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Liver receptor homolog-1, an emerging metabolic modulator

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1. ABSTRACT

The liver receptor homolog-1 (LRH-1; NR5A2) belongs to nuclear hormone receptor superfamily, and is expressed mainly in the liver, intestine, exocrine pancreas, and ovary. It binds DNA as a monomer, and is best known as a regulator of hepatic expression of the key bile acid biosynthetic enzyme cholesterol 7alpha hydroxylase (Cyp7A1). It is also expressed in embryonic stem cells and the initial stages of embryonic development, and the very early lethality of LRH-1 knockout mice highlights its essential developmental role. Recent crystal structures of LRH-1 and its closest relative steroidogenic factor-1 (SF-1; NR5A1) identified phospholipids as potential ligands. This intriguing discovery raises the possibility of an unexpected new class of nuclear receptor signaling molecules, but the broader functional roles of LRH-1 and these new ligands remain to be established.

2. INTRODUCTION

Nuclear hormone receptors are a group of transcription factors, most of which exert their transcriptional activities through ligand binding and subsequent coactivator recruitment to coordinate development, proliferation, and metabolism. Those without known ligands are categorized as orphan nuclear receptors. Most bind DNA as either homodimers or heterodimers with the common partner RXR. However, some NRs are not dependent on dimerization and directly bind DNA as monomers. Since the initial cloning of cDNAs encoding the classical nuclear receptors more than 20 years ago, many former orphan receptors have been associated with physiologic ligands, including the peroxisome proliferator-activated receptors (PPARs) (1, 2), the liver X receptor (LXR) (3, 4) and farnesoid X receptor (FXR) (5-7). The identification of new ligands has helped

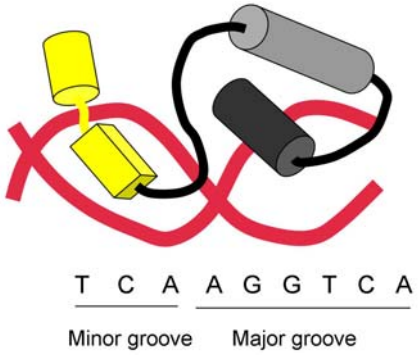

Figure 1. DNA binding model of LRH-1. Adapted from the crystal structure solved by Solomon et al. (40). The DNA double helix is depicted as red lines. The P box helix (dark grey cylinder) occupies the major groove of the 5'AGGTCA 3' hexamer, as in many other nuclear receptor DNA binding domains. The C terminal extension of the DNA binding domain (yellow) recognizes the 5' extension of the consensus recognition element (TCA) and allows LRH-1 monomer binding. The unique Ftz-F1 helix (yellow cylinder) is important for monomer binding and coactivator recruitment.

in the discovery of novel signaling pathways and provided key insights into the regulation of glucose and lipid metabolism.

Recently, crystallographic studies have contributed to the characterization of the potential ligands for orphan receptors. For example, the NGFI-B ligand binding pocket is completely filled by bulky hydrophobic residues, leaving no room for an exogenous ligand (8), while the small pocket of hepatocyte nuclear factor-4 (HNF-4) contains an apparently non-exchangeable fatty acid (9, 10). In contrast, ROR-alpha may be activated by cholesterol derivatives, including cholesterol sulfate (11). Interestingly, several different structures of LRH-1 and its close relative SF-1 have revealed either a stabilized conformation without any molecule in the ligand binding pocket (12, 13), or the presence of phospholipids in the pocket (14-17). Here we discuss molecular and physiological aspects of LRH-1 function.

3. MOLECULAR DETAILS

3.1. DNA binding

Mammalian othologues of the Drosophila transcription factor and developmental regulator Fushi Tarazu-Factor 1 (Ftz-F1) (18, 19) were independently isolated by a number of groups and have received many names. The most generally used is LRH-1, but others include pancreas hormone receptor (PHR-1), fetoprotein transcription factor (FTF), human B1-binding factor (hB1F), or CYP7A promoter binding factor (CPF) (20-23). LRH-1 is predominantly expressed in tissues of endodermal origin such as liver, small intestine, exocrine pancreas, and also ovary and testis (24-27).

In functional tests, LRH-1 shows relatively modest, apparently constitutive transactivation. It binds as a monomer to the consensus 5'-(T/C)(C/A)AAGGX(C/T)X-3', which consists of a receptor binding hexamer with a 5' extension, found in a large number of genes (21-23, 28-38). By sequence and direct structural analysis, LRH-1 is highly related to the orphan receptor SF-1 (NR5A1). Since LRH-1 and SF-1 share more than 95% amino acid sequence identity in their DNA binding domain they recognize the same DNA sequences. Both receptors contain a distinctive 26-aa extension, called the Ftz-F1 domain, in their DNA binding domain, which is a critical determinant for the specificity of DNA binding (39). As diagrammed in Figure 1, the x-ray crystal structure of LRH-1 bound to its monomeric site shows that the "P box" helix interacts with the major groove of the AGGX(C/T)X hexamer, as in other nuclear receptors, while the C-terminal extension of the DNA binding domain contacts the minor groove of the adjacent (T/C)(T/C)A motif (40).

3.2. Crystal structures and potential ligands

LRH-1 exists as multiple isoforms in different species, though specific in vivo roles of the isoforms have not been elucidated (21, 23, 41). However, the differences in the major isoform of human LRH-1 documented in HeLa cells, and mouse form lacking 49 amino acids at the N-terminus raise the possibility of species specific roles of LRH-1 (23).

The initial crystal structure of the mouse LRH-1 ligand binding domain revealed that it can form a stable active monomeric structure with a large unoccupied hydrophobic pocket in the absence of ligand, coregulator peptide, or heterodimeric receptor partner, corroborating the observed constitutive activity of LRH-1 in many different cellular contexts (13). Single amino acid substitutions directed at hindering potential ligand binding failed to reduce transcriptional activity, supporting the potential for ligand-independent transactivation. In addition, Sablin et al. suggested that LRH-1 can interact with SMRT and, paradoxically, is strongly activated by the corepressor. This observation is striking since SF-1 also interacts strongly with SMRT but is repressed by the corepressor (42). The functional significance of this apparently differential interaction remains to be defined.

Following the initial report on mouse LRH-1, human LRH-1 and SF-1 from both species have been reported to crystallize with bacterially derived phospholipids in their ligand binding pockets (14-17). These phospholipids are predominantly phosphatidylgycerol and phosphatidylethanolamine, with 16-18 carbon tails in their fatty acid moieties. In these structures, the phospholipid head groups extend out of the pocket without directly contacting the receptor, suggesting that phospholipids with distinct head groups could interact with the NR5A receptors. Consistent with this, phosphatidyl inositols were found to bind hLRH-1 in a membrane immobilized lipid binding assay and were suggested as endogenous ligands (15).

A crucial issue that remains to be resolved is whether phospholipids actually function as physiologically

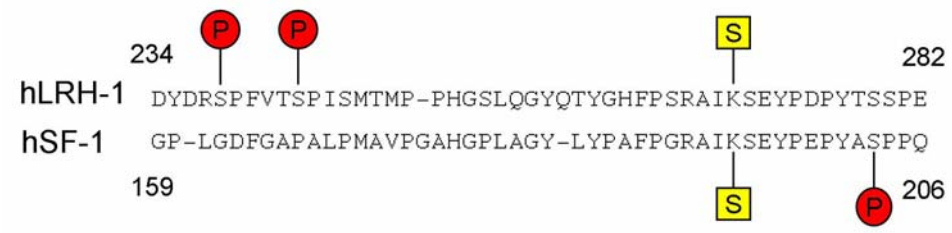

Figure 2. Posttranslational modification targets in the hinge domains of human LRH-1 and SF-1. The sumoylation site is conserved in LRH-1 and SF-1, while phosphorylation sites are not. This approximately 50 amino acid segment of the hinge region has 38% sequence identity, which is less than the 91% and 61% identity in the DNA binding and ligand binding domains.

relevant modulators of LRH-1 and SF-1 function, or perhaps simply as cofactors to stabilize the receptors as seems the case for fatty acids and HNF4-alpha (43). Support for this possibility is provided by the observation of decreased transcriptional activity with a series of mutant derivatives of LRH-1 that decrease phospholipid binding (14), and also by the identification of synthetic agonists for both LRH-1 and SF-1 (44). More directly, a tandem mass spectrometry analysis of SF-1 expressed in a human adrenal cell line revealed that it was bound by a series of phospholipids, including a relatively low molecular weight form of phosphatidic acid, and functional studies showed that an unusual form of phosphatidic acid with two saturated 14 carbon acyl chains could increase transactivation by SF-1, but not LRH-1 (45). These results extended those of a similar mass spectrometry and functional analysis of SF1 that identified the sphingolipids sphingosine and lyso-sphingomyelin as inhibitors of SF-1 transactivation (46). Overall, it seems likely that diverse biologically active lipids modulate NR5A function.

3.3. Modulations of transcriptional activity

In certain promoter contexts, LRH-1 alone is not enough to activate transcription, and appears to function as a competence factor to enhance promoter activity driven by other transcription factors (21, 33, 47-49). Consistent with this, there are not many reports on the direct interaction of LRH-1 with coactivators. Although interactions with SRC/p160 family members and PGC-1alpha have been reported (14, 50), it has also been suggested that LRH-1 strongly interacts with the C-terminal glutamine rich domain of SRC-1 (51) but not the LxxLL motif containing receptor interaction domain generally required for coactivator interaction.

In contrast to the weak interaction with known coactivators, LRH-1 shows a strong interaction with another orphan receptor, small heterodimer partner (SHP; NR0B2), which acts as a corepressor (47, 50, 52). Crystal structure analysis defined the interaction between LRH-1 and SHP NR box and explained the preference of SHP for interaction over other coregulators (14, 53). As described in more detail below, it is thought that SHP is a dominant regulator of LRH-1 activity in liver, and potentially other tissues.

Like many other nuclear hormone receptors, LRH-1 has also been reported to undergo posttranslational modification to regulate its transcriptional activity. The two major modifications identified are sumoylation (54) and phosphorylation (55), which have opposite effects on transactivation. SUMO modification of the hinge region localizes LRH-1 to promyelocytic leukemia protein (PML) nuclear bodies, thereby excluding the transcription factor from active chromatin and blocking its transcriptional activity (54). The major sumoylation site (Lys^{224}; Lys^{270} on hLRH-1 variant 1) is conserved in SF-1 at Lys^{194} (Figure 2). The underlying repression mechanism also appears highly conserved in these two receptors (56, 57). Although aimed at the same hinge domain, modification by phosphorylation targets non-conserved sites on the two proteins and enhances their transcriptional activities (Figure 2). Human LRH-1 has two serine residues at 238 and 243, missing in SF-1, which can be phosphorylated by protein kinase C (PKC) dependent pathways (55). Ser^{469} of hLRH-1 LBD domain has also been suggested as a potential target for protein kinase A dependent activation of human aromatase PII promoter (58). However, activation of LRH-1 by PKC or PKA dependent pathways appears to be tissue specific, since direct activation of mLRH-1 by these two pathways has not been observed in different tissue systems (31). In the case of SF-1, only Ser^{203} is phosphorylated, thereby stabilizing the protein and enhancing coactivator recruitment (42). The potential functional interactions of hinge domain phosphorylation and sumoylation are an important issue that remains to be explored.

4. PHYSIOLOGICAL ROLES

4.1. Development and bile acid/cholesterol homeostasis

The first suggested physiological role of LRH-1 was in expression of $alpha_1$-fetoprotein, a marker of endodermal specification during early liver development and a member of the albumin gene family (21). Another important association with endodermal differentiation emerged from the demonstrations that the mouse LRH-1 gene promoter has binding sites for transcription factors important for endodermal determination and hepatic differentiation such as GATA, Nkx, and HNF4-alpha, and that LRH-1 in turn contributes to expression of genes encoding transcription factors critical to early hepatic differentiation such as HNF3-beta, HNF4-alpha, and HNF1-alpha (59).

An even earlier developmental function of LRH-1 is its ability to activate expression of Oct4, which is

required to maintain pluripotence at the earliest stages of both embryonic development and in ES cell differentiation (60). The importance of LRH-1 in development is confirmed by its broad expression in the early embryo and especially by the very early embryonic lethal phenotype in homozygous LRH-1 knock out mice (26).

A careful expression profile of numerous adult mouse tissues revealed that LRH-1 mRNA is most abundant in ovary (27). This is consistent with several additional studies in mice and other species (25, 32, 61, 62). Expression in ovary appears to be confined to granulosa cells, corpus luteum and luteinized follicles, where the ovarian steroid hormones are synthesized by the action of cytochrome P450 steroid hydroxylases. Interestingly, aromatase, which converts androgens into estrogens, and side chain cleavage P450 ($P450_{scc}$), which catalyses the conversion of cholesterol to pregnenolone, have been proposed as potential ovarian LRH-1 target genes, suggesting a regulatory role for LRH-1 in ovarian steroidogenesis (31, 38). The elucidation of the impact of LRH-1 expression in ovarian development and ovulation awaits the development of ovarian specific LRH-1 null mice.

As expected from its name, the role of LRH-1 has been best characterized in the liver, where it has been identified as a key regulator of Cyp7A1, the first and rate-limiting enzyme in the classic or neutral pathway of bile acid biosynthesis (23, 47, 52). Tight control of Cyp7A1 gene expression is very important in not only bile acid homeostasis but also cholesterol homeostasis, since bile acid synthesis is the major pathway to remove cholesterol from the body (63). Thus, loss of Cyp7A1 function in humans results in decreased bile acid excretion and elevated hepatic and serum cholesterol, highlighting its critical role in cholesterol homeostasis (64). Consistent with this, mice lacking LXR-alpha, a feed forward regulator of Cyp7A1 gene expression, showed massive hepatic accumulation of cholesterol when fed a high cholesterol diet (65). A number of studies suggest that LRH-1 is essential for proper Cyp7A1 promoter activity (23, 47, 52). In mice carrying an LRH-1 transgene controlled by the zinc inducible metallothionein I promoter, Cyp7A1 gene expression was clearly stimulated 6 hours after zinc injection (26). However, Cyp7A1 gene expression was increased, rather than decreased as expected, in heterozygous LRH-1 null mice (26, 66). Making the matter more complicated, adenovirus mediated overexpression of LRH-1 in wild type mice strongly suppressed Cyp7A1 gene expression 3-5 days after infection (66), sharply contrasting not only with the observations in the transgenic mice, but also with increased Cyp7A1 expression at an earlier time point (48hrs) after adenoviral infection (36).

The role of LRH-1 as a positive regulator of expression of the orphan receptor SHP (67) likely explains at least some of these discrepancies. In an elegant nuclear receptor cascade, LRH-1 combines with the bile acid receptor FXR to activate SHP expression when bile acid levels are elevated (47, 52). This results in potent inhibition of Cyp7A1 expression via repression of transactivation by LRH-1, HNF4-alpha and potentially other targets, which promotes the normalization of bile acid levels. It is apparent that the level of LRH-1 expression is only one of several factors that control Cyp7A1 expression. In particular, it would be expected that acute upregulation of LRH-1, for example via adenoviral overexpression, would result initially in the observed increase in Cyp7A1 expression, followed by decreased levels due to the negative effects of SHP.

It is important to emphasize that this "simple" LRH-1 – FXR – SHP loop is only one of several regulatory inputs in the increasingly complex area of bile acid homeostasis. Thus, SHP is also required for the negative regulation of Cyp7A1 expression in response to Fibroblast Growth Factor (FGF) 15/19 activation of its receptor FGFR4 (68). In this loop, it is activation of FXR in the gut that results in increased FGF15/19 expression and decreased hepatic bile acid production. The existence of additional, SHP-independent pathways was clearly demonstrated by the ability of dietary bile acids, but not synthetic FXR ligands, to repress Cyp7A1 expression in SHP null mice (69, 70).

In addition to Cyp7A1, LRH-1 also directly controls expression of a number of additional enzymes and transporters involved in cholesterol and bile acid metabolism, including sterol 12alpha hydroxylase (Cyp8B1), multidrug resistance protein 3 (MRP3), cholesteryl ester transfer protein (CETP), Scavenger receptor class B type I (SR-BI), mouse apical sodium dependent sodium-dependent bile acid transporter (ASBT), and human Apolipoprotein AI (ApoAI) (28-30, 32, 34, 36, 71). All of these genes contain LRH-1 response element in their promoter and are regulated positively by LRH-1.

ApoAI, an initiator of high density lipoprotein (HDL) biogenesis, functions as an acceptor molecule for phospholipids and cholesterol effluxed from peripheral tissues, assembling pre-HDL particles. The mature HDL particles in plasma are transported into hepatocytes via the SR-BI receptor. In an alternative pathway, cholesteryl esters from HDL in plasma can be transferred into apolipoprotein B-containing lipoproteins to form VLDL by CETP, and eventually taken up by the liver through LDL receptors. In liver, cholesterols and cholesteryl esters are converted to bile acids by the series of enzymatic action by Cyp7A1 and Cyp8B1 to be secreted out of the liver via bile. Therefore, all these LRH-1 potential targets are involved in cholesterol transfer to liver and eventual elimination to bile acids, suggesting that LRH-1 is a crucial regulator for cholesterol metabolism (Figure 3). The regulation of Asbt and MRP3 by LRH-1 suggests that LRH-1 also plays an important role in bile acid recycling and homeostasis (34). Although regulation of Asbt by LRH-1 was manifested only in mouse not in rat, regulation of Mrp3 has been demonstrated in both human and mouse system (29, 71). The upregulation of Mrp3 by LRH-1 is protective for the liver uploaded with bile acids upon bile duct ligation. This regulation is achieved by an increased expression of LRH-1 and requires intact TNF-alpha

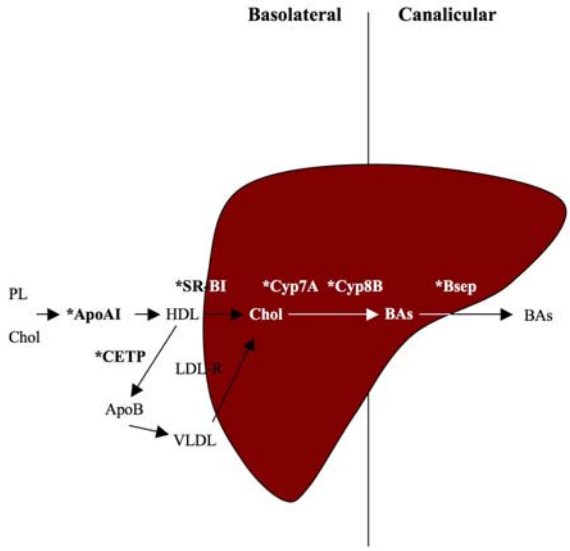

Figure 3. Reverse cholesterol transport systems. Typical cholesterol efflux pathways from peripheral tissues to liver are depicted with key enzymes and transporters potentially regulated by LRH-1, which are highlighted with asterisks. PL; phospholipids, Chol; cholesterol, BAs; bile acids.

signaling because TNF-alpha null mice failed to increase Mrp3 expression upon bile duct ligation (71). Overall, it seems clear that LRH-1 is a major contributor to the complex network of bile acid and cholesterol homeostasis, but this awaits confirmation by appropriate genetic studies using, for example, liver specific ablation of LRH-1 expression.

4.2. Pathologic roles in colon and breast cancer

Besides its important function in cholesterol and bile acid homeostasis, LRH-1 is also involved in cancer development, especially colon and breast cancer (31, 35, 37). In colon cancer, LRH-1 has a unique role in regulating target gene expression. In this paradigm, LRH-1 functionally interacts with the beta-catenin/Tcf4 signaling pathway to increase expression of cyclin D1 and cyclin E1 (37). Haploinsufficiency of LRH-1 in mice reduces the level of G1 cyclins, BrdU incorporation, and length of the crypt in the intestine (37). Interestingly, LRH-1 promotes expression of the G1 cyclins indirectly via an interaction with beta-catenin, which is recruited to target gene promoters through interaction with Tcf4. The LRH-1 DNA binding domain is dispensable for the cyclin D1 activation. In contrast, in the cyclin E1 promoter LRH-1 directly binds a perfect consensus site to activate gene transcription, and beta-catenin potentiates the transcriptional impact by direct interaction with LRH-1. A similar interaction has also been demonstrated in transcriptional effects mediated by SF-1 (72-75), suggesting that beta-catenin serves as a coactivator for the Ftz-F1 subfamily of nuclear receptors.

The increased expression of these proliferative targets eventually contributes to development of colon cancer, indicated by a subsequent study using $Apc^{min/+}$ mice, a genetic model of intestinal tumorigenesis (24). Thus, LRH-1 haploinsufficiency in the $Apc^{min/+}$ background reduced the number of intestinal tumors, and a reduced incidence of colon carcinogenesis was also observed in azoxymethane induced mouse colon carcinogenesis. However, the expression of LRH-1 in tumors was reduced in the two tumorigenic mouse models regardless of the number of functional LRH-1 alleles (24), leaving the direct linkage of tumor development with LRH-1 somewhat uncertain.

LRH-1 also plays a quite different role in breast cancer development and progression. Local estrogen concentration is an important factor for the growth of breast cancers (76), and LRH-1 stimulates expression of Cyp19, which encodes aromatase, a key estrogen biosynthetic enzyme (31). In turn, LRH-1 is apparently directly induced by activated estrogen receptor in breast cancer cells, suggesting the existence of a positive feedback loop (77). In different tissues such as placenta, ovary, testis, brain, bone, and adipose, expression of Cyp19 gene is controlled by alternative promoters, which are regulated by distinct factors (78). In adipose tissues surrounding breast tumors, Cyp19 expression is dependent on promoter II, instead of normal promoter I.4, and conditions that increase promoter II activity can enhance the growth and aggravate the progression of adjacent tumors. LRH-1 binds directly to promoter II and synergistically activates it in combination with PKC/PKA activators including forskolin and phorbol ester, suggesting that LRH-1 is positively involved in breast cancer development (31). Indeed, adenoviral overexpression of LRH-1 in such adipose tissue potentiates effects of prostaglandin E_2 (PGE_2), a major hormone contributing to promoter II activity through PKC and PKA pathways (79).

5. PERSPECTIVE

Although the elucidation of the physiological functions of LRH-1 has been hampered by the early embryonic lethal phenotype of LRH-1 homozygous null mice, it is clear that LRH-1 plays important regulatory role in cholesterol and bile acid homeostasis in conjunction with SHP. In mice, LRH-1 functions with LXR-alpha to control Cyp7A1 expression, but humans lack the LXR response element in the Cyp7A1 promoter and thus lack the ability to increase Cyp7A1 expression in response to hypercholesterolemia (80). It seems possible that activation of LRH-1, via either an agonist ligand such as a phospholipid, or post-translational modification such as phosphorylation, could have therapeutic value in promoting cholesterol catabolism. From an opposite perspective, an LRH-1 antagonist could be useful in treating or preventing colon or breast cancer. The identification of the endogenous LRH-1 ligand(s) and the further characterization of this important nuclear receptor will certainly lead to new insights into its impact on normal physiology and pathologic states, and will undoubtedly suggest additional therapeutic opportunities.

6. ACKNOWLEDGMENT

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Abbreviations: LRH-1: liver receptor homolog-1, Cyp7A1: cholesterol 7alpha hydroxylase, SF-1: steroidogenic factor-1, LXR: liver X receptor , FXR: farnesoid X receptor, HNF-4: hepatocyte nuclear factor-4, Ftz-F1: Fushi Tarazu-Factor 1, SHP: small heterodimer partner, Cyp8B1: sterol 12alpha hydroxylase, MRP3: multidrug resistance protein 3, CETP: cholesteryl ester transfer protein, SR-BI: Scavenger receptor class B type I, ASBT: apical sodium dependent sodium-dependent bile acid transporter, ApoAI: Apolipoprotein AI, HDL: high density lipoprotein.

Key Words: Liver Receptor Homolog-1, Steroidogenic Factor-1, Cholesterol 7alpha Hydroxylase, Crystal Structure, Bile Acid, Cholesterol, Phospholipid, Review

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