Therapeutic potential of Liver Receptor Homolog-1 modulators

ARTICLE *in* THE JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY · JANUARY 2012

Impact Factor: 3.63 · DOI: 10.1016/j.jsbmb.2011.12.017 · Source: PubMed

Journal of Steroid Biochemistry & Molecular Biology 130 (2012) 138–146

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Review

Therapeutic potential of Liver Receptor Homolog-1 modulators

Kyren A. Lazarus[a,1], Dhilushi Wijayakumara[a,1], Ashwini L. Chand[a], Evan R. Simpson[b], Colin D. Clyne[b,*]

[a] Prince Henry's Institute, Clayton, Victoria 3168, Australia
[b] Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3168, Australia

ARTICLE INFO

Article history:
Received 10 February 2011
Received in revised form
14 November 2011
Accepted 27 December 2011

Keywords:
Orphan nuclear receptor
NR5A2
LRH-1

ABSTRACT

Liver Receptor Homolog-1 (LRH-1; NR5A2) belongs to the orphan nuclear receptor superfamily, and plays vital roles in early development, cholesterol homeostasis, steroidogenesis and certain diseases, including cancer. It is expressed in embryonic stem cells, adult liver, intestine, pancreas and ovary. It binds to DNA as a monomer and is regulated by various ligand-dependent and -independent mechanisms. Recent work identified synthetic ligands for LRH-1; such compounds may yield useful therapeutics for a range of pathologic conditions associated with aberrant expression and activity of LRH-1.

Contents

1. Introduction

Nuclear receptors (NRs) comprise a large superfamily of mammalian transcription factors that are essential in embryonic development, differentiation, metabolism and cell death [1–3]. NRs mediate cellular signals to the nucleus by directly interacting with DNA sequences known as hormone response elements (HREs) [3,4]. Unlike most NRs, orphan NRs regulate transcription independent of known ligands. Orphan receptors play a diverse and important biological role in development and adult physiology [1,5–7].

Liver Receptor Homolog-1 (LRH-1; NR5A2; FTZ-F1; FTF; CPF) is one such orphan nuclear receptor that plays vital roles in early development and is important for bile acid synthesis, cholesterol metabolism and steroidogenesis in the adult [8–14] (see Fig. 1).

* Corresponding author. Tel.: +61 3 9594 4372; fax: +61 3 9594 6125.
E-mail address: colin.clyne@princehenrys.org (C.D. Clyne).
[1] Equal contribution.

0960-0760/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2011.12.017

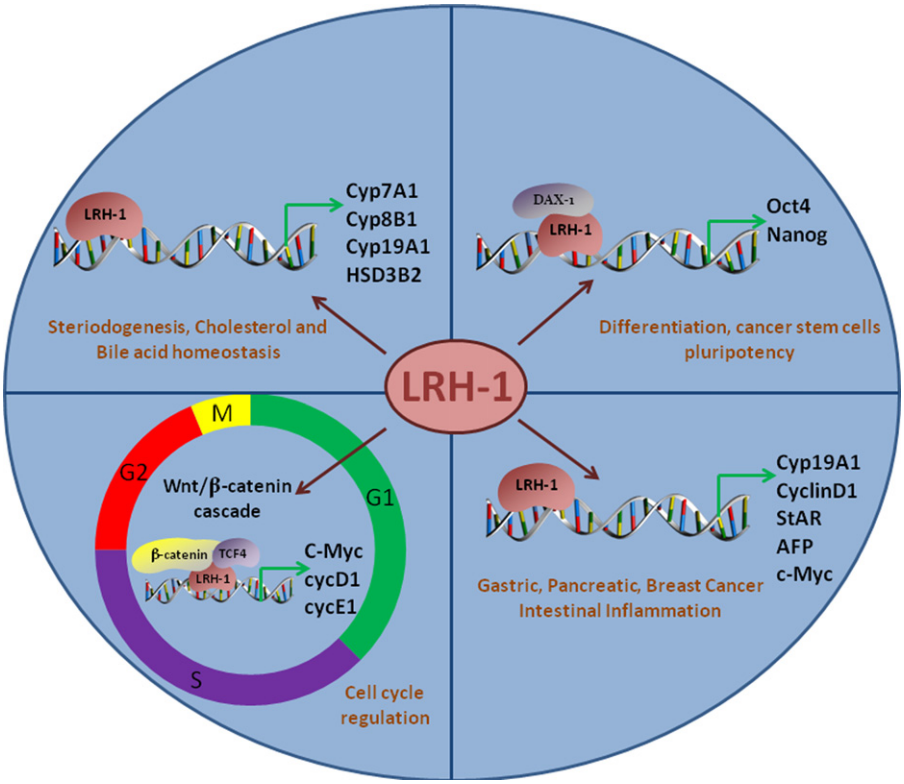

Fig. 1. Physiological actions of LRH-1.

LRH-1 and its mammalian homolog Steroidogenic Factor-1 (SF-1) binds to DNA as monomers to nuclear receptor half site sequences [1,15,16]. As LRH-1 is constitutively active [8], its regulation of function occurs via interactions of co-activators and co-repressors [17–19]. Furthermore, interactions with other orphan receptors including small heterodimer partner (SHP) [9,20–24] and dosage sensitive sex reversal-adrenal hypoplasia congenital gene on the X chromosome, gene 1 (DAX-1) cause the repression of LRH-1 transcriptional activity [25,26]. Ligands identified for LRH-1 and its homologue, SF-1 include small drug-like molecules [8] and endogenous phospholipids [27–30]. With its wide implication for disease progression in various organs, this review will focus on the therapeutical potential of LRH-1 modulators.

2. LRH-1: structure

The human gene encoding LRH-1 spans more than 150 kb of chromosome 1q32.11 and has eight exons. LRH-1 binding is dictated by the Ftz-F1 box and it binds as a monomer to the extended half site YCAAGG YCR (where Y is any pyrimidine and R is any purine), the recognition motif for the NR5A subfamily of nuclear receptors [8,16,31,32]. The ligand binding domain (LBD) of most NRs consists of twelve α-helical regions, folded into a three-layered, anti-parallel helical sandwich with a conserved β-turn between H5 and H6 [8]. The LBD also contains a pocket to which a ligand binds causing a reversible conformational change. This repositions H12 allowing for co-activator recruitment [8,32]. However, in orphan receptors, the key to constitutive activation may lie in the N-terminal region of the LBD. This is strongly conserved amongst orphan receptors and shares limited sequence amongst other nuclear receptors [8]. The presence of an additional structure pertaining to an extension of an H2 layer may provide an explanation of the stabilised conformation, by stabilising H12 which contains the C-terminal activation helix (AF-2) [8]. The folding over of the AF-2 region allows the LBD to be held in an active conformation without the need for a ligand.

3. Established roles of LRH-1: cholesterol metabolism

A key biological function of LRH-1 is the regulation of cholesterol metabolism via its effect on bile acid homeostasis. LRH-1 regulates enterohepatic development and function via the expression of key genes involved in the regulation of bile acid synthesis, cholesterol homeostasis and transport (Table 1) [8,10,15,20,33–38]. LRH-1 activates gene transcription of the rate-limiting enzyme in bile acid biosynthesis, cytochrome P450 family 7A1 or Cholesterol 7 α-hydroxylase (Cyp7A1) [20,33,39,40]. In humans, the loss of function of Cyp7A1 results in a decrease in bile acid excretion and an increase in hepatic and serum cholesterol levels [41]. However, LRH-1 ± heterozygous mice have elevated CYP7A1 and CYP8B1 mRNA levels [42]. This may be due to a dominant effect of LRH-1 on Cyp7A1 and Cyp8B1 transcription. Interestingly, hepatocytes and intestinal epithelium (IE) specific LRH-1 knockout mice showed no overt abnormalities [43]. Furthermore, LRH-1 deficiency in the IE, had no effect on Cyp7A1 levels. This discrepancy in observations may be due to the minor role LRH-1 plays in Cyp7A1 regulation, or there might be an alternative redundant factor regulating Cyp7A1 in the absence of LRH-1 [43]. Further work need to be undertaken in determining the role of LRH-1 in the feedback mechanism of bile acid synthesis. In contrast to Cyp7A1, Cyp8B1 levels were significantly decreased with LRH-1 deficiency, consistent with previous findings.

LRH-1 positively regulates expression of other enzymes and transporters involved in reverse transport of cholesterol and bile acid synthesis pathways. These genes which contain the LRH-1 response element (nuclear receptor half site) in their promoters

include cytochrome P450 family 8B1 (Cyp8B1) or Sterol 12α hydroxylase, multidrug resistance protein 3 (MRP3), cholesteryl ester transfer protein (CETP), scavenger receptor class B type I (SR-BI), mouse apical sodium-dependent bile acid transporter (ASBT) and human Apolipoprotein A1 (ApoA1) [8,20,33,34,37,44,38,45]. These findings are consistent with the selective knockout of LRH-1 in hepatocytes where expression of these targets were significantly ablated [43]. By activating gene expression, LRH-1 impacts various functions outlined. Increase in ApoA1 allows the initiation of high density lipoprotein (HDL) biosynthesis and for ApoA1 to act as an acceptor of cholesterol and phospholipids effluxed from peripheral tissues. Up regulation of SR-BI receptors allows the transfer of mature HDL particles from plasma into hepatocytes [46]. CETP transfers the cholesteryl esters from plasma HDL into Apolipoprotein B-containing triglyceride-rich very low density lipoproteins (VLDL). These VLDL particles are acted upon by lipoprotein lipase and the fatty acids are taken up by the adipose and skeletal muscle. The cholesterol-rich remnants are then taken up by the liver [37,47].

Within hepatocytes, cholesterol and cholesteryl esters are converted to bile acids by Cyp7A1 and Cyp8B1 for secretion out of the liver in bile. Furthermore MRP3 and ASBT are involved in bile acid recycling, indicating that LRH-1 is important for bile acid homeostasis [44,48]. Major subsets of LRH-1 gene targets are involved in the transfer of cholesterol to the liver and subsequent elimination into bile acids, and in bile acid synthesis, highlighting the importance of LRH-1 in cholesterol metabolism [41].

4. Emerging roles of LRH-1 in disease

4.1. Embryonic stem cells: pluripotency and differentiation

LRH-1 is a critical factor in early embryonic development, and LRH-1 null mice die at embryonic day 6.5–7.5 with features typical of visceral endoderm dysfunction [42]. One of the key factors essential for maintaining pluripotency in embryonic stem cells (ESCs), Oct4 is regulated by LRH-1 [49,50]. Its role in Oct4 regulation is confirmed with the loss of Oct4 expression in the absence of LRH-1 [49]. In addition to Oct4, LRH-1 is known to regulate other factors required to maintain pluripotency [51,52].

A recent report suggests that LRH-1 regulates Oct4 and Nanog induction and its expression is regulated via the Wnt signalling pathway, via binding of β-catenin to activate an embryonic-specific LRH-1 promoter [50] thus identifying a new pathway for the regulation of self renewal in ESC.

Somatic cells can be reprogrammed to induced pluripotent stem cells (iPSCs) with the introduction of Oct4, Sox2 Klf4, and c-Myc [53]. Of these factors, Oct4 cannot be replaced by other factors to allow for the generation of iPSC from somatic cells [54]. A study entailing a screen of nuclear receptors for their ability to enhance reprogramming efficiency led to the discovery that LRH-1, and to a lesser extent SF-1 could replace Oct4 in the reprogramming of mouse embryonic fibroblasts to iPSCs further indicating its vital role in the regulation of Oct4 and maintaining pluripotency [49,55].

A point mutation within the LRH-1 LBD (A368M) that interferes with the binding of endogenous phospholipids [56] did not abrogate its reprogramming capacity, suggesting that phospholipid binding is not essential for this function. However, since LRH-1 is constitutively active, and its activity appears to be regulated primarily by binding of protein co-regulators (see below), further studies into looking at the exact function of phospholipids in terms of regulating LRH-1 function are required. The possibility remains that pharmacological modulation of co regulator interactions could impact LRH-1 function in stem cells.

Recent work suggests that a transcriptional partner of LRH-1 to activate Oct4 expression is DAX-1. DAX-1 is abundant in ESCs and it plays a role in maintaining pluripotency [57]. DAX-1 expression is controlled by LRH-1 and Nanog [58]. In mouse ESC, DAX-1 interaction with LRH-1 does not inhibit but rather activates Oct4 gene transcription [46]. This is thought to be due to the interaction of steroid receptor RNA activator (SRA) with DAX-1 to allow an activation function [59]. This interaction of LRH-1 with DAX-1 is evidence of importance of co-regulator interaction in the modulation of LRH-1 activity. It also raises the possibility that co-regulator interaction allows for tissue specific LRH-1 functions.

4.2. Ovarian function: steroidogenesis and luteinisation

LRH-1 is expressed at relatively high levels in the ovary, with highest expression in granulosa cells and corpora lutea, with no expression observed in theca cells [60]. Although SF-1 is also present in the ovary its expression is lower in LRH-1 expressing cells and highest in the theca [60]. The two nuclear receptors are differentially regulated, SF-1 mRNA and protein increased in granulosa cells by estradiol, whereas LRH-1 expression was increased by FSH in granulosa cells and by prolactin in luteal cells [63]. Both receptors have been shown to regulate ovarian steroid synthesis although the relative roles of each are still unclear, since they both share common target genes including aromatase, steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase (HSD3B2) and inhibin α subunit [61–65]. Recent work has

Table 1
LRH-1 target genes and functions.

	Target genes	Organ/tissue	Function
Embryonic	AFP-1, Prox1, HNF3β, HNF4α, HNF1α	Liver	Early enterohepatic development [34,42,110,111]
	Oct4	ES cells	Early embryonic development [49,55]
Adult	CYP19A1	Preadipocytes	Steroidogenesis [14,22,75,23]
	APOA1, SR-B1, CETP, SHP	Liver	Cholesterol transport [46,112]
	CYP7A1, CYP8B1, CEL, MRP3	Liver	Bile acid homeostasis [8,20,34,44]
	ABST Cyclin D1, TCF4/β-cantenin	Intestine	Bile acid homeostasis [37]
			Intestinal epithelium renewal [69]
	CYP11A1, CYP19, HSD3B2	Ovary	Ovary steroidogenesis [8,65]
	SR-BI		Cholesterol transport [8]
	LHβ	Gonadotrope	Normal reproductive function [113]
Disease	Cyclin E1, TCF4, β-cantenin	Intestine	Gastric cancer [69,95]
			Intestinal inflammation [69,72]
			Pancreatic cancer [89]
	CYP19A1, Cyclin D1, StAR, AFP	Breast	Tumour progression and carcinogenesis
	C-Myc	Pancreas	Pancreatic cancer [89]
			Pancreatic cancer [89]

suggested that LRH-1 preferentially regulates progesterone over oestrogen production [12,66,67]. Consistent with this, granulosa cell-specific LRH-1 null mice exhibit anovulation due to failure of cumulus expansion, luteinisation, and follicular rupture [68]. Loss of LRH-1 in granulosa cells also impaired progesterone synthesis due to reduced expression of StAR and cytochrome P450 side-chain cleavage (P450scc). Interestingly aromatase expression was unaltered. This dramatic phenotype highlights the potential of LRH-1 antagonists as novel contraceptives, since loss of LRH-1 inhibits both ovulation and luteinisation [68].

4.3. Colon cancer and inflammation

Recent evidence indicates a role of LRH-1 in colon cancer development and progression. LRH-1 is highly expressed in intestinal crypts where it is involved in the control of cell proliferation and renewal [69]. Botrugno et al. have demonstrated that LRH-1 promotes intestinal cell proliferation by stimulating the expression of the G_1 cyclins D1 and E1 [69]. In these cells, LRH-1 co-activates β-catenin/Tcf4 to induce cyclin D1 and c-Myc expression, and also binds directly to the cyclin E1 promoter to promote transcription in synergy with β-catenin [69]. Consistent with this, haplo-insufficiency of LRH-1 markedly protects against tumour development in both genetic ($Apc^{Min/+}$) and chemical (azoxymethane) induced models of intestinal cancer [70]. Alterations in LRH-1 expression and localisation are also seen in human colon cancer, with increased LRH-1 expression observed in surface epithelial cells that are normally LRH-1 negative and non-proliferative [70]. However, the lack of significant proliferative phenotype in intestinal specific LRH-1 knock-out mice [41] raises questions as to the role of endogenous LRH-1 in vivo.

LRH-1 also plays an important role in glucocorticoid synthesis within the intestine by inducing expression of steroidogenic enzymes (cholesterol side chain cleavage enzyme and 11β-hydroxylase that catalyse the first and last steps in glucocorticoid formation, respectively) in intestinal epithelial cell lines [71]. This is likely of physiological relevance since haplo-insufficiency of LRH-1 in mice reduced expression of these enzymes and glucocorticoid synthesis in response to immunological stress [71]. Similar effects were seen in mice with an inducible intestinal epithelial deletion of LRH-1 [72]. Consistent with these findings, expression of both LRH-1 and its steroidogenic target genes were reduced in patients with Crohn's disease and ulcerative colitis [72].

4.4. Breast cancer

In human breast adipose, LRH-1 is expressed in low levels, localising specifically in the stromal fraction [13]. However in breast cancer, LRH-1 expression is high and localised both in tumour epithelial cells and intra-tumoural stroma [73,74]. High expression is observed in primary invasive breast carcinoma and ductal carcinoma in situ [74]. Although the effects of LRH-1 in breast cancer are not fully understood, evidence suggests that its roles are tightly integrated with the oestrogen signalling pathway (Fig. 2).

One of the first identified target genes of LRH-1 in breast adipose stromal cells is *CYP19A1* [13,14,75], encoding for cytochrome P450 family 19A1 or aromatase. Aromatase is a critical enzyme required for the conversion of androgens to oestrogens. After menopause,

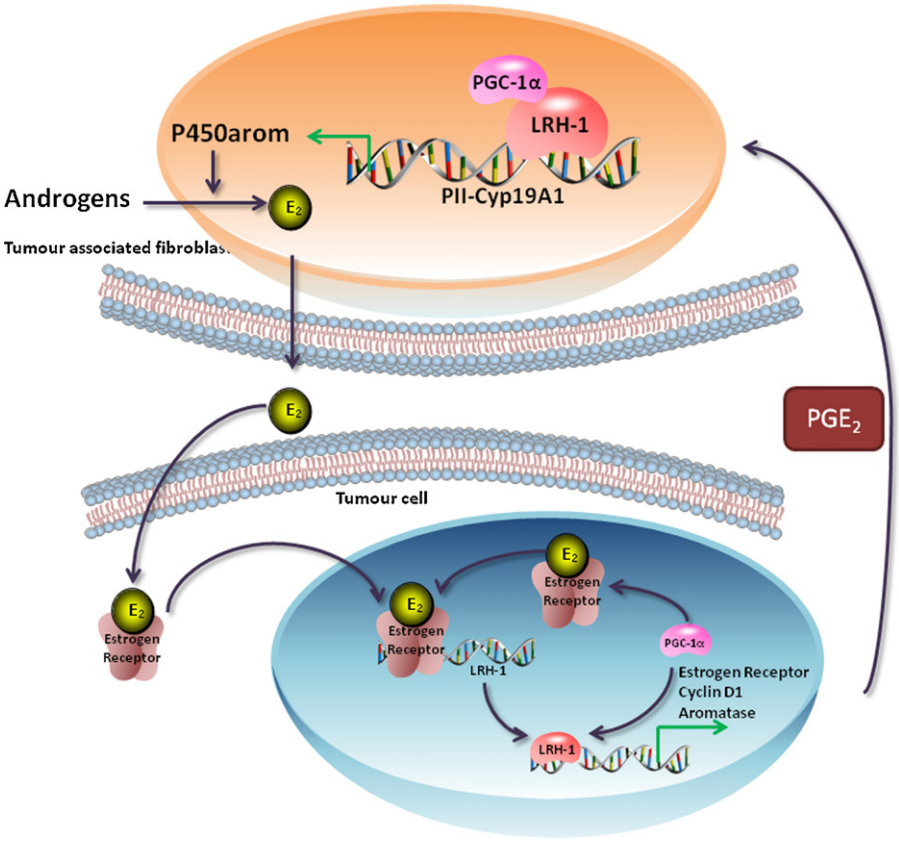

Fig. 2. LRH-1 actions in a breast cancer cell. LRH-1 activates aromatase transcription in cancer associated adipose fibroblasts leading to increased oestrogen synthesis, thus promoting tumour growth. The oestrogen receptor (ERα) activates LRH-1 expression in malignant epithelial cells. LRH-1 in turn further up regulates ERα and aromatase expression. Cyclin D1 a known target gene is also up regulated by LRH-1. LRH-1 is positively regulated by co-activator PGC-1α, leading to the activation of target genes such as ERα and aromatase. Therefore through its involvement in ERα expression and co-regulators and repressors also act on the ER, suggesting that the LRH-1 signalling pathway is tightly linked with the oestrogen signalling pathway.

when the risk of developing oestrogen-dependent breast cancer is greatest, local aromatase expression in adipose tissue represents the major source of oestrogen in women (as well as in men). Aromatase activity is regulated primarily by transcription of the *CYP19A1* gene via tissue specific promoters [76–78]. By activiating *CYP19A1* transcription, LRH-1 most likely regulates the availability of mitogenic oestrogen for tumour growth [14]. In the tumour context, the aberrant expression of LRH-1 allows the activation of the gonadal-specific aromatase promoter (promoter II). LRH-1 expression in adipose stromal cells is positively regulated by breast tumour derived factors such as prostaglandin E_2 [75]. In addition to stromal cells, aromatase is present in breast cancer epithelium [79], and its expression also regulated by LRH-1 in breast cancer epithelial cells [80].

A second level of cross-talk is evident in that expression of LRH is itself regulated by oestrogen in breast cancer cells. Annicotte et al. reported that the human LRH-1 promoter contains a near-perfect palindromic oestrogen response element (ERE) [74], to which ERα binds to stimulate promoter activity. This is consistent with the observed correlation between LRH-1 mRNA levels and ERα status in a variety of breast cancer cell lines [74], as well as the known positive association between LRH-1 positivity and ERα status in primary human breast carcinoma [11,81]. Knockdown of LRH-1 expression with siRNA in MCF-7 cells inhibits the proliferative effect of oestrogen [74], suggesting that the mitogenic effects of oestrogen may be mediated, in part, via LRH-1.

Furthermore LRH-1 regulates expression of ERα in breast cancer cells; siRNA knockdown of LRH-1 in MCF-7 cells inhibits expression of both ERα, and of ERα target genes such as pS2, whereas transfection of LRH-1 into these cells stimulates ERα expression [81]. This effect is mediated by LRH-1 binding directly to the major ERα promoter used in breast cancer cells [81]. Therefore, LRH-1 both regulates, and is regulated by ERα in addition to regulating the synthesis of its ligand.

Although the above evidence indicates that LRH-1 induces proliferation by stimulating oestrogen signalling, LRH-1 also has oestrogen independent effects on breast cancer cells. siRNA-mediated knockdown of LRH-1 inhibited breast cancer cell motility, invasion and colony formation in both ER +ve MCF-7 and ER −ve MDA-MB-231, as well as the non-tumourigenic MCF-10A mammary epithelial cell line [82]. Over-expression of LRH-1 (in the absence of oestrogen) produced the opposite effects.

In addition, over-expression of LRH-1 resulted in the post-translation cleavage of mature 120 kDa E-Cadherin to its inactive 97 kDa form. This effect may be mediated by matrix metalloproteases (MMPs) since LRH-1 was also shown to induce MMP9 mRNA expression in MCF-7 cells [82], consistent with the known role of LRH-1 as a regulator of MMP9 expression in the ovary [68]. LRH-1 was also shown to influence actin remodelling in breast cancer cell lines [82] which, taken with its effects on E-Cadherin and MMP9, may suggest a role for LRH-1 in promoting epithelial to mesenchymal transition (EMT). Given that LRH-1 has well-characterised roles in mouse and human embryonic stem cells [49,83–85], and that breast cancer stem cells possess many of the characteristics of cells undergoing EMT [86,87], the potential role of LRH-1 in promoting EMT is an intriguing possibility that merits further investigation.

4.5. Pancreatic cancer

Recent genome-wide association studies have linked mutations in the LRH-1 gene and its up-stream promoter regions with pancreatic cancer [88]. In addition, LRH-1 expression is found to be elevated in human pancreatic ductal adenocarcinomas [89]. In pancreatic cancer cells, LRH-1 also mediates tumour cell proliferation via the up regulation of cyclins D1, E1 and c-Myc. Conversely knockdown of LRH-1 expression resulted in cell cycle arrest but not apoptosis in pancreatic cancer cells. This study implicates an important role for LRH-1 in mediating proliferation and differentiation pancreatic ductal adenocarcinomas and therefore a potential therapeutic target for this cancer [89].

4.6. Metabolic disorders

One of the key sites of LRH-1 expression and function is in the hepatocytes where it regulates transcription of genes such as Cyp7A1, Cyp8B1, encoding enzymes critical in bile acid and cholesterol synthesis; scavenger receptor class B member 1 (SR-B1) important for cholesterol transport and SHP, an LRH-1 co-repressor with known roles in obesity and diabetes [20,46,90]. Whilst these roles in the liver are well characterised, until recently the effect of LRH-1 in metabolic disorders was not well understood. A recent study [91] describes two phosphatidylcholine phospholipids, dilauroyl phosphatidylcholine (DPLC) and diundecanoyl phosphatidylcholine (DUPC) as potent agonists of LRH-1, binding to the ligand binding pocket of the human receptor. This is an extension to previous findings where structural analysis of LRH-1 identified bacterial phospholipids bound in the ligand binding pocket [24,92]. These two lipid ligands, DPLC and DUPC potently activate NR5A receptors: LRH-1 (human and mouse homologues) and SF-1 (mouse homologue), however, show no effects on other nuclear receptor function. Lee and colleagues hypothesised that the activation of liver-specific activity of LRH-1 would increase bile acid production thereby having a positive metabolic effect in diabetic and obese disease models. In diet induced obese mice, oral administration of DPLC resulted in increased bile acid production, decreased hepatic steotosis and improved glucose utilisation [91]. Additionally in liver specific LRH-1 knockout animals, DPLC treatment did not show any improvement in the high fat diet induced metabolic phenotype. This suggests a direct effect on LRH-1 receptor activity and/or the downstream activation of LRH-1 dependent pathways in regulating insulin sensitivity. Due to the absorption and clearance of these lipid ligands, the primary site of LRH-1 activation appeared to be the liver and SF-1 activity in the adrenal was not affected. This study therefore reveals a novel, LRH-1 dependent phosphatidylcholine pathway that could be targeted for metabolic disorders such as diabetes.

5. LRH-1 modulators as a novel therapeutic target

Since its identification in 1996, there is accumulating evidence of LRH-1 as an important regulator of pathways involved in metabolism, steroidogenesis, cancer and regulation of pluripotency. Given its expression and functions in various tumours including invasive breast carcinomas, ductal carcinomas in situ, colon, gastric and pancreatic cancers, the potential impact of LRH-1 modulators as therapeutic targets should be considered. Recent progress with the identification of small molecule agonists [93,94] and phospholipid ligands will eventually lead to the development of sensitive LRH-1 modulators. There appears to be therapeutic benefits for both the activation of LRH-1 (in the liver) where it would be beneficial in the treatment of metabolic disease such as diabetes; and in the inactivation of LRH-1 in tumour cells. Suppression of LRH-1 activity in tumour cells (gastric, pancreatic, intestinal, breast) would potentially have an anti-proliferative effect [70,82,89,95]. As LRH-1 is expressed in inflammatory cells such as lymphocytes [89] and in surrounding breast adipose stromal cells, blocking of LRH-1 function would have additional anti-tumour effects such as down-regulating inflammatory pathways and reducing oestrogen synthesis within the tumour milieu. Given the importance of LRH-1 in metabolism and cancer, the

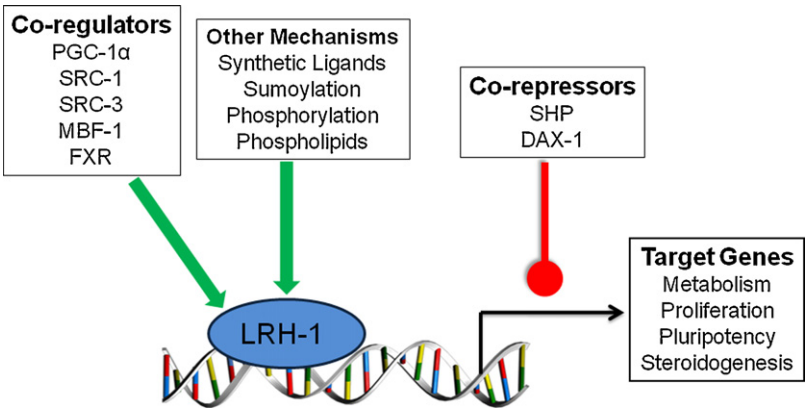

Fig. 3. LRH-1 activity can be regulated by various mechanisms in a tissue specific manner. Regulation mechanisms include synthetic ligands, post-translational modification, phospholipids. Co-regulators and co-repressors also play a role in regulating LRH-1, which then modulates expression of target genes that are involved in metabolism, proliferation, pluripotency and steroidogenesis.

regulation of its activity could potentially lead to important breakthroughs in treatment of these diseases.

The regulation of LRH-1 function occurs via interaction with intracellular phospholipids, transcriptional co-regulators (CoRs) and post-translational modifications such as phosphorylation and sumoylation (Fig. 3). By considering these modes of regulation, new approaches to block or enhance LRH-1 activity may emerge.

5.1. Regulation of LRH-1 activity

5.1.1. Phospholipids as LRH-1 activators

Crystallisation of the LRH-1 ligand binding domain (LBD) (mouse and human isoforms) identified bacterial phospholipids in the ligand binding pocket. Subsequently phospholipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol were shown to bind with human LRH-1 and SF-1 from both mouse and human [24,27,56,92,96] identifying them as endogenous ligands. Functional studies of mouse LRH-1 LBD show that ligands are dispensable for activity [56]. Disruption of the size and shape of the hydrophobic ligand binding pocket does not appear to affect its transcriptional activity. However mutations in the human LRH-1 LBD reduce phospholipid binding, and prevent recruitment of co-activators causing an inhibition of transcriptional activity of LRH-1 [24,96]. In support of this idea is the recent identification of DPLC and DUPC which act as strong LRH-1 activators in vitro and in vivo models [91].

5.1.2. Co-activators of LRH-1

Structurally LRH-1 is permanently held in an active conformation and binds to DNA as a monomer [1,8,15]. LRH-1 activity is primarily regulated by transcriptional co-regulators. LRH-1 can also act as a competence factor by binding to other orphan nuclear receptors and transcriptional complexes to enhance transcription of target genes. Peroxisome proliferator-activated receptor-γ-co-activator-1 α (PGC-1α) is a key co-activator of LRH-1 in the ovary, facilitating LRH-1 mediated differentiation of granulosa cells into progesterone producing luteal cells [12]. This interaction is blocked by a known repressor of LRH-1 activity, DAX-1 in granulosa cells [12]. Since both DAX-1 and PGC-1α can bind to LRH-1 [12], this indicates that the binding affinity of DAX-1 for LRH-1 is stronger than PGC-1α [12]. Furthermore, the interaction between LRH-1 and PGC-1α is also evident in bile acid homeostasis [9]. However this interaction in the liver is blocked by SHP-1 [9]. Recently a genomic-wide interrogation of hepatic faresoid X nuclear receptor (FXR), revealed that FXR binds to LRH-1, and LRH-1 is required for the FXR mediated activation of SHP, Rdh9, Pcx and Pemt [97].

In human adipose stromal cells, PGC-1α enhances LRH-1 dependent aromatase promoter II transcription [13]. The p-160 family members steroid receptor co-activators (SRC-1 and SRC-3) also regulate LRH-1 activity [98]. These co-activators contain an LXXLL motif in the receptor interaction domain; short peptide sequences derived from these co-activators are shown to bind LRH-1 LBD [19]. Multi-protein bridging factor (MBF-1) which also interacts with LRH-1 does not possess the LXXLL motif, typical for most co-activators, but interacts with the TATA-binding protein (TBP) [99]. This interaction is either in isolation or through the recruitment of transcription factor IID complex [99].

5.1.3. Co-repressors of LRH-1

Nuclear receptors SHP and DAX-1 act as repressors of many NRs including LRH-1 by inhibiting co-activator binding [35]. The repression of LRH-1 by SHP has been well-defined due to its high interaction with the nuclear receptor [20,31,33]. Like most co-repressors, SHP binds to the AF-2 region of LRH-1 [8]. The LRH-1–SHP interaction occurs via interaction with LRH-1 residues Arg361 and Glu534 which form an atypical charge clamp [24]. Upon SHP interaction, p160 co-activators are competed out for binding to the CoR domain of LRH-1. In addition to the N-terminal receptor interaction domain, SHP includes a C-terminal domain with autonomous repression function [100]. In breast adipose stromal cells and in hepatocytes, SHP represses LRH-1 activity with the blocking of PGC-1α and LRH-1 interactions [40]. This action in hepatocytes is shown to be mediated via the recruitment of SIRT-1 by SHP [21]. SHP is a well known target gene of LRH-1 and and recent reports show a cooperative action with FXR to activate SHP transcription [97]. DAX-1 colocalises with LRH-1 and SF-1 in granulosa cells [12] and in mouse embryonic stem cells [59]. Crystallisation of mouse DAX-1 with LRH-1 LBD indicates that its N-terminus LXXLL-related motifs interact directly with LRH-1 [26,101,102]. It has also been reported that the C-terminus end is essential for DAX-1 mediated repression [103]. DAX-1 binds to the AF-2 domain as a dimer, binding with high affinity in contrast to most repressors which interact via their single LXXLL motif [25]. It is hypothesised that the DAX-1 dimer binds LRH-1 in a "claw-like" fashion, one of the DAX-1 structures extending into the ligand binding pocket. This feature may act as a sensor for ligand binding, and or additional interactions with components of the transcriptional machinery [25,26].

LRH-1 function is also inhibited by the protein inhibitor of activated signal transducer and activator of transcription-γ (PIASγ) [104]. This inhibition occurs due to the competitive binding of PIAS γ on the AF2 domain, which is involved in the binding of co-activator SRC-1. However, the over-expression of SRC-1 could

partially overcome the LRH-1 mediated induction of CYP11A1 [104]. The silencing mediator for retinoid and thyroid receptors (SMRT) is also shown to represses LRH-1 through an indirect mechanism currently unknown [8].

The role of transcriptional CoRs is critical in the modulation of transcription factor activity. In the case of orphan nuclear receptors, co-regulators may well be the most important mode of functional regulation. Evidence of co-localisation and interaction between CoRs and LRH-1 suggest tissue and cell specific modulation of LRH-1 action. Could targeting the disruption of these CoR interactions provide a means to selectively repressing LRH-1 activity? Due to the presence of SHP and DAX-1 in breast cancer [105,106], it is tempting to postulate their roles in repressing LRH-1 in breast cancer cells. However this possibility requires further investigation.

5.1.4. Post-transcriptional regulation of LRH-1

In addition to CoRs, the activity of LRH-1 is modulated by post-translational modification. Phosphorylation of the serine residues 238 and 243 located within the hinge region of LRH-1 via phorbol 12-myristate 13-acetate (PMA), was found to be important for LRH-1 transactivation [107]. Sumoylation of LRH-1 also occurs in the hinge region, allowing for additional control of its activity via regulation of its subcellular localisation [108]. Sumoylated LRH-1 is localised to the transcriptionally inactive nuclear bodies, away from active chromatin. Interestingly, the newly identified phosphatidylcholine agonists of LRH-1, DPLC and DUPC activated LRH-1 mutants lacking phosphorylation sites (S238, 243A) or the sumoylation site (K270R) [91]. The double mutants F342 W and I426 W targeting the ligand binding pocket where not activated by DPLC or DUPC highlighting the importance of the ligand binding pocket in regulating LRH-1 activity.

5.1.5. Synthetic ligands

The identification of synthetic agonists for LRH-1 and SF-1 supports the notion of ligand dependent activity of LRH-1 [93]. GSK8470, a substituted cis-bicyclo[3.3.0]-oct-2-ene, was identified as a high affinity ligand for both LRH-1 and SF-1. This molecule led to increased expression of the LRH-1 target gene SHP in liver cells [93]. Modifications to this molecule at 3 different sites also led to the identification of other agonists with varying degree of potency and efficacy. One such molecule, RWJ101 was shown to be selective for LRH-1, however further work is being undertaken to improve its efficiency [94]. Recently an inverse agonist was identified for SF-1; 4-(heptyloxy)phenol (AC-45594) and its analogues were shown to regulate SF-1 activity and subsequently down regulate SF-1 target genes [109]. This molecule had no effect on LRH-1 activity indicating that despite their structural similarities, distinct ligands for LRH-1 and SF-1 could be synthesized.

6. Future perspectives

Since LRH-1 is implicated in important cellular functions and plays a role in various diseases, it represents an attractive therapeutic target in infertility, cancer and metabolic disorders. NRs have been targeted by pharmaceutical agents for decades, although the majority of drugs act at the ligand binding pocket to mimic or antagonise endogenous ligands. For orphan receptors, the challenges are greater. Since co-regulators exert profound effects on LRH-1 activity, the possibility of modulating LRH-1: co-regulator interactions should be considered in addition to searching for compounds that bind to the phospholipid/ligand binding pocket. In addition to tissue-specific coregulators, there may be tissue-specific endogenous ligands adding to the complexity of the regulation of orphan nuclear receptors such as LRH-1. Identification of ligands for LRH-1 will not only uncover its true biological roles but provide an avenue for new treatment opportunities for a range of diseases.

References

[1] G. Benoit, A. Cooney, V. Giguere, H. Ingraham, M. Lazar, G. Muscat, T. Perlmann, J.-P. Renaud, J. Schwabe, F. Sladek, M.-J. Tsai, V. Laudet, International union of pharmacology. LXVI. Orphan nuclear receptors, Pharmacological Reviews 58 (2006) 798–836.
[2] P. Germain, B. Staels, C. Dacquet, M. Spedding, V. Laudet, Overview of nomenclature of nuclear receptors, Pharmacological Reviews 58 (2006) 685–704.
[3] A. Schweitzer, S.K. Knauer, R.H. Stauber, Therapeutic potential of nuclear receptors, Expert Opinion on Therapeutic Patents 18 (2008) 861–888.
[4] J.M. Olefsky, Nuclear receptor minireview series, Journal of Biological Chemistry 276 (2001) 36863–36864.
[5] J. Sonoda, L. Pei, R.M. Evans, Nuclear receptors: decoding metabolic disease, FEBS Letters 582 (2008) 2–9.
[6] W. Xie, R.M. Evans, Orphan nuclear receptors: the exotics of xenobiotics, Journal of Biological Chemistry 276 (2001) 37739–37742.
[7] K. Schoonjans, G. Martin, B. Staels, J. Auwerx, Peroxisome proliferator-activated receptors, orphans with ligands and functions, Current Opinion in Lipidology 8 (1997) 159–166.
[8] E. Fayard, J. Auwerx, K. Schoonjans, LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis, Trends in Cell Biology 14 (2004) 250–260.
[9] D.-J. Shin, T.F. Osborne, Peroxisome proliferator-activated receptor-γ-coactivator-1α activation of CYP7A1 during food restriction and diabetes is still inhibited by small heterodimer partner, Journal of Biological Chemistry 283 (2008) 15089–15096.
[10] E. Fayard, K. Schoonjans, J.-S.b. Annicotte, J. Auwerx, Liver Receptor Homolog 1 controls the expression of carboxyl ester lipase, Journal of Biological Chemistry 278 (2003) 35725–35731.
[11] Y. Miki, C.D. Clyne, T. Suzuki, T. Moriya, R. Shibuya, Y. Nakamura, T. Ishida, N. Yabuki, K. Kitada, S.-i. Hayashi, H. Sasano, Immunolocalization of liver receptor homologue-1 (LRH-1) in human breast carcinoma: possible regulator of in situ steroidogenesis, Cancer Letters 244 (2006) 24–33.
[12] T. Yazawa, Y. Inaoka, R. Okada, T. Mizutani, Y. Yamazaki, Y. Usami, M. Kuribayashi, M. Orisaka, A. Umezawa, K. Miyamoto, PPAR-γ coactivator-1α regulates progesterone production in ovarian granulosa cells with SF-1 and LRH-1, Molecular Endocrinology 24 (2010) 485–496.
[13] C.D. Clyne, C.J. Speed, J. Zhou, E.R. Simpson, Liver receptor homologue-1 (LRH-1) regulates expression of aromatase in preadipocytes, Journal of Biological Chemistry 277 (2002) 20591–20597.
[14] C.D. Clyne, A. Kovacic, C.J. Speed, J. Zhou, V. Pezzi, E.R. Simpson, Regulation of aromatase expression by the nuclear receptor LRH-1 in adipose tissue, Molecular and Cellular Endocrinology 215 (2004) 39–44.
[15] L.A. Freeman, A. Kennedy, J. Wu, S. Bark, A.T. Remaley, S. Santamarina-Fojo, H.B. Brewer, The orphan nuclear receptor LRH-1 activates the ABCG5/ABCG8 intergenic promoter, Journal of Lipid Research 45 (2004) 1197–1206.
[16] H. Ueda, G.C. Sun, T. Murata, S. Hirose, A novel DNA-binding motif abuts the zinc finger domain of insect nuclear hormone receptor FTZ-F1 and mouse embryonal long terminal repeat-binding protein, Molecular and Cellular Biology 12 (1992) 5667–5672.
[17] R.B. Riggins, M.M. Mazzotta, O.Z. Maniya, R. Clarke, Orphan nuclear receptors in breast cancer pathogenesis and therapeutic response, Endocrine Related Cancer 17 (2010) R213–R231.
[18] L.H. Wang, X.Y. Yang, X. Zhang, P. An, H.-J. Kim, J. Huang, R. Clarke, C.K. Osborne, J.K. Inman, E. Appella, W.L. Farrar, Disruption of estrogen receptor DNA-binding domain and related intramolecular communication restores tamoxifen sensitivity in resistant breast cancer, Cancer Cell 10 (2006) 487–499.
[19] R. Safi, A. Kovacic, S. Gaillard, Y. Murata, E.R. Simpson, D.P. McDonnell, C.D. Clyne, Coactivation of liver receptor homologue-1 by peroxisome proliferator-activated receptor γ coactivator-1α on aromatase promoter II and its inhibition by activated retinoid × receptor suggest a novel target for breast-specific antiestrogen therapy, Cancer Research 65 (2005) 11762–11770.
[20] B. Goodwin, S.A. Jones, R.R. Price, M.A. Watson, D.D. McKee, L.B. Moore, C. Galardi, J.G. Wilson, M.C. Lewis, M.E. Roth, P.R. Maloney, T.M. Willson, S.A. Kliewer, A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1, Represses Bile Acid Biosynthesis 6 (2000) 517–526.
[21] D. Chanda, Y.-B. Xie, H.-S. Choi, Transcriptional corepressor SHP recruits SIRT1 histone deacetylase to inhibit LRH-1 transactivation, Nucleic Acids Research (2010) 227.
[22] C.D. Clyne, C.J. Speed, A. Kovacic, J. Zhou, E.R. Simpson, Regulation of aromatase expression in preadipocytes by liver receptor homologue-1 (LRH-1) and small heterodimer partner (SHP), Breast Cancer Research and Treatment 76 (2002) 366.
[23] A. Kovacic, C.J. Speed, E.R. Simpson, C.D. Clyne, Inhibition of aromatase transcription via promoter II by short heterodimer partner in human preadipocytes, Molecular Endocrinology 18 (2004) 252–259.
[24] E.A. Ortlund, Y. Lee, I.H. Solomon, J.M. Hager, R. Safi, Y. Choi, Z. Guan, A. Tripathy, C.R.H. Raetz, D.P. McDonnell, D.D. Moore, M.R. Redinbo, Modulation of human nuclear receptor LRH-1 activity by phospholipids and SHP, Nature Structural and Molecular Biology 12 (2005) 357–363.

[25] E.P. Sablin, A. Woods, I.N. Krylova, P. Hwang, H.A. Ingraham, R.J. Fletterick, The structure of corepressor Dax-1 bound to its target nuclear receptor LRH-1, Proceedings of the National Academy of Sciences 105 (2008) 18390–18395.
[26] T. Suzuki, M. Kasahara, H. Yoshioka, K.-i. Morohashi, K. Umesono, LXXLL-related motifs in Dax-1 have target specificity for the orphan nuclear receptors Ad4BP/SF-1 and LRH-1, Molecular and Cell Biology 23 (2003) 238–249.
[27] W. Wang, C. Zhang, A. Marimuthu, H.I. Krupka, M. Tabrizizad, R. Shelloe, U. Mehra, K. Eng, H. Nguyen, C. Settachatgul, B. Powell, M.V. Milburn, B.L. West, The crystal structures of human steroidogenic factor-1 and liver receptor homologue-1, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 7505–7510.
[28] E.P. Sablin, R.D. Blind, I.N. Krylova, J.G. Ingraham, F. Cai, J.D. Williams, R.J. Fletterick, H.A. Ingraham, Structure of SF-1 bound by different phospholipids: evidence for regulatory ligands, Molecular Endocrinology 23 (2009) 25–34.
[29] T. Balla, Found in the crystal: phospholipid ligands for nuclear orphan receptors, Trends in Endocrinology and Metabolism 16 (2005) 289–290.
[30] T. Zhang, J.-H. Zhou, L.-W. Shi, R.-X. Zhu, M.-B. Chen, Molecular dynamics simulation study for LRH-1: interaction with fragments of SHP and function of phospholipid ligand, Proteins: Structure, Function, and Bioinformatics 70 (2008) 1527–1539.
[31] Y.-K. Lee, D.D. Moore, Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner, Journal of Biological Chemistry 277 (2002) 2463–2467.
[32] I.H. Solomon, J.M. Hager, R. Safi, D.P. McDonnell, M.R. Redinbo, E.A. Ortlund, Crystal structure of the human lrh-1 dbd-dna complex reveals Ftz-F1 domain positioning is required for receptor activity, Journal of Molecular Biology 354 (2005) 1091–1102.
[33] T.T. Lu, M. Makishima, J.J. Repa, K. Schoonjans, T.A. Kerr, J. Auwerx, D.J. Mangelsdorf, Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors, Molecular Cell 6 (2000) 507–515.
[34] M. Nitta, S. Ku, C. Brown, A.Y. Okamoto, B. Shan, CPF: an orphan nuclear receptor that regulates liver-specific expression of the human cholesterol 7alpha-hydroxylase gene, Proceedings of the National Academy of Sciences of the United States of America 96 (1999) 6660–6665.
[35] G.A. Francis, E. Fayard, F.d.r. Picard, J. Auwerx, Nuclear Receptors and the control of Metabolism, Annual Review of Physiology 65 (2003) 261–311.
[36] A. del Castillo-Olivares, G. Gil, a-Fetoprotein transcription factor is required for the expression of sterol 12a-hydroxylase, the specific enzyme for cholic acid synthesis, Journal of Biological Chemistry 275 (2000) 17793–17799.
[37] F. Chen, L. Ma, P.A. Dawson, C.J. Sinal, E. Sehayek, F.J. Gonzalez, J. Breslow, M. Ananthanarayanan, B.L. Shneider, Liver receptor homologue-1 mediates species- and cell line-specific bile acid-dependent negative feedback regulation of the apical sodium-dependent bile acid transporter, Journal of Biological Chemistry 278 (2003) 19909–19916.
[38] Z.N. Wang, M. Bassett, W.E. Rainey, Liver receptor homologue-1 is expressed in the adrenal and can regulate transcription of 11 beta-hydroxylase, Journal of Molecular Endocrinology 27 (2001) 255–258.
[39] A. del Castillo-Olivares, J.A. Campos, W.M. Pandak, G. Gil, The role of a-Fetoprotein transcription factor/LRH-1 in bile acid biosynthesis, Journal of Biological Chemistry 279 (2004) 16813–16821.
[40] D.-J. Shin, T.F. Osborne, Peroxisome proliferator-activated receptor-γ coactivator-1a activation of Cyp7a1 during food restriction and diabetes is still inhibited by small heterodimer partner, Journal of Biological Chemistry 283 (2008) 15089–15096.
[41] C.R. Pullinger, C. Eng, G. Salen, S. Shefer, A.K. Batta, S.K. Erickson, A. Verhagen, C.R. Rivera, S.J. Mulvihill, M.J. Malloy, J.P. Kane, Human cholesterol 7α-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype, Journal of Clinical Investigation 110 (2002) 109–117.
[42] J.-F.o. Pare, D. Malenfant, C. Courtemanche, M.v. Jacob-Wagner, S. Roy, D. Allard, L. Balanger, The fetoprotein transcription factor (FTF) gene is essential to embryogenesis and cholesterol homeostasis and is regulated by a DR4 element, Journal of Biological Chemistry 279 (2004) 21206–21216.
[43] Y.-K. Lee, D.R. Schmidt, C.L. Cummins, M. Choi, L. Peng, Y. Zhang, B. Goodwin, R.E. Hammer, D.J. Mangelsdorf, S.A. Kliewer, Molecular Endocrinology 22 (2008) 1345–1356.
[44] A. Bohan, W.-S. Chen, L.A. Denson, M.A. Held, J.L. Boyer, Tumor necrosis factor a-dependent up-regulation of Lrh-1 and Mrp3(Abcc3) reduces liver injury in obstructive cholestasis, Journal of Biological Chemistry 278 (2003) 36688–36698.
[45] T. Yazawa, Y. Inanoka, T. Mizutani, M. Kuribayashi, A. Umezawa, K. Miyamoto, Liver receptor homolog-1 regulates the transcription of steroidogenic enzymes and induces the differentiation of mesenchymal stem cells into steroidogenic cells, Endocrinology 150 (2009) 3885–3893.
[46] K. Schoonjans, J.-S. Annicotte, T. Huby, O.A. Botrugno, Elisabeth Fayard, Yukihiko Ueda, J. Chapman, J. Auwerx, Liver Receptor Homolog 1 controls the expression of the scavenger receptor class B type I, EMBO Reports 3 (2002) 1181–1187.
[47] C.J. Packard, T. Demant, J.P. Stewart, D. Bedford, M.J. Caslake, G. Schwertfeger, A. Bedynek, J. Shepherd, D. Seidel, Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions, Journal of Lipid Research 41 (2000) 305–317.
[48] A. Inokuchi, E. Hinoshita, Y. Iwamoto, K. Kohno, M. Kuwano, T. Uchiumi, Enhanced expression of the human multidrug resistance protein 3 by bile salt in human enterocytes, Journal of Biological Chemistry 276 (2001) 46822–46829.
[49] P. Gu, B. Goodwin, A.C.K. Chung, X. Xu, D.A. Wheeler, R.R. Price, C. Galardi, L. Peng, A.M. Latour, B.H. Koller, J. Gossen, S.A. Kliewer, A.J. Cooney, Orphan nuclear receptor LRH-1 is required to maintain Oct4 expression at the epiblast stage of embryonic development, Molecular and Cell. Biology 25 (2005) 3492–3505.
[50] R.T. Wagner, X. Xu, F. Yi, B.J. Merrill, A.J. Cooney, Canonical Wnt/β-catenin regulation of liver receptor homolog-1 (Lrh-1) mediates pluripotency gene expression, Stem Cells (2010), N/A-N/A.
[51] K. Miyamoto, T. Yazawa, T. Mizutani, Y. Imamichi, S.-y. Kawabe, M. Kanno, T. Matsumura, Y. Ju, A. Umezawa, Stem cell differentiation into steroidogenic cell lineages by NR5A family, Molecular and Cellular Endocrinology 336 (2011) 123–126.
[52] G. Guo, A. Smith, A genome-wide screen in EpiSCs identifies Nr5a nuclear receptors as potent inducers of ground state pluripotency, Development 137 (2010) 3185–3192.
[53] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[54] M. Nakagawa, M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, S. Yamanaka, Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, Nature Biotechnology 26 (2008) 101–106.
[55] J.-C.D. Heng, B. Feng, J. Han, J. Jiang, P. Kraus, J.-H. Ng, Y.L. Orlov, M. Huss, L. Yang, T. Lufkin, B. Lim, H.-H. Ng, The nuclear receptor Nr5a2 can replace Oct4 in the reprogramming of murine somatic cells to pluripotent cells, Cell Stem Cell 6 (2010) 167–174.
[56] E.P. Sablin, I.N. Krylova, R.J. Fletterick, H.A. Ingraham, Structural basis for ligand-independent activation of the orphan nuclear receptor LRH-1, Molecular Cell 11 (2003) 1575–1585.
[57] J. Wang, S. Rao, J. Chu, X. Shen, D.N. Levasseur, T.W. Theunissen, S.H. Orkin, A protein interaction network for pluripotency of embryonic stem cells, Nature 444 (2006) 364–368.
[58] V.R. Kelly, G.D. Hammer, LRH-1 and Nanog regulate Dax1 transcription in mouse embryonic stem cells, Molecular and Cellular Endocrinology 332 (2011) 116–124.
[59] V.R. Kelly, B. Xu, R. Kuick, R.J. Koenig, G.D. Hammer, Dax1 up-regulates Oct4 expression in mouse embryonic stem cells via LRH-1 and SRA, Molecular Endocrinology 24 (2010) 2281–2291.
[60] M.M. Hinshelwood, J.J. Repa, J.M. Shelton, J.A. Richardson, D.J. Mangelsdorf, C.R. Mendelson, Expression of LRH-1 and SF-1 in the mouse ovary: localization in different cell types correlates with differing function, Molecular and Cellular Endocrinology 207 (2003) 39–45.
[61] J. Weck, K.E. Mayo, Switching of NR5A proteins associated with the inhibin α-subunit gene promoter after activation of the gene in granulosa cells, Molecular Endocrinology 20 (2006) 1090–1103.
[62] J.W. Kim, J.C. Havelock, B.R. Carr, G.R. Attia, The orphan nuclear receptor, liver receptor homolog-1, regulates cholesterol side-chain cleavage cytochrome P450 enzyme in human granulosa cells, The Journal of Clinical Endocrinology and Metabolism 90 (2005) 1678–1685.
[63] A.E. Falender, R. Lanz, D. Malenfant, L. Belanger, J.S. Richards, Differential expression of steroidogenic factor-1 and FTF/LRH-1 in the rodent ovary, Endocrinology 144 (2003) 3598–3610.
[64] D.L. Liu, W.Z. Liu, Q.L. Li, H.M. Wang, D. Qian, E. Treuter, C. Zhu, Expression and functional analysis of liver receptor homologue 1 as a potential steroidogenic factor in rat ovary, Biology of Reproduction 69 (2003) 508–517.
[65] N. Peng, J.W. Kim, W.E. Rainey, B.R. Carr, G.R. Attia, The role of the orphan nuclear receptor, liver receptor homologue-1, in the regulation of human corpus luteum 3β-hydroxysteroid dehydrogenase type II, The Journal of Clinical Endocrinology and Metabolism 88 (2003) 6020–6028.
[66] D. Saxena, R. Safi, L. Little-Ihrig, A.J. Zeleznik, Liver receptor homolog-1 stimulates the progesterone biosynthetic pathway during follicle-stimulating hormone-induced granulosa cell differentiation, Endocrinology 145 (2004) 3821–3829.
[67] D. Saxena, R. Escamilla-Hernandez, L. Little-Ihrig, A.J. Zeleznik, Liver receptor homolog-1 and steroidogenic factor-1 have similar actions on rat granulosa cell steroidogenesis, Endocrinology 148 (2007) 726–734.
[68] R. Duggavathi, D.H. Volle, C. Mataki, M.C. Antal, N. Messaddeq, J. Auwerx, B.D. Murphy, K. Schoonjans, Liver Receptor Homolog 1 is essential for ovulation, Genes & Development 22 (2008) 1871–1876.
[69] O.A. Botrugno, E. Fayard, J.-S. Annicotte, C. Haby, T. Brennan, O. Wendling, T. Tanaka, T. Kodama, W. Thomas, J. Auwerx, K. Schoonjans, Synergy between LRH-1 and β-catenin induces G1 cyclin-mediated cell proliferation, Molecular Cell 15 (2004) 499–509.
[70] K. Schoonjans, L. Dubuquoy, J. Mebis, E. Fayard, O. Wendling, C.L. Haby, K. Geboes, J. Auwerx, Liver Receptor Homolog 1 contributes to intestinal tumor formation through effects on cell cycle and inflammation, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 2058–2062.
[71] M. Mueller, I. Cima, M. Noti, A. Fuhrer, S. Jakob, L. Dubuquoy, K. Schoonjans, T. Brunner, The nuclear receptor LRH-1 critically regulates extra-adrenal glucocorticoid synthesis in the intestine, The Journal of Experimental Medicine 203 (2006) 2057–2062.
[72] A. Coste, L. Dubuquoy, R. Barnouin, J.-S. Annicotte, B. Magnier, M. Notti, N. Corazza, M.C. Antal, D. Metzger, P. Desreumaux, T. Brunner, J. Auwerx, K. Schoonjans, LRH-1-mediated glucocorticoid synthesis in enterocytes protects

against inflammatory bowel disease, Proceedings of the National Academy of Sciences 104 (2007) 13098–13103.
[73] Y. Miki, C.D. Clyne, T. Suzuki, T. Moriya, R. Shibuya, Y. Nakamura, T. Ishida, N. Yabuki, K. Kitada, S. Hayashi, H. Sasano, Immunolocalization of liver receptor homologue-1 (LRH-1) in human breast carcinoma: possible regulator of in situ steroidogenesis, Cancer Letters 244 (2006) 24–33.
[74] J.-S. Annicotte, C. Chavey, N. Servant, J. Teyssier, A. Bardin, A. Licznar, E. Badia, P. Pujol, F. Vignon, T. Maudelonde, G. Lazennec, V. Cavailles, L. Fajas, The nuclear receptor liver receptor homolog-1 is an estrogen receptor target gene, Oncogene 24 (2005) 8167–8175.
[75] J. Zhou, T. Suzuki, A. Kovacic, R. Saito, Y. Miki, T. Ishida, T. Moriya, E.R. Simpson, H. Sasano, C.D. Clyne, Interactions between prostaglandin E2, liver receptor homologue-1, and aromatase in breast cancer, Cancer Res 65 (2005) 657–663.
[76] E. Simpson, M. Mahendroo, G. Means, M. Kilgore, C. Corbin, C. Mendelson, Tissue-specific promoters regulate aromatase cytochrome P450 expression, Clinical Chemistry 39 (1993) 317–324.
[77] M.S. Mahendroo, C.R. Mendelson, E.R. Simpson, Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue, Journal of Biological Chemistry 268 (1993) 19463–19470.
[78] G.D. Means, M.W. Kilgore, M.S. Mahendroo, C.R. Mendelson, E.R. Simpson, Tissue-specific promoters regulate aromatase cytochrome P450 gene expression in human ovary and fetal tissues, Molecular Endocrinology 5 (1991) 2005–2013.
[79] Y. Miki, T. Suzuki, C. Tazawa, Y. Yamaguchi, K. Kitada, S. Honma, T. Moriya, H. Hirakawa, D.B. Evans, S.-i. Hayashi, N. Ohuchi, H. Sasano, Aromatase localization in human breast cancer tissues: possible interactions between intratumoral stromal and parenchymal cells, Cancer Research 67 (2007) 3945–3954.
[80] M.F. Bouchard, H. Taniguchi, R.S. Viger, Protein kinase A-dependent synergism between gata factors and the nuclear receptor, liver receptor homolog-1, regulates human aromatase (CYP19) PII promoter activity in breast cancer cells, Endocrinology 146 (2005) 4905–4916.
[81] P. Thiruchelvam, C.-F. Lai, H. Hua, R. Thomas, A. Hurtado, W. Hudson, A. Bayly, F. Kyle, M. Periyasamy, A. Photiou, A. Spivey, E. Ortlund, R. Whitby, J. Carroll, R. Coombes, L. Buluwela, S. Ali, The liver receptor homolog-1 regulates estrogen receptor expression in breast cancer cells, Breast Cancer Research and Treatment 127 (2011) 385–396.
[82] A. Chand, K.A. Herridge, E.W. Thompson, C. Clyne, The orphan nuclear receptor LRH-1 promotes breast cancer motility and invasion, Endocrine Related Cancer 17 (2010) 965–975.
[83] G. Da-ming, W. Lin-fang, L. Jing, K. Yu-ying, W. Yuan, X. You-hua, Expression of mouse liver receptor homologue 1 in embryonic stem cells is directed by a novel promoter, FEBS letters 580 (2006) 1702–1708.
[84] Q. Zhou, H. Chipperfield, D.A. Melton, W.H. Wong, A gene regulatory network in mouse embryonic stem cells, Proceedings of the National Academy of Sciences 104 (2007) 16438–16443.
[85] C.-Q. Xie, Y. Jeong, M. Fu, A.L. Bookout, M.T. Garcia-Barrio, T. Sun, B.-h. Kim, Y. Xie, S. Root, J. Zhang, R.-H. Xu, Y.E. Chen, D.J. Mangelsdorf, Expression profiling of nuclear receptors in human and mouse embryonic stem cells, Molecular Endocrinology 23 (2009) 724–733.
[86] T. Blick, H. Hugo, E. Widodo, M. Waltham, C. Pinto, S. Mani, R. Weinberg, R. Neve, M. Lenburg, E. Thompson, Epithelial mesenchymal transition traits in human breast cancer cell lines parallel the CD44 (hi/) CD24 (lo/) stem cell phenotype in human breast Cancer, Journal of Mammary Gland Biology Neoplasia 15 (2010) 235–252.
[87] S.A. Mani, W. Guo, M.-J. Liao, E.N. Eaton, A. Ayyanan, A.Y. Zhou, M. Brooks, F. Reinhard, C.C. Zhang, M. Shipitsin, L.L. Campbell, K. Polyak, C. Brisken, J. Yang, R.A. Weinberg, The epithelial-mesenchymal transition generates cells with properties of stem cells, Cell 133 (2008) 704–715.
[88] G.M. Petersen, L. Amundadottir, C.S. Fuchs, P. Kraft, R.Z. Stolzenberg-Solomon, K.B. Jacobs, A.A. Arslan, H.B. Bueno-de-Mesquita, S. Gallinger, M. Gross, K. Helzlsouer, E.A. Holly, E.J. Jacobs, A.P. Klein, A. LaCroix, D. Li, M.T. Mandelson, S.H. Olson, H.A. Risch, W. Zheng, D. Albanes, W.R. Bamlet, C.D. Berg, M.-C. Boutron-Ruault, J.E. Buring, P.M. Bracci, F. Canzian, S. Clipp, M. Cotterchio, M. de Andrade, E.J. Duell, J.M. Gaziano, E.L. Giovannucci, M. Goggins, G. Hallmans, S.E. Hankinson, M. Hassan, B. Howard, D.J. Hunter, A. Hutchinson, M. Jenab, R. Kaaks, C. Kooperberg, V. Krogh, R.C. Kurtz, S.M. Lynch, R.R. McWilliams, J.B. Mendelsohn, D.S. Michaud, H. Parikh, A.V. Patel, P.H.M. Peeters, A. Rajkovic, E. Riboli, L. Rodriguez, D. Seminara, X.-O. Shu, G. Thomas, A. Tjonneland, G.S. Tobias, D. Trichopoulos, S.K. Van Den Eeden, J. Virtamo, J. Wactawski-Wende, Z. Wang, B.M. Wolpin, H. Yu, K. Yu, A. Zeleniuch-Jacquotte, J.F. Fraumeni, R.N. Hoover, P. Hartge, S.J. Chanock, A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1 1q32.1 and 5p15.33, Nature Genetics 42 (2010) 224–228.
[89] C. Benod, M.V. Vinogradova, N. Jouravel, G.E. Kim, R.J. Fletterick, E.P. Sablin, Nuclear receptor liver receptor homologue 1 (LRH-1) regulates pancreatic cancer cell growth and proliferation, Proceedings of the National Academy of Sciences 108 (2011) 16927–16931.
[90] Y.-K. Lee, K.L. Parker, H.-S. Choi, D.D. Moore, Activation of the promoter of the orphan receptor SHP by orphan receptors that bind DNA as monomers, Journal of Biological Chemistry 274 (1999) 20869–20873.
[91] J.M. Lee, Y.K. Lee, J.L. Mamrosh, S.A. Busby, P.R. Griffin, M.C. Pathak, E.A. Ortlund, D.D. Moore, A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects, Nature 474 (2011) 506–510.
[92] I.N. Krylova, E.P. Sablin, J. Moore, R.X. Xu, G.M. Waitt, J.A. MacKay, D. Juzumiene, J.M. Bynum, K. Madauss, V. Montana, L. Lebedeva, M. Suzawa, J.D. Williams, S.P. Williams, R.K. Guy, J.W. Thornton, R.J. Fletterick, T.M. Willson, H.A. Ingraham, Structural analyses reveal phosphatidyl inositols as ligands for the NR5 orphan receptors SF-1 and LRH-1, Cell 120 (2005) 343–355.
[93] R.J. Whitby, S. Dixon, P.R. Maloney, P. Delerive, B.J. Goodwin, D.J. Parks, T.M. Willson, Identification of small molecule agonists of the orphan nuclear receptors liver receptor homolog-1 and steroidogenic factor-1, Journal of Medicinal Chemistry 49 (2006) 6652–6655.
[94] R.J. Whitby, J. Stec, R.D. Blind, S. Dixon, L.M. Leesnitzer, L.A. Orband-Miller, S.P. Williams, T.M. Willson, R. Xu, W.J. Zuercher, F. Cai, H.A. Ingraham, Small molecule agonists of the orphan nuclear receptors steroidogenic factor-1 (SF-1, NR5A1) and liver receptor homologue-1 (LRH-1, NR5A2), Journal of Medicinal Chemistry 54 (2011) 2266–2281.
[95] S.-L. Wang, D.-Z. Zheng, F.-H. Lan, X.-J. Deng, J. Zeng, C.-J. Li, R. Wang, Z.-Y. Zhu, Increased expression of hLRH-1 in human gastric cancer and its implication in tumorigenesis, Molecular and Cellular Biochemistry 308 (2008) 93–100.
[96] Y. Li, M. Choi, G. Cavey, J. Daugherty, K. Suino, A. Kovach, N.C. Bingham, S.A. Kliewer, H.E. Xu, Crystallographic identification and functional characterization of phospholipids as ligands for the orphan nuclear receptor steroidogenic factor-1, Molecular Cell 17 (2005) 491–502.
[97] H.K. Chong, A.M. Infante, Y.-K. Seo, T.-I. Jeon, Y. Zhang, P.A. Edwards, X. Xie, T.F. Osborne, Genome-wide interrogation of hepatic FXR reveals an asymmetric IR-1 motif and synergy with LRH-1, Nucleic Acids Research 38 (2010) 6007–6017.
[98] P.-L. Xu, Y.-Q. Liu, S.-F. Shan, Y.-Y. Kong, Q. Zhou, M. Li, J.-P. Ding, Y.-H. Xie, Y. Wang, Molecular mechanism for the potentiation of the transcriptional activity of human Liver Receptor Homolog 1 by steroid receptor coactivator-1, Molecular Endocrinology 18 (2004) 1887–1905.
[99] C. Brendel, L. Gelman, J. Auwerx, Multiprotein bridging factor-1 (MBF-1) is a cofactor for nuclear receptors that regulate lipid metabolism, Molecular Endocrinology 16 (2002) 1367–1377.
[100] Y.K. Lee, D.D. Moore, Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner, J. Biol. Chem 277 (2002) 2463–2467.
[101] T. Suzuki, M. Kasahara, H. Yoshioka, K. Umesono, K. Morohashi, LXXLL motifs in Dax-1 have target specificity for the orphan nuclear receptors Ad4BP/SF-1 and LRH-1, Endocrine Research 28 (2002) 537.
[102] M. Ito, R. Yu, J.L. Jameson, DAX-1 inhibits SF-1-mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenita, Molecular and Cell. Biology 17 (1997) 1476–1483.
[103] D. Lopez, W. Shea-Eaton, M.D. Sanchez, M.P. McLean, DAX-1 represses the high-density lipoprotein receptor through interaction with positive regulators sterol regulatory element-binding protein-1a and steroidogenic factor-1, Endocrinology 142 (2001) 5097–5106.
[104] H.-T. Hsieh, C.-H. Wang, M.-L. Wu, F.-M. Yang, Y.-C. Tai, M.-C. Hu, PIASy inhibits LRH-1-dependent CYP11A1 expression by competing for SRC-1 binding, The Biochemical Journal 419 (2009) 201–209.
[105] L. Insabato, I. Amelio, M. Quarto, A. Zannetti, F. Tolino, G. de Mauro, L. Cerchia, P. Riccio, D. Baumhoer, G. Condorelli, L. Terracciano, V. de Franciscis, Elevated expression of the tyrosine phosphatase SHP-1 defines a subset of high-grade breast tumors, Oncology 77 (2009) 378–384.
[106] I. Conde, J. Alfaro, B. Fraile, A. Ruiz, R. Paniagua, M. Arenas, DAX-1 expression in human breast cancer: comparison with estrogen receptors ER-alpha, ER-beta and androgen receptor status, Breast Cancer Research 6 (2004) R140–R148.
[107] Y.-K. Lee, Y.-H. Choi, S. Chua, Y.J. Park, D.D. Moore, Phosphorylation of the hinge domain of the nuclear hormone receptor LRH-1 stimulates transactivation, Journal of Biological Chemistry 281 (2006) 7850–7855.
[108] F.M. Yang, C.T. Pan, H.M. Tsai, T.W. Chiu, M.L. Wu, M.C. Hu, Liver receptor homolog-1 localization in the nuclear body is regulated by sumoylation and cAMP signaling in rat granulosa cells, The Febs Journal 276 (2009) 425–436.
[109] A.L. Del Tredici, C.B. Andersen, E.A. Currier, S.R. Ohrmund, L.C. Fairbain, B.W. Lund, N. Nash, R. Olsson, F. Piu, Identification of the first synthetic steroidogenic factor 1 inverse agonists: pharmacological modulation of steroidogenic enzymes, Molecular Pharmacology 73 (2008) 900–908.
[110] L. Galarneau, J.F. Pare, D. Allard, D. Hamel, L. Levesque, J.D. Tugwood, S. Green, L. Belanger, The alpha1-fetoprotein locus is activated by a nuclear receptor of the Drosophila FTZ-F1 family, Molecular Cell Biology 16 (1996) 3853–3865.
[111] J.-F. Pare, S. Roy, L. Galarneau, L. Belanger, The mouse fetoprotein transcription factor (FTF) gene promoter is regulated by three GATA elements with tandem E Box and Nkx motifs, and FTF in turn activates the Hnf3b, Hnf4a, and Hnf1a gene promoters, Journal of Biological Chemistry 276 (2001) 13136–13144.
[112] P. Delerive, C.M. Galardi, J.E. Bisi, E. Nicodeme, B. Goodwin, Identification of liver receptor homolog-1 as a novel regulator of apolipoprotein AI gene transcription, Molecular Endocrinology 18 (2004) 2378–2387.
[113] W. Zheng, J. Yang, Q. Jiang, Z. He, L.M. Halvorson, Liver receptor homologue-1 regulates gonadotrope function, J Molecular Endocrinology 38 (2007) 207–219.