

Fig. 3. LRH-1 regulates glucose and glutamine metabolism, leading to the cooperation of different pathways in the regulation of metabolic adaptations required for cell growth. The enhanced availability of the intermediate G6P through LRH-1 mediated regulation of GCK modulates several pathways, such as glycogen storage, glycolysis, pentose phosphate pathway and *de novo* lipogenesis. On the other hand, the action of LRH-1 on glutamine anaplerosis affects mTORC1 signaling through the production of α-KG. LRH-1 also promotes the production of NADPH, a biological reductant essentially required for the biosynthesis of biomolecules. Established direct target genes of LRH-1 are indicated in red, while relevant genes indirectly regulated by LRH-1 are depicted in green.

directly from the diet, from the hydrolysis of fat in the adipose tissue, or indirectly from *de novo* lipogenesis in the liver. The latter process uses glucose as initial substrate and requires glycolysis to yield acetyl-CoA as substrates for fatty acid biosynthesis.

Several studies using different genetically modified models have assessed the contribution of LRH-1 in the etiology of dyslipidemia and steatosis (Fig. 4). Initial studies using germline *Lrh-1*$^{+/-}$ mice reported reduced triglyceride and fatty acid accumulation in the liver [107]. In line with these effects, several genes involved in lipogenesis were also down-regulated in the liver of these mice. Furthermore, *Lrh-1* heterozygosity was also correlated with lower levels of serum triglycerides and cholesterol, pointing to a major involvement of LRH-1 in systemic and liver lipid homeostasis [107]. Interestingly, while *Lrh-1*$^{hep-/-}$ mice display reduced *de novo* lipogenesis during the early postprandial phase [82] (discussed in Section 4.4), they develop prominent steatosis after an overnight fasting (non-published communication). This phenotype results from a different mechanism and does not involve *de novo* lipogenesis, which is only relevant shortly after a meal ingestion. Fasting-induced steatosis is usually indicative of hepatic mitochondrial dysfunction, a phenotype that was previously demonstrated in *Lrh-1*$^{hep-/-}$ mice [97]. In another study, hepatic triglycerides are unchanged in the *Lrh-1*$^{hep-/-}$ mice after a short-term fasting (6 h) compared to that in *Lrh-1*$^{hep+/+}$ mice, while acutely disrupting LRH-1 function in adult hepatocytes by means of an AAV8 virus (AAV8-CRE), increases circulating free fatty acids and promotes hepatic lipid accumulation, leading to hepatic steatosis and liver injury after a high-fat dietary challenge [108]. Several reasons, including enhanced susceptibility to CRE toxicity or developmental compensatory mechanisms, could be invoked to explain these differences [108]. Altogether, these findings corroborate the critical importance of LRH-1 in the regulation of liver fat distribution.

Activation of LRH-1 by pharmacological ligand exposure or by genetic annihilation of its SUMOylation acceptor sites has led to intriguing findings. In an elegant study conducted in genetic and dietary models of diabesity, the group of David Moore showed that treatment with the phospholipid DLPC protects mice from developing NAFLD and insulin resistance [10]. Importantly, both the antidiabetic and lipotropic effects of DLPC were lost in the *Lrh-1*$^{hep-/-}$ mice [10]. More recently, the contribution of LRH-1 SUMOylation to the development of NAFLD was also assessed [88]. *Lrh-1*^{K289R} mice develop NAFLD and early signs of NASH when challenged with a lipogenic, high-fat, high-sucrose diet. The LRH-1 K289R mutation induces the expression of OSBPL3, enhances SREBP-1 processing, and promotes *de novo* lipogenesis (Fig. 4). Remarkably, *Osbpl3* clusters with genes involved in inflammation and fibrosis in mice and humans with NAFLD demonstrating that the LRH-1-OSBPL3 signaling axis might be a driver of NASH [88]. While in apparent contradiction, these results could be explained by the fact that the K289R mutation represents a selective GOF that does not induce the expression of *Shp*, the well-known repressor of LRH-1 [88] while DLPC treatment in mice strongly represses SHP expression [10]. This DLPC-induced repression of SHP is reminiscent of the *Shp* knockout mice that are also protected from the development of NAFLD in obese mouse models, and at least in part due to increased hepatic VLDL secretion [86].

DLPC also binds to human LRH-1 and activates human LRH-1-mediated transcription [9,10,97]. Mechanistically, the activation is due to the dynamic binding of DLPC allowing human LRH-1 to undergo structural fluctuations and thus alter its ability to interact with co-regulators [9]. Functionally, DLPC specifically activates LRH-1 target genes in human C3A/HepG2 cells [10,97]. Clinically, genomic

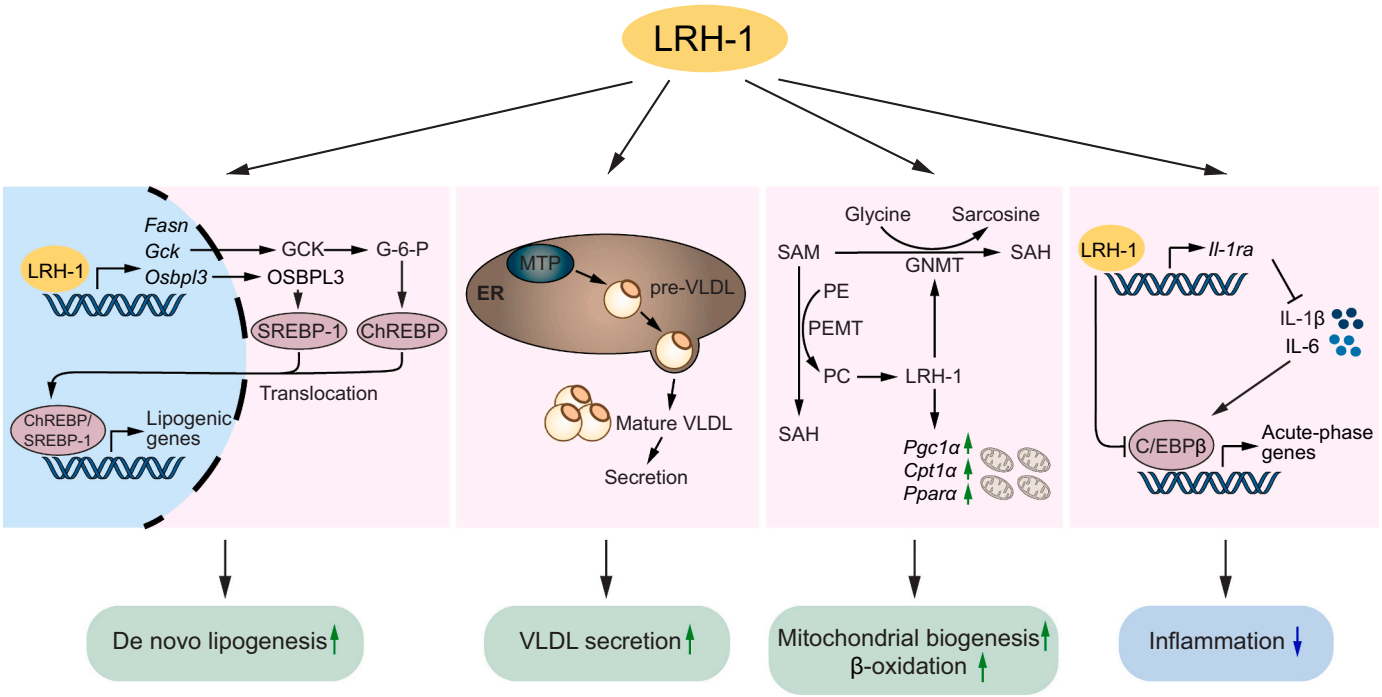

Fig. 4. Role of LRH-1 in processes affecting NAFLD/NASH outcome. Activation of liver LRH-1 promotes *de novo* lipogenesis through direct regulation of the lipogenic target gene *Fasn*, and by mediating the nuclear translocation of the master lipogenic transcription factors SREBP-1 and ChREBP. MTP-mediated VLDL secretion is regulated by the LRH-1/SHP axis. LRH-1 senses intracellular SAM levels *via* the SAM-dependent PEMT pathway, and modulates methyl group metabolism thereby improving hepatic mitochondrial function. LRH-1 inhibits liver inflammation *via* induction of *Il-1ra* expression and repression of IL-1b- and IL-6-elicited inflammatory response.

expression profiling reported that LRH-1 is significantly downregulated in patients (N = 102) with either NAFLD or NASH [109]. Taken together, these findings suggest LRH-1 as a potential target for the prevention and treatment of NAFLD, even though its exact contribution and its tight link to SHP remain to be further explored.

7. LRH-1 and hepatocellular carcinoma

LRH-1 promotes the development of several cancers and its oncogenic properties of LRH-1 can be partially explained by its role to coordinate development and differentiation. While LRH-1 is vital in the very early stages of development, in part because of its role in maintaining embryonic stem cell pluripotency [11,12], it is also a critical modulator of endodermic organ differentiation [14]. In the liver, LRH-1 is involved in the regulation of a set of genes essential for its proper development. A number of transcription factors critical for early hepatic development and differentiation, such as HNF-1α, HNF-3β, and HNF-4α also contain an LRH-1 binding site in their promoters and are directly regulated by LRH-1 [110]. LRH-1 controls the early expression of α-fetoprotein (AFP) [111], hence the alternative name FTF (α-fetoprotein transcription factor) for LRH-1 (Fig. 5). AFP is the most abundant serum protein in the developing embryo and strongly expressed throughout the fetal development of the liver before disappearing around the perinatal period. Healthy adults have no circulating AFP, while patients with chronic liver disease or HCC display elevated AFP levels [112]. Thus, a blood test that measures the level of AFP is normally used to confirm or rule out a diagnosis of liver cancer or to monitor cancer treatment.

Chronic infection with hepatitis virus is one of the dominant risk factors for the development of HCC. Chronic hepatitis B virus (HBV) infection accounts for at least 50% of global HCC cases. LRH-1 is involved in the regulation of HBV gene transcription and DNA replication [113,114]. Mechanistically, LRH-1, cooperating with HNF1, binds to Enhancer II (ENII), one of the critical *cis*-elements in the HBV genome that plays an important role in regulating the transcription of all HBV genes. This synergism upregulates the liver-specific activity of ENII, which consequently modulates hepatic viral gene transcription and DNA replication (Fig. 5).

Another indication in support of a role for LRH-1 in tumor development is its involvement in cell proliferation and cell cycle progression, which in a cancer prone context could culminate in uncontrolled proliferation (Fig. 5). Earlier work showed that LRH-1 acts in synergy with the β-catenin pathway to induce G1 cyclin-mediated proliferation in intestinal crypts by inducing the *Ccnd1* (cyclin D1), *Ccne1* (cyclin E1), and several other genes involved in cell proliferation [22]. Moreover, LRH-1 expression conferred a selective growth advantage to cells and favored tumor formation and progression in nude mice [22] and in mouse models of colorectal cancer [115]. Increasing evidence indicates that hepatic LRH-1 may have a similar function in the liver. Although most of the studies have been limited to hepatoblastoma cell lines, overexpression of LRH-1 is usually associated with enhanced proliferation [22], while knockdown of *LRH-1* using RNA interference [116], transcription activator-like effector nucleases (TALENs) [117] or lentiviral-based shRNAs [118] attenuates cell proliferation and colony formation. In line with its action in the gut, suppression of LRH-1 in hepatoblastoma cells dampens the expression of *Ccnd1*, *Ccne1* and *C-myc*, while inducing *p21*, resulting in cell cycle arrest at G1 phase [117,118]. Moreover, treatment with the recently developed LRH-1 antagonist LRA [32] inhibits cell proliferation and colony formation in a dose-dependent manner [118]. Furthermore, one microRNA, *miR-381*, directly targeting and negatively modulating LRH-1 expression, functions as a tumor suppressor to repress HCC cell growth and invasion. The expression of *miR-381* itself is significantly decreased in HCC tissues and cell lines [119]. Of note, *miR-381* is located in the cluster of imprinted genes delineated by the delta-like non-canonical Notch ligand 1 gene and the iodothyronine deiodinase type III gene (*Dlk1-Dio3*). While several studies indicate that the miRNAs within this region are involved in the development of liver cancer [120,121], further research is needed

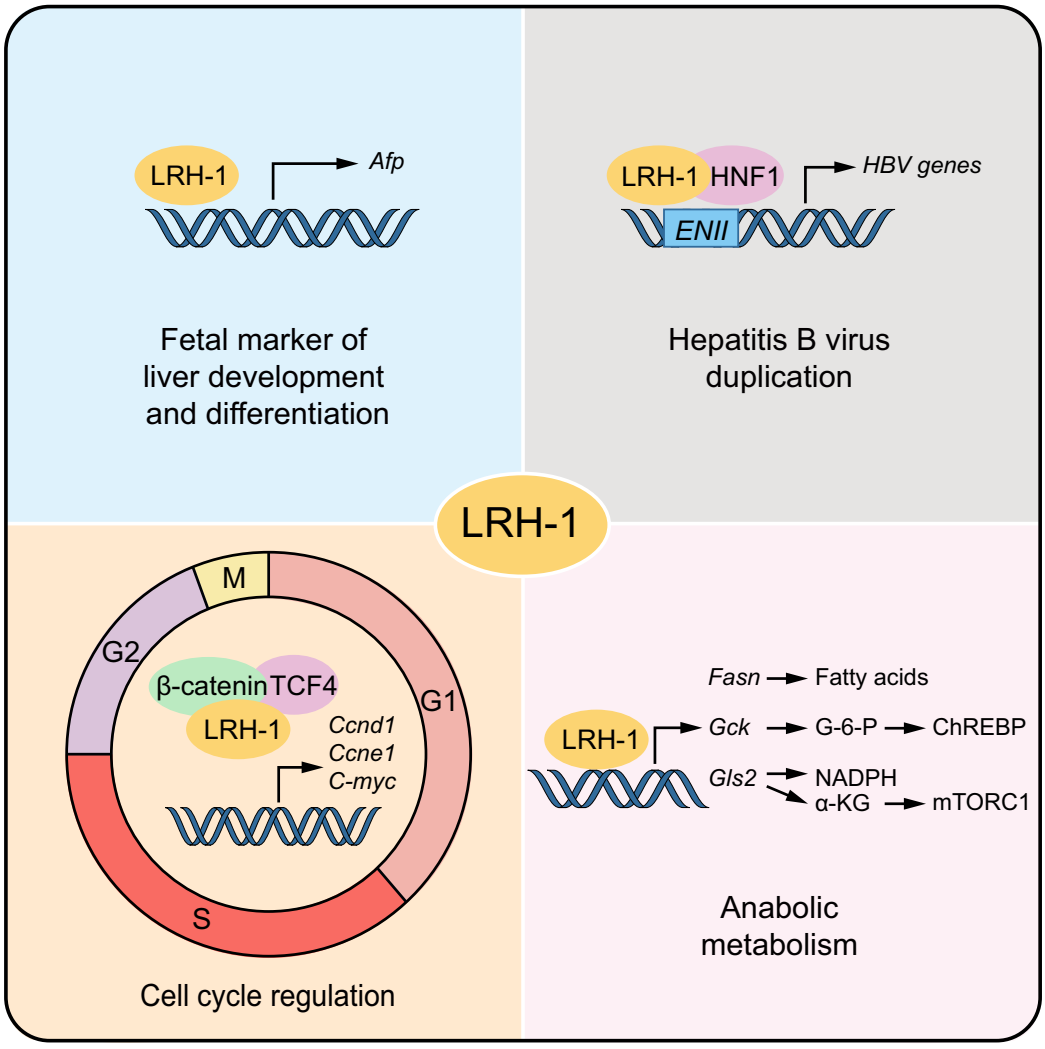

Fig. 5. LRH-1 plays important roles in the development of HCC. LRH-1 promotes the development of HCC by regulating proliferation, gene expression of the fetal liver marker, hepatitis B virus duplication and several anabolic pathways.

to determine its exact biological role in the liver.

Evidence is emerging that LRH-1 also acts as a key regulator of hepatic cancer cell metabolism (Figs. 3 and 5). Cancer cells display enhanced and unusual metabolic activities to fuel proliferation. While all cancer cells increase their uptake and breakdown of glucose to ensure sufficient production of biomass, some cancer cells also rely on glutamine to replenish the TCA cycle. Glutamine breakdown or glutaminolysis is accomplished *via* a two-step deamination reaction in which cells convert glutamine to glutamate and further to the TCA intermediate, α-ketoglutarate (α-KG). While the first step is catalyzed by glutaminase, the second step is usually completed by glutamate dehydrogenase, however, a non-canonical pathway comprised of aminotransferases exist for pancreatic ductal adenocarcinoma cells that provides additional NADPH reductive power to support pancreatic cancer growth and to keep oxidative stress in check [122]. Interestingly, Xu and colleagues found that in the liver, LRH-1 coordinates a similar non-canonical glutamine pathway reliant on mitochondrial GLS2, the first and rate-limiting step of the glutaminolysis pathway in the liver, and a series of mitochondrial and cytosolic transaminases, glutamate pyruvate transaminase 2 (GPT2) and glutamate oxaloacetate transaminase 1 (GOT1) (Fig. 3). Disruption of hepatic LRH-1 markedly blunts glutamine-dependent anaplerosis due to a reduced flux through GLS2. The resulting reduction in α-KG availability furthermore dampens mTORC1 (mammalian target of rapamycin complex 1) signaling to eventually block cell growth and proliferation. Moreover, while regulating the noncanonical glutamine processing, LRH-1 also promotes the production of NADPH [106], which provides the reducing power for reductive biosynthesis and for counteracting oxidative stress. NADPH is an essential cofactor for anabolic pathways, in particular for the biosynthesis of fatty acids and cell membrane constituents, such as phospholipids (Fig. 3). In addition, LRH-1 also contributes to the production of biomass required for cell proliferation through the regulation of *Gck* and *Fasn* [82,85] (Fig. 5). In accordance with these data, *in vivo* studies revealed that *Lrh-1*$^{hep-/-}$ mice are protected against diethylnitrosamine (DEN)-induced liver cancer [106]. Clinically, immunohistochemistry analysis of 50 pairs of HCC clinical samples and corresponding adjacent tissues shows that the expression of LRH-1 is dramatically increased in HCC tissues, especially in the nuclei [117]. Overall, these studies suggest the important role of LRH-1 in the development and progression of HCC and raise the possibility that targeting LRH-1 may provide an effective and attractive prospect in the treatment of liver cancers.

8. Conclusions and future perspectives

LRH-1 was initially identified as a regulator of cholesterol and bile acid homeostasis. Over the last decade, however, multiple new functions of LRH-1 in the liver emerged, ranging from control of intermediary

metabolism to regulation of cellular stress responses, inflammation, growth and proliferation. Significant progress has been made in elucidating the molecular events that determine its active or repressive state. Consistent with the paradigm that human LRH-1 acts as a constitutive nuclear receptor, phospholipids have been found to permanently reside in the ligand binding pocket. However, in the last couple of years, studies have highlighted the possibility to further activate its activity by specific medium-chain phospholipid species, such as DLPC. Other reports have unveiled the complexity of its regulation by posttranslational modifications, such as SUMO modification, and led to the intriguing possibility to modulate its activity in a selective co-repressor-dependent manner.

Given the prominent effects on hepatic lipid metabolism and insulin tolerance with the endogenous agonist DLPC, LRH-1 seems to be involved in the development and progression of NAFLD. The exciting discovery of DLPC as an antidiabetic and lipotropic agent may provide a window of therapeutic opportunity. However, it will be important to fully characterize and optimize the pharmacological characteristics of this unusual phosphatidyl choline specimen and potentially other LRH-1 ligands. With regard to DLPC, many questions remain about its bioavailability and subcellular distribution, as well as its off-target effects. Further work will also be required to fully understand the mechanistic differences between pharmacologic and genetic activation in the context of NAFLD/NASH. In contrast to DLPC, which triggers a global induction of LRH-1 target genes, genetic ablation of the SUMO acceptor motif in LRH-1 only leads to a selective activation of LRH-1 target genes in the liver without affecting the expression of its target and co-repressor SHP. On the other hand, repression of LRH-1 by PROX1 or NCOR is eliminated. A better understanding of the relative contribution of each of these molecular actors in healthy and diseased liver will be informative for the design and fine-tuning of selective modulators. It will also be of interest to understand the exact contribution of LRH-1 in non-parenchymal cell populations in the liver, especially in the context of NASH.

A final question relates to the long-term effects of LRH-1 agonists. This is particularly relevant in view of the role of LRH-1 in liver carcinogenesis. Antagonists or inverse agonists of the receptor are potentially equally interesting avenues to pursue, but will be subject to similar pharmacological constraints as LRH-1 agonists for their development as anti-cancer therapeutics. The one thing that is certain given the wide-ranging activities of the receptor is that the development of any compound inhibiting or activating LRH-1 will be a balancing act between beneficial and harmful effects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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