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CRITICAL REVIEW

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Nuclear-mitochondrial crosstalk: On the role of the nuclear receptor liver receptor homolog-1 (NR5A2) in the regulation of mitochondrial metabolism, cell survival, and cancer

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Abstract

Liver receptor homolog-1 (LRH-1, NR5A2) is an orphan nuclear receptor with widespread activities in the regulation of development, stemness, metabolism, steroidogenesis, and proliferation. Many of the LRH-1-regulated processes target the mitochondria and associated activities. While under physiological conditions, a balanced LRH-1 expression and regulation contribute to the maintenance of a physiological equilibrium, deregulation of LRH-1 has been associated with inflammation and cancer. In this review, we discuss the role and mechanism(s) of how LRH-1 regulates metabolic processes, cell survival, and cancer in a nuclear-mitochondrial crosstalk, and evaluate its potential as a pharmacological target.

KEYWORDS

apoptosis, cancer, metabolism, mitochondria, nuclear receptors

Abbreviations: AF, activation function; ALL, acute lymphoblastic leukemia; AR, androgen receptor; ATF2, activating transcription factor 2; BH, Bcl-2 homology; Cpd3, compound 3; Cpd3d2, compound 3d2; CPT, carnitine palmitoyltransferase; CRPC, castration-resistant prostate cancer; CYP, cytochrome P450; DAX-1, dosage-sensitive sex reversal, adrenal hypoplasia critical region on chromosome X, gene 1; DBD, DNA-binding domain; DL, death ligand; DLPC, dilauroyl phosphatidylcholine; DR, death receptor; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinases; ERα, estrogen receptor α; FAO, fatty acid β-oxidation; FasL, Fas ligand; Ftz-F1, Drosophila fushi tarazu factor 1; GATA6, GATA-binding factor 6; GC, glucocorticoid; GLS2, glutaminase 2; GLUT, glucose transporter; HCC, hepatocellular carcinoma; HDAC, histone deacetylases; LBD, ligand-binding domain; LRH-1, liver receptor homolog-1; LRH-$1^{+/-}$, heterozygous LRH-1 deletion; MAPK, mitogen-activated protein kinase; MBF-1, multiprotein-bridging factor-1; MDC1, DNA damage checkpoint 1; MOMP, mitochondrial outer membrane permeabilization; NF-κB, nuclear factor κB; NR, nuclear receptor; NR5A, NR subclass 5A; NSCLC, nonsmall cell lung cancer; OAC, esophageal adenocarcinoma; P450scc, cytochrome P450 side-chain cleavage; PDAC, pancreatic ductal adenocarcinoma; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PGE_2, prostaglandin E2; Plk3, Polo-like kinase 3; PROX-1, prospero homeobox protein 1; PTF1-L, pancreas transcription factor 1-L; PTM, post-translational modification; ROS, reactive oxygen species; SF-1, steroidogenic factor-1; SHP, small heterodimer partner; SOD2, superoxide dismutase 2; StAR, steroidogenic acute regulatory protein; SUMO, small ubiquitin-related modifier; TCA, tricarboxylic acid; TNFR, tumor necrosis factor receptor; TNFα, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; UPR, unfolded protein response; WNT, Wingless and INT-1.

1 | INTRODUCTION: LIVER RECEPTOR HOMOLOG-1 IN HEALTH

Liver receptor homolog-1 (LRH-1, NR5A2) is a member of the nuclear receptor (NR) superfamily, a diverse set of evolutionary-related transcription factors. The human genome encodes for 48 NRs that are characterized by their ability to bind specific small hydrophobic ligands, including steroid and thyroid hormones, certain vitamins as well as fatty acids.[1] Given that these lipophilic ligands are able to cross cellular membranes, NRs act as sensors of extracellular stimuli and link them to intercellular signaling by regulating the expression of critical signaling molecules or enzymes. They are key regulators of a plethora of genes that are implicated in diverse physiological and pathophysiological processes, many of them involving mitochondrial processes.[2] Targeting the activity of NRs with known ligands and inhibitors has therefore been a successful therapeutic approach to treat metabolic, autoimmune, and cancerous diseases for many years.

The human LRH-1 gene consists of eight exons spanning a 150 kb region located on chromosome 1q32.11.[3,4] Belonging to the NR subclass 5A (NR5A), the LRH-1 protein harbors a Drosophila fushi tarazu factor 1 (Ftz-F1) box. This highly conserved stretch of 26 amino acids is a unique feature of the Ftz-F1 subfamily that accounts for the specificity of DNA binding.[5] Systematic anatomical profiling of NR expression by Bookout et al. confirmed initial findings that LRH-1 is highly expressed in epithelial tissues of endodermal origin, including the liver, pancreas, and intestine as well as the ovaries.[6] There is, however, increasing evidence for LRH-1 expression in other organs, including the hematopoietic system.[7,8] Also, recent studies of our group provided substantial evidence for LRH-1 expression at comparably low levels, but responsible for vital functions, in different immune cell types.[9–11]

1.1 | LRH-1 in physiological processes

As a result of its expression in different organs and context-dependent regulation of target genes, LRH-1 plays a dominant role in a wide variety of physiological processes, which are summarized in Figure 1 (left). A crucial role of LRH-1 in embryogenesis is emphasized by expression of LRH-1 during morphogenesis of the liver,

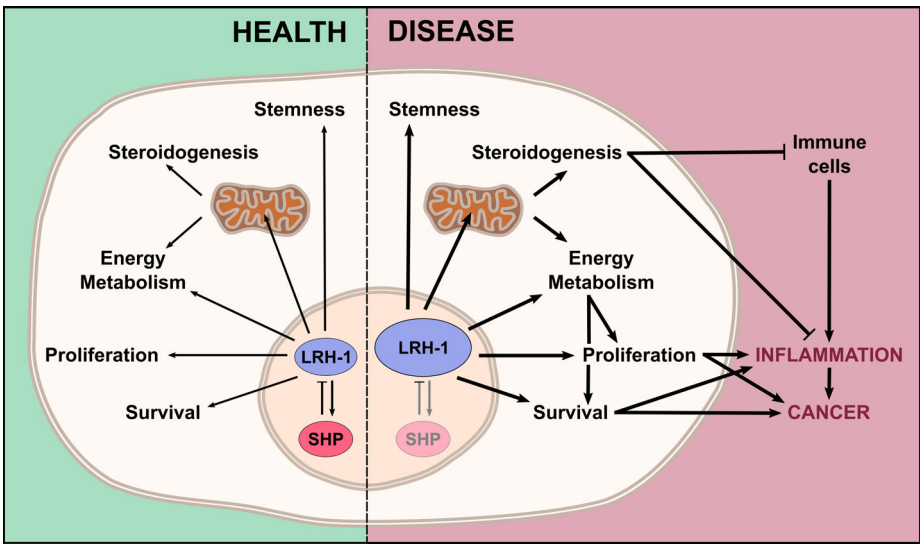

FIGURE 1 Functions of LRH-1 in health and disease. LRH-1 regulates the expression of target genes involved in diverse physiological processes in a context- as well as tissue-specific manner (left). Constantly exhibiting nuclear localization and transcriptional activity, LRH-1 is primarily regulated by interaction with comodulatory factors. One well-described corepressor inhibiting the transcriptional activity of LRH-1 is the atypical nuclear receptor SHP. Under physiological conditions, SHP is a direct LRH-1 target, providing a negative feedback-loop mechanism for their reciprocal regulation. Overexpression or deregulation of LRH-1, as for example caused by a reduced abundance of SHP, results in the enhanced expression of LRH-1 target genes. A consequent selective proliferation advantage and improved survival is known to promote cancer-stimulating inflammation and the transformation of healthy into malignant cells (right). These processes are likely supported by the simultaneous increase in mitochondrial metabolic enzyme expression and overall cellular energy production. The LRH-1-regulated expression of mitochondrial steroidogenic enzymes and hence synthesis of immunosuppressive steroid hormones could further dampen cancer-directed immune responses and thus allow escape from tumor immune surveillance. LRH-1, liver receptor homolog-1; SHP, small heterodimer partner

pancreas, and intestine, and early embryonic lethality of LRH-1-deficient mice.[12,13] Promoting the gene expression and activity of self-renewal and pluripotency factors, such as Nanog and Oct-4, LRH-1 is part of genetic regulatory networks fundamentally involved in maintaining stemness and pluripotency of embryonic stem cells.[14,15] In the liver and pancreas of adult organisms, LRH-1 mainly regulates metabolic processes. It governs the expression of several hepatic enzymes that are involved in bile acid metabolism, reverse cholesterol transport, and glucose homeostasis, as reviewed by Stein et al.[16] In the pancreas, LRH-1 and pancreas transcription factor 1-L (PTF1-L) coregulate synthesis and secretion of the digestive fluid by controlling the expression of a set of genes encoding for pancreatic digestive enzymes and secretory proteins.[17] LRH-1 further plays an important role in reproduction, as it is a regulator of multiple mechanisms essential for maturation of ovarian follicles and ovulation. This includes the transcriptional control of mitochondrial steroidogenic acute regulatory protein (StAR) and cytochrome P450 side-chain cleavage (P450scc, Cyp11a1). Both proteins being crucial for mitochondrial steps of steroidogenesis,[18] this provides a direct functional link between the nuclear transcriptional activities of LRH-1 and mitochondrial functions. Similarly, LRH-1 is responsible for the expression of the mitochondrial steroidogenic enzymes Cyp11b1 (P450c11) and Cyp11a1 in the intestinal epithelium. Combining in vitro and in vivo approaches, a study by Mueller et al. showed that LRH-1 is essential for the synthesis of immunoregulatory glucocorticoids (GCs) in the intestinal epithelium, and is thus of particular importance for the regulation of local immune homeostasis and intestinal tissue integrity.[19] This is highlighted by the findings that LRH-1-mediated production of intestinal GCs regulates antiviral immune responses as well as the pathogenesis of inflammatory bowel disease.[20,21] In the intestine, LRH-1 does, however, not only promote extra-adrenal GC synthesis, but also plays an essential role in the regulation of intestinal stem cell renewal, ensuring intestinal epithelial barrier integrity. LRH-1 induces proliferation of intestinal crypt cells by synergistically controlling transcription of the positive cell cycle regulators c-Myc, and cyclin D1 and E1 in cooperation with beta-catenin. LRH-1 potently coactivates beta-catenin on the c-Myc and cyclin D1 promoter, whereas beta-catenin is a coactivator of LRH-1 on the cyclin E1 promoter.[22] One of our recent studies demonstrates that LRH-1 is also a critical regulator of activation-induced proliferation in T lymphocytes, and thus T cell-regulated immune responses. Similar as in intestinal cells, LRH-1 promotes T cell proliferation via the transcriptional regulation of positive cell cycle regulators, including c-Myc and cyclin E1.[10]

1.2 | LRH-1 signaling and regulation

In contrast to most NRs, LRH-1 is known to constantly exhibit nuclear localization and transcriptional activity. It binds to DNA as a monomer with high affinity for specific response elements with the Ftz-F1-consensus binding sequence ((T/C)CAAGG(T/C)C(A/G)).[23,24] The protein structure of LRH-1, however, follows the common structural NR organization, comprising four conserved motifs: (a) an N-terminal A/B domain that contains a ligand-independent activation function 1 (AF-1); (b) a highly structured DNA-binding domain (DBD) that contains the Ftz-F1 box; (c) a flexible hinge region that links the DBD to (d) the terminal hydrophobic, highly structured and multi-functional ligand-binding domain (LBD).[25,26] The LBD does not only include the ligand-dependent activation function 2 (AF-2), but also serves as the major comodulator association region.[27] It is well established that ligand binding leads to a conformational change in the LBD as well as the whole receptor structure that allows for the interaction of NRs with coactivator proteins.[27] However, for some so-called "orphan" receptors, no physiological ligands have been identified yet. Based on the facts that LRH-1 is constitutively active and lacks known endogenous ligands, it has been classified as an orphan NR. Crystal structure analysis revealed that due to structural rearrangements in its LBD, LRH-1 exhibits a ligand-independent active conformation permitting constant interaction with NR coactivator proteins.[28] This active state is mainly conferred by a constant repositioning of helix H12, being tightly packed against further helices of the LBD core.[29] LRH-1 activity is thus primarily regulated by interaction with comodulatory factors as well as post-translational modifications (PTMs) in a context- as well as tissue-specific manner. Well-described coactivators able to interact with and increase LRH-1 activity include steroid receptor coactivator histone acetyltransferases,[30] peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)[31] and multiprotein-bridging factor-1.[32] Various studies further showed that LRH-1 also physically interacts with other transcription factors to cooperatively stimulate distinct target gene transcription. Examples are the already mentioned cooperation of LRH-1 and beta-catenin in intestinal epithelium proliferation,[22] as well as LRH-1 and PTF1-L in pancreas-specific gene expression.[17] LRH-1 further interacts with the corepressors small heterodimer partner (SHP) in enterohepatic tissues[23] and dosage-sensitive sex reversal, adrenal hypoplasia critical region on chromosome X, and gene 1 (DAX-1) in the ovaries.[33,34] Binding of these two closely related atypical NRs to the LBD of LRH-1 precludes binding of coactivators and thereby inhibits its

transcriptional activity in a tissue-specific manner.[35] Noteworthy, SHP is simultaneously a direct transcriptional target of LRH-1, providing a negative feedback-loop mechanism for their reciprocal regulation.[36] Furthermore, interactions of LRH-1 with prospero homeobox protein 1 (PROX-1)[37] as well as certain corepressor complexes that are associated with histone deacetylases (HDAC) potently repress its transcriptional activity.[24] Also worth mentioning is a tumor necrosis factor alpha (TNFα) induced inhibitory interaction of LRH-1 with the transcription factors c-Jun and nuclear factor κB (NF-κB) in intestinal epithelial cells.[38]

In addition to interaction with comodulatory proteins, LRH-1 is subject to PTMs, which do not only affect its activity, but also subcellular localization and protein turnover. Phosphorylation of the LRH-1 hinge region by extracellular signal-regulated kinases (ERK1/2) is known to stimulate LRH-1 activity.[39] ERK1/2 are members of the mitogen-activated protein kinase (MAPK) superfamily and critically involved in cell proliferation and survival. It is well established that the MAPK signaling cascade is one of the most commonly deregulated pathways in human cancers.[40] Aberrant ERK 1/2 activity, for example, in epidermal growth factor receptor or Ras mutant tumors, may possibly result in constitutively phosphorylated and thus active LRH-1 that might contribute to mitogenic signaling in tumors cells.

Contrary, covalent modification with small ubiquitin-related modifier (SUMO) peptides reduces LRH-1 activity by different mechanisms. On one hand, SUMOylation leads to translocation of LRH-1 from the active chromatin regions to nuclear bodies[41] and on the other hand stabilizes interaction with corepressors, such as PROX1.[42] In a similar way, a complex composed of SHP and the HDAC sirtuin 1 was shown to bind acetylated LRH-1, suppressing its transactivation capacity.[43] A recent publication further demonstrated that LRH-1 protein stability is regulated via ubiquitination and subsequent proteasomal degradation.[44]

Since LRH-1 is considered an orphan NR, extensive research had been conducted in recent years to identify endogenous as well as synthetic LRH-1 ligands. Krylova et al. were the first to discover that different types of phospholipids, including phosphatidyl choline and phosphatidyl inositols, can bind to a conserved cluster of residues in the LBD of LRH-1 in vitro, which is required for maximal LRH-1 activity.[45] It was further shown that specific mutations within the ligand-binding pocket of LRH-1 reduce the binding of phospholipid agonists and the subsequent interaction with coactivators, resulting in diminished LRH-1 activity.[46] These first structural analyses from 2005 were followed by several publications that confirmed initial findings and identified a number of natural phospholipids that enhance the transcriptional activity of LRH-1 in vitro as well as in vivo by altering interaction with coregulators. Of particular interest in this context is dilauroyl phosphatidylcholine (DLPC), a potent LRH-1 agonist in vitro.[47] Furthermore, in an LRH-1-dependent manner, DLPC exhibits strong anti-diabetic effects in two independent in vivo mouse models of obesity-associated insulin resistance.[48] In addition to phospholipids, several synthetic small molecules, such as substituted cis-bicyclo[3.3.0]-oct-2-enes (RWJ101),[49] have also been identified that bind to the LBD of LRH-1 and enhance its activity in vitro. The small LRH-1 agonist BL001, which was recently developed by Cobo-Vuilleumier et al., does not only attenuate but also prevent development of chemically and autoimmune-induced diabetes in mice, while apparently not having any undesirable side effects. Furthermore, BL001 protects beta cells in islets of type 2 diabetic patients from apoptosis and enhances their insulin secretion capacity in vitro.[50]

Together with recent advantages in the development of even more potent and selective LRH-1 agonists,[51] all these findings highlight the promising perspective that LRH-1 could be a druggable target in treating human metabolic diseases, such as diabetes. Also low molecular substances that similarly bind to the LBD of LRH-1, but lead to inactivating structural rearrangements, could be highly interesting tools to modulate LRH-1 activity. It would thus not only be possible to stimulate LRH-1 in a specific context, such as diabetes, where its enhanced activity has beneficial effects, but also to inhibit LRH-1 in diseases were its activity is undesired. Already several small molecules have been discovered that potently inhibit LRH-1 and possibly have great potential in LRH-1-based cancer therapy, which will be discussed in detail in the following. However, keeping in mind that this transcription factor has multiple tissue-specific functions, it is difficult to predict whether stimulation or repression of LRH-1 activity could be sufficiently fine-tuned to have therapeutic benefits, while not causing adverse side effects.

2 | LRH-1 AS AN ONCOGENE AND ASSOCIATED FUNCTIONS IN CANCER

Many of the above-described functions of LRH-1 that are important for normal embryonic development and physiology in the adult organism are also associated with cancer when deregulated. As summarized in Figure 1 (right), overexpression or deregulation of LRH-1 activity can result in enhanced cellular proliferation and survival.

Defects in these regulatory circuits are known to contribute to the transformation of healthy into malignant cells, and acquisition of functions that are considered as "hallmarks of cancer."[52] A selective proliferative advantage as a consequence of elevated LRH-1 activity likely favors progression, aggressiveness, and growth of solid tumors. Simultaneously, an altered response to cellular stress improves the overall survival of tumor cells that are challenged with cytotoxic stimuli, including cell death-inducing drugs or attack by infiltrating immune cells. The LRH-1-regulated tumor-specific synthesis of immunosuppressive GCs would further shape the tumor microenvironment, dampen immune responses, and thus allow escape from tumor immune surveillance.[53] In addition, the effect of enhanced metabolic enzyme expression due to elevated LRH-1 activity might not be underestimated in the context of cancer progression. The consequential increase in the overall cellular energy metabolism is closely related to modulation of mitochondrial functions. It is perfectly conceivable that this process ensures an energy supply that is sufficient to meet the massive anabolic demands of cancer cells, concomitant with all of the above-described processes.

Considering all possible consequences of increased LRH-1 activity, it comes as no surprise that indeed a great number of clinical as well as scientific studies were able to provide evidence for overexpression and implication of LRH-1 in different types of human cancer, including pancreatic, breast, liver, gastric, and colon cancer.[54–58] In this context, there has recently been growing interest in the role of certain small noncoding RNA molecules that under physiological conditions repress LRH-1 expression. Reduced levels of such tumor-suppressing microRNAs inversely correlate with enhanced LRH-1 transcription and activity in breast[59] and colon cancer.[60,61] Similar correlations were, however, also established in cancers where only little is known about the contribution of LRH-1, such as hepatocellular carcinoma (HCC),[62] non-small cell lung cancer (NSCLC)[63] and osteosarcoma.[64] With a particular focus on proliferation, evidence for the rather well studied role of LRH-1 in human pancreatic, breast and intestinal cancers will be reviewed in the following.

In pancreatic cancer, single nucleotide polymorphisms in the LRH-1 gene were linked to increased cancer risk,[65–67] and high LRH-1 mRNA levels were identified as a prognostic biomarker for poor survival rates.[68] Immunohistochemistry revealed LRH-1 overexpression in pancreatic tumors in comparison to adjacent healthy pancreas, and LRH-1 was similarly overexpressed as well as activated in tumor cells in comparison to healthy pancreatic epithelial cells.[8,69] Several independent research groups provided evidence that this promotes growth of pancreatic cancer cells by increased expression of LRH-1 target genes involved in proliferation, including c-Myc, and cyclin D1 and E1.[8,54] It was further shown that LRH-1 overexpression increases the metastatic potential as well as invasion of pancreatic cancer cells, contributing to a more aggressive malignant phenotype.[70]

A similar correlation had been established between LRH-1 and breast cancer, which in the majority of cases is driven by deregulated estrogen signaling that promotes breast cancer cell proliferation.[71] Interestingly, LRH-1 is not only abnormally expressed in over 40% of breast cancer cases, but correlates also positively with the status of estrogen receptor α (ERα).[72] This might be explained by the findings that LRH-1 is a direct target gene of the ERα[73] and the ERα vice versa an LRH-1 target gene,[74] likely resulting in a positive feedback loop. ERα-responsive genes often comprise shared binding sites for both transcription factors and are thus coregulated by ERα and LRH-1.[75] Thus, enhancing the expression of ERα target genes, such as growth regulation by estrogen in breast cancer 1, is one mechanism by which LRH-1 promotes proliferation of breast cancer cells.[76] In addition, LRH-1 contributes to ERα activation and estrogen-dependent proliferation by regulating the expression of aromatase (CYP19), a steroidogenic enzyme that is required for the estrogen production.[72] The LRH-1-mediated aromatase upregulation seems to be at least partially induced by tumor-derived prostaglandin E_2 (PGE_2), a major driver of aromatase expression in breast cancer.[77] Together with sphingosine-1-phophate, a bioactive lipid implicated in breast cancer,[78,79] PGE_2 induces LRH-1 mRNA expression by recruitment of multiple transcriptional activators to the LRH-1 promoter.[80] Interestingly, these also include LRH-1, which was shown to significantly induce its own expression at least in this cellular context.[80] In line with this, the presence of a potential LRH-1 response element located within its own promoter region 89 bp upstream of the start codon could be confirmed.[81] Apart from contributing to the continuous estrogen biosynthesis in breast cancer, this suggests a positive feedback loop for LRH-1 expression and activity. One could certainly assume that this also promotes other oncogenic functions of LRH-1 in breast cancer, but possibly also other types of cancer. in vitro silencing and overexpression studies revealed that LRH-1 further promotes breast cancer cell proliferation by cyclin D1 induction[82] and p21 repression.[83] The universal cyclin-dependent kinase inhibitor p21 is a major target and mediator of p53 signaling that induces growth arrest, differentiation, or senescence upon cellular and especially DNA damage.[84–86] Interfering with these tumor-suppressive functions of p21, LRH-1 favors the proliferation of cells

that might contain damaged DNA, which is thought to be an underlying cause for cancer initiation.[87] Additionally, increased LRH-1 activity promotes the migratory properties and invasiveness of breast cancer cells in vivo.[88] A clear association between LRH-1 expression patterns with breast cancer phenotype and aggressiveness was discovered only recently by immunohistochemistry analysis of patient samples.[55]

Being involved in proliferation and renewal of intestinal epithelial cells, implication and enhanced expression of LRH-1 was unsurprisingly also confirmed in gastric[57] and colon cancer.[89] Quantification of LRH-1 expression in colon cancer tissue by Wu et al. revealed that LRH-1 expression was not only abnormally elevated, but also correlated with stage, invasiveness, and metastasis in colon cancer. They were further able to prove that LRH-1 expression is associated with poor prognosis and a low 5-year survival rate, and proposed LRH-1 as a prognostic marker in colon cancer.[58] It is well established that one of the key determinants of colon cancer pathogenesis is beta-catenin.[90] It is part of the Wingless and INT-1 (WNT) signaling cascade, a pathway that is hyperactivated in almost all colon cancer cases.[91] The described interaction of LRH-1 with beta-catenin contributes to intestinal tumor formation by synergistic induction of the cell cycle-initiating cyclins D1 and E1, as well as c-Myc resulting in an enhanced proliferative potential of intestinal cancer cells.[92,93] All three of these cell cycle regulators are frequently overexpressed in human gastrointestinal tumors, with especially c-Myc being a classic proto-oncogene promoting the generation of cancer cells with a massive self-renewal capacity.[94–97] In their cutting-edge paper of 2005, Schoonjans et al. further showed that LRH-1 haploinsufficieny mitigates tumorigenesis and inflammatory TNFα expression in genetic or chemical mouse models of human colon carcinogenesis.[98] Confirming the pro-proliferative role of LRH-1 in intestinal tumors, RNA interference-mediated LRH-1 knockdown impairs proliferation and prolongs the G0/G1 phase of colorectal cancer cells.[99] Similar to observations made in the context of breast cancer, deregulated LRH-1 activity promotes colon cancer by the transcriptional repression of p21.[89]

Even though less well studied up to now, LRH-1 deregulation is also associated with tumorigenesis of HCC,[100–102] gastric cancer,[92,93] Barrett's-associated adenocarcinoma,[103] prostate cancer,[104] and NSCLC.[63,105,106] However, the mechanisms that underlie LRH-1-regulated proliferation seem to be common among all cancer types analyzed: induction of c-Myc, cyclin D1/E1, and WNT/beta-catenin signaling. Simultaneously, by repressing p21 transcription, LRH-1 promotes carcinogenesis and development of cancerous cells. Because of these critical functions in multiple types of tumors, LRH-1 represents an attractive therapeutic target in cancer therapy. In particular, because it further improves survival of tumor cells in a cell autonomous as well as indirect, cell nonautonomous manner, which will be discussed in detail in the following chapters. Targeting LRH-1 will thus not only be useful to decelerate proliferation but also specifically induce cell death of cancer cells.

3 | LRH-1 AS A REGULATOR OF CELL SURVIVAL AND MITOCHONDRIAL APOPTOSIS

Apart from sustained proliferative signaling and insensitivity to growth suppression also evasion of programmed cell death (apoptosis) is, among others, a classical "hallmark of cancer" as defined in the seminal article by Weinberg and Hanahan.[107] Beyond important functions during development and tissue homeostasis, apoptosis ensures the elimination of cells, which are damaged beyond repair or overexpress oncogenes, to prevent their malignant transformation. In contrast to necrosis, a rather uncontrolled form of cell death, apoptosis is a tightly regulated process that can be triggered by different stimuli and is thus a highly important target in cancer therapy. Apoptotic cell death is either initiated by the mitochondrial (intrinsic) or the extrinsic pathway, both resulting in the activation of apoptosis-executing proteases, called caspases.[108] The extrinsic apoptosis pathway is initiated by interaction of death ligands with their respective cell-surface death receptors (DRs) of the tumor necrosis factor (TNF) receptor (TNFR) family, including TNFR1, Fas and TNF-related apoptosis-inducing ligand (TRAIL) receptors 1/2. Ligand-binding results in the recruitment of a caspase-activating platform that brings multiple monomers of the initiator caspase 8 into close proximity, at the intracellular region of DRs. This stimulates the proteolytic activity of recruited caspase 8 molecules, ultimately resulting in their auto-proteolytic activation.[109] The intrinsic apoptosis pathway is initiated upon various cellular stress stimuli, including DNA damage, endoplasmic reticulum (ER) stress or absence of growth factors. In a cell-autonomous manner, these processes trigger mitochondrial outer membrane permeabilization (MOMP), which is the key event and (usually) point of no-return of mitochondrial apoptosis.[110] MOMP is a highly controlled process that is mainly orchestrated by proteins of the Bcl-2 family. These are characterized by containing at least one or more eponymous Bcl-2 homology (BH) domains facilitating their interaction. In general, the Bcl-2 family can be subdivided into three groups: (a) antiapoptotic Bcl-2 proteins, including Bcl-2, Bcl-xL, and Mcl1, that counteract

MOMP by binding to and thus inhibiting pro-apoptotic family members; (b) the pro-apoptotic effectors Bax and Bak, that upon activation integrate, oligomerize, and form pores in the mitochondrial outer membrane; (c) BH3-only proteins like Bim, Bid, Puma, and Noxa, which are direct activators of Bax/Bak and/or block anti-apoptotic Bcl-2 family members.[111,112] Various signaling pathways regulate the relative abundance as well as activity of BH3-only proteins, making them important upstream sensors that fine-tune the antagonizing forces between antiapoptotic and pro-apoptotic Bcl-2 proteins during apoptotic signaling. Pro- and antisurvival signals are thus integrated by the highly complex interactome and balance of Bcl-2 family members, deciding over the cellular fate by controlling integrity of mitochondria.[113] Activation of Bax/Bak and subsequent MOMP is associated with the release of certain pro-apoptotic mitochondrial proteins, such as cytochrome *c*.[110] Once in the cytosol, cytochrome *c* facilitates the ATP-dependent assembly of another caspase-activating platform, called apoptosome. Similar to the DISC, the apoptosome facilitates the spatial proximity of pro-caspase 9 monomers and subsequent auto-proteolysis-induced activation.[114] In simplified terms, activation of initiator caspases 8 or 9 culminates in the processing and thereby activation of caspases 3 and 7. These effector caspases themselves can process hundreds of intracellular proteins, ultimately leading to the coordinated fragmentation of the cell.[115] It is further widely accepted that there is a crosstalk between the extrinsic and intrinsic apoptosis pathway. This is particularly important in certain cell types (type 2 cells), like hepatocytes, that rely on the amplification of the extrinsic, DR-mediated signal via the mitochondrial pathway to undergo apoptosis.[113] Being absolutely central in stress-induced apoptotic signaling, mitochondria, the Bcl-2 family and other components of the intrinsic apoptosis pathway are thus classic targets in cancer chemotherapy, commonly aiming to trigger mitochondrial apoptosis in highly proliferating cells. Prominent examples are cytostatic compounds, such as doxorubicin, that cause DNA damage and genotoxic stress during replication.[116] The concomitantly activated tumor suppressor p53 directly regulates the transcription of the BH3-only proteins Puma and Noxa.[117] All processes and factors that in any way influence elements of the apoptotic signaling cascades have apoptosis-regulating function and could thus be considered as potential therapeutic targets in cancer therapy. This also includes nuclear transcription factors that regulate the expression and thus abundance of for example, Bcl-2 family members and other pro- or antiapoptotic proteins.

Reviewing the effects of LRH-1 inhibition and deletion on the entire organism, certain tissues or specific cell types, indicates that LRH-1 has to be such a regulator of apoptotic cell death and survival. As mentioned before, systemic homozygous LRH-1 deletion results in embryonic lethality with embryos dying at E.6.5–7.5. At this time point, striking anomalies and malformations can be observed that are indicative of visceral endoderm dysfunction.[12] Tissue-specific or heterozygous deletion of LRH-1 does, however, not affect embryonic development, but results in a functional deficiency, exacerbated inflammatory response and increased cell death in certain tissues, including the intestine[21,118] and liver.[119–123] In 2018, Bayrer et al. were not only able to demonstrate a crucial role of LRH-1 in maintenance of the intestinal epithelium, but also its involvement in cell death and survival pathways. Using different ex vivo and in vivo approaches, they showed that intestine-specific LRH-1 knockout reduces Notch signaling, a pathway important for renewal of adult tissues,[124] while simultaneously increasing spontaneous intestinal crypt cell death. Upon acute deletion of LRH-1, a rapid induction of cell death was observed in murine intestinal organoids. Interestingly, this was accompanied by elevated levels of activated caspase 3, suggesting induction of apoptotic signaling. And indeed, LRH-1 deletion evoked significant changes in genes implicated in survival as well as apoptosis signaling pathways.[118]

In T cells, LRH-1 deletion results, apart from causing prominent proliferation defects, in elevated basal cell death as well as increased cell death in response to mitogenic stimuli.[10] Moreover, we recently identified Fas ligand (FasL), a cytotoxic T cell effector molecule and death ligands, as an LRH-1 target gene. We observed reduced activation-induced FasL expression in response to pharmacological LRH-1 inhibition. This not only limits the capacity of cytotoxic T cells to activate the extrinsic apoptotic pathway in other target cells but also prevents FasL-mediated, cell-autonomous activation-induced T cell apoptosis.[9] In primary murine macrophages and macrophage cell lines, we observed that LRH-1 inhibition and knockdown impairs the production of pro-inflammatory cytokines. Similarly, the macrophage-dependent production of TNFα and concomitant triggering of hepatocyte apoptosis was markedly reduced after LRH-1 inhibition in a murine hepatitis model in vivo.[11] Taken together these findings suggest that LRH-1 is regulating apoptotic processes also in cells of the hematopoietic system. On one hand, LRH-1 seems to regulate apoptosis directly, in a cell-autonomous manner, but on the other hand also affects the expression of death ligands, and thus induction of the extrinsic apoptotic pathway in neighboring or target cells.

A role of LRH-1 in apoptotic signaling was also confirmed by studies that vice versa analyzed the protective

effects mediated by experimental LRH-1 upregulation. For example, overexpression of LRH-1 protected intestinal organoids from cell death induced by Fluorouracil, a common chemotherapeutic drug promoting intestinal toxicity. Similarly, TNFα-induced inflammation and apoptosis can be suppressed by LRH-1 overexpression in intestinal organoids derived from tissue samples of healthy individuals and patients with Crohn's disease.[118]

Based on the facts that reduced LRH-1 activity triggers spontaneous apoptosis, whereas enhanced LRH-1 levels protect from cell death-inducing stimuli, it can be assumed that LRH-1 is critically involved in the survival and apoptosis protection of different cell types. It thus seems very likely that there is a link between LRH-1 and mitochondria, which can be seen as a central element in ensuring cellular energy supply and survival. Discussing the concept of LRH-1 being a regulator of mitochondrial functions and apoptotic signaling will help to explain how overexpression of LRH-1 contributes to cancer cell survival.

3.1 | Cell-autonomous role of LRH-1 in mitochondrial functions and apoptotic signaling

3.1.1 | Metabolic reprogramming

Certainly, resistance to apoptotic cell death induction is directly or indirectly coupled to a deregulation of cellular energetics and metabolism, another "hallmark of cancer."[52] Being a critical regulator of diverse metabolic processes, it is thus only reasonable to hypothesize that LRH-1 overexpression, resp. deregulation could be involved in this process. Enhanced energy production and metabolic reprogramming is indispensable in order to meet the massive energy demands that come along with the increased anabolic requirements in cancer cells. This ensures synthesis of proteins that are vital for a rapid proliferation and survival, which in turn also opens perspectives for therapeutic targeting of cancer cells. One interesting example is thiazolides. Although initially developed as antiparasitic drugs, they also have anticancer activity by promoting energy depletion in colorectal cancer cells via direct inhibition of mitochondrial oxidative phosphorylation. This interference with bioenergetic processes selectively induces cell cycle arrest, prior to Bim-mediated apoptosis in tumor cells, thus contributing to the anticancer activity of thiazolides.[53,125]

In the context of metabolic transformation of tumor cells, the Warburg effect is probably the best described phenomenon. It is defined as a switch from ATP generation via mitochondrial oxidative phosphorylation to aerobic glycolysis that refers to the fermentation of glucose to lactate in the presence of oxygen.[126] Even though many cancer cells run on Warburg metabolism, most use also and/or are even depending on mitochondrial respiration. Hosting the machinery for the tricarboxylic acid (TCA) cycle, fatty acid β-oxidation (FAO), and oxidative phosphorylation, mitochondria are best known for their functions in cellular bioenergetics and ATP production. However, mitochondria are also important biosynthetic and signaling organelles. They act as sensors for cellular stress and allow eukaryotic cells to rapidly adapt to changes in the environment. Mitochondria are thus associated with survival, transformation and tumorigenesis.[127] Alteration or more specifically increase in cellular mitochondrial mass and ATP production is one aspect that contributes to survival of cancer cells.[127] Mitochondrial biogenesis is primarily regulated by PGC-1α, a known interaction partner and transcriptional coactivator of LRH-1.[128] Depending on the cancer type, upregulation of PGC-1α is associated with increased mitochondrial mass and dependence of cancer cells on mitochondrial respiration.[129] Only recently, it could be shown that liver-specific LRH-1 deletion results in reduced mitochondrial biogenesis, DNA copy number, oxygen consumption rate, and ATP-producing capacity. Even more intriguing is the finding that activation of LRH-1 increases the expression of PGC-1α.[130] This suggests a positive feedback loop, in which LRH-1 induces its own coactivator to directly enhance mitochondrial biomass and function, a process that once deregulated likely contributes to tumorigenesis. In this regard, it is interesting that also the LRH-1 target gene c-Myc contributes to an increase in mitochondrial mass.[77]

Another important trait of cancer cells is a general metabolic reprogramming that includes changes in lipid and amino acid metabolism, as well as glucose utilization.[131] Some cancer types, as for example leukemia, heavily rely on FAO to fuel the TCA cycle for energy production.[132] This type of metabolic transformation is associated with upregulation of carnitine palmitoyltransferase (CPT), an enzyme required for mitochondrial fatty acid import. CPT1C expression promotes FAO and ATP production in cancer cell lines as well as growth of tumors in vivo. It further confers resistance to therapy and metabolic stress, such as hypoxia or glucose depletion.[133] Interestingly, also in this context, there is a clear link to LRH-1. Knockout and pharmacological gain of function studies using the LRH-1 ligand DLPC revealed that LRH-1 directly controls the transcription of CPT in primary murine hepatocytes and cell lines. LRH-1 thus also seems to be a critical regulator of mitochondrial FAO, which could contribute to the metabolic transformation, survival, and apoptosis resistance in certain cancer types.[130]

As a result of the Warburg effect and the limited availability of pyruvate that feeds into mitochondrial metabolism, cancer cells rely on alternative substrates that feed into the TCA cycle, and are in this context particularly depend on glutamine. Despite fueling the TCA cycle, glutamine serves as an important building block for the synthesis of amino acids, nucleotides, lipids, and glutathione. Consequently, glutaminolysis is elevated in glutamine-addicted tumors. This is frequently driven by upregulation of glutaminases that are involved in the two-step conversion of glutamine to α-ketoglutarate, the first and rate-limiting step during glutaminolysis.[134] Directly linking LRH-1 to carcinogenesis, Xu et al. demonstrated that LRH-1 is a regulator of mitochondrial glutaminase 2 (GLS2), thus promoting hepatic glutamine processing, mitochondrial energy metabolism, and cancer cell proliferation. Importantly, the associated increase in α-ketoglutarate further stimulates cell growth by activation of mammalian target of rapamycin complex 1 (mTORC1) signaling, which contributes to LRH-1 driven liver carcinogenesis.[56]

Furthermore, deregulated LRH-1 signaling also appears to enhance the overall availability of glucose by promoting the expression of glucose transporters (GLUT), which promote cellular glucose uptake. First evidence for this hypothesis was provided by a study that analyzed the role of LRH-1 in muscle metabolism. There, it was reported that DLPC-induced activation of LRH-1 selectively increases expression of GLUT4 and thus glucose uptake promoting glucose preference in muscle cells.[135] On top of that, LRH-1 is a direct transcriptional regulator of tissue-specific hexokinases.[121,135] After uptake of glucose by GLUTs, these enzymes catalyze the phosphorylation of glucose to glucose-6-phophate, the rate-limiting and first step of glycolysis.[136] Whereas hepatocyte-specific LRH-1 deletion reduced the expression of the hepatic hexokinase glucokinase and glycolysis rates,[121] DLPC-mediated LRH-1 activation increased the expression of hexokinase in muscle cells.[135] In line with these findings, pharmacological inhibition of LRH-1 impaired mitochondrial ATP production through a decrease in glucokinase and also GLS2 expression in macrophages.[11] Unsurprisingly, upregulation of GLUTs, including GLUT4,[137] as well as increased expression of glycolytic enzymes, such as hexokinases, has been reported in different cancer cells that highly depend on glucose as an energy source.[138] Despite their critical role in glycolysis, the expression and mitochondrial association of hexokinases has further been linked to cellular growth, survival, and prevention of mitochondrial cell death in healthy, but above all also in malignant cells.[136,139]

Taken together, LRH-1 likely contributes to the metabolic transformation in certain cancer types by facilitating the increased uptake and utilization of glucose. LRH-1-mediated upregulation of hexokinases might simultaneously prevent mitochondrial apoptosis. Most importantly, however, LRH-1 promotes proliferation and survival of cancer cells by regulating target genes involved in metabolic functions of mitochondria. LRH-1 seems to be particularly important to stimulate metabolic pathways, like FAO and glutaminolysis, that feed into the TCA cycle, ensuring oxidative phosphorylation for ATP generation runs smoothly.

3.1.2 | Protection from ER stress, and oxidative and DNA damage

During tumorigenesis and metastasis, cancer cells are typically facing a hostile environment and increased intrinsic stress. Hypoxia, changes in pH, nutrient limitation, treatment with chemotherapeutics, oncogene activation, increased glycolysis, and protein translation rates as well as mutations impeding correct protein folding collectively add pressure on the ER and secretory pathway.[140] Deregulation of ER homeostasis and permanent ER stress had been reported in various types of cancer.[141] The accumulation of mis- or unfolded proteins in the ER promotes the activation of adaptive signaling pathways called unfolded protein response (UPR). It regulates communication between the ER and the nucleus, crosstalk with mitochondrial pro-apoptotic signaling pathways, as well as protein degradation and export.[142] Increasing evidence suggests that deregulated activation of the UPR machinery promotes the ability of cancer cells to adapt for cell survival and evade mitochondrial apoptosis. This process has been implicated in tumor growth, metastasis, metabolism and chemotherapy resistance.[140,141] Also in this context, there is a direct link between LRH-1 and ER stress resolution pathways, however, independent of the classic UPR machinery.[143] In response to ER stress, LRH-1 induces the expression of its direct target gene Polo-like kinase 3 (Plk3), a stress response protein that, depending on the cancer type, can have tumor-suppressive or -promoting functions.[144] The Plk3-mediated phosphorylation of activating transcription factor 2 (ATF2) subsequently drives ER stress resolution through unknown underlying mechanisms. Pharmacological activation of LRH-1 increases activation of Plk3 and ATF2, the ability to resolve high levels of ER stress and protects from mitochondrial apoptosis induction.[123] Similar to Plk3, ATF2 involved in a wide variety of cellular processes, including cell cycle control, DNA repair, immune responses, as well as metabolism. Altered expression and phosphorylation state of ATF2 is also associated with various cancer types and resistance to genotoxic stress-inducing therapeutic agents.[145] Due to these diverse functions of Plk3 and ATF2, the LRH-

1-Plk3-ATF2 axis might, apart from ER resolution, contribute to carcinogenesis through multiple signaling pathways.

The high energy demand and consequently extreme upregulation of metabolic processes, including mitochondrial respiration, of cancer cells results in the production of large amounts of reactive oxygen species (ROS). Contributing to the transformation of cells, ROS are modulating signaling pathways, like NF-κB, that drive several aspects of carcinogenesis, including proliferation, apoptosis evasion, invasion, and metastasis.[146] However, if ROS levels exceed the cellular capacity of antioxidants, it can have fatal effects as cellular components, including DNA, RNA, proteins and lipids, become oxidized. In order to adapt, cancer cells develop different ways to establish a protective antioxidant system and new redox potential.[147] Superoxide dismutase 2 (SOD2), which is a key antioxidant enzyme located within the mitochondria, was shown to be a direct transcriptional target of LRH-1. The pharmacological activation of LRH-1 was reported to increase SOD2 expression, while simultaneously reducing ROS production.[148] A protein stabilizing SOD2 polymorphism has been implicated in the development of various cancer types[149] and its expression levels are associated with a poor prognosis of ovarian cancer.[150] Thus, enhanced expression as well as activity of LRH-1 that increase SOD2 levels may contribute to the development of ROS resistance in certain cancer cells.

LRH-1 further contributes to survival and chemotherapy resistance of cancer cells by regulating stress sensors implicated in DNA damage responses. It was reported that RNA interference-based LRH-1 downregulation results in reduced expression of the growth arrest and DNA damage-inducible pro-survival factor Gadd45beta correlating with induction of apoptosis in HCC cells.[100] LRH-1 further attenuates the cytotoxicity of DNA damage inducing chemotherapeutic drugs by directly promoting the expression of mediator of DNA damage checkpoint 1 (MDC1). Resulting in the mitigation of chemotherapy-induced DNA damage by increasing DNA damage repair processes, upregulation of MDC1 is another underlying mechanism of how LRH-1 confers chemoresistance to certain cancer cell types.[151]

3.1.3 | Direct regulation of mitochondrial apoptosis signaling

Apart from indirectly contributing to the fate and survival of cancer cells by governing metabolic and general stress responses, LRH-1 might also directly affect the mitochondrial apoptosis pathway. So far, there is, however, only little clear molecular proof for the involvement of LRH-1 in the regulation of classical antiapoptotic and pro-apoptotic factors. Nonetheless, some studies confirm that LRH-1 contributes to the viability and chemoresistance of cancer cells by transcriptional activation of proteins with antiapoptotic functions, but also repression of certain pro-apoptotic factors. Depletion and pharmacological inhibition of LRH-1 in esophageal adenocarcinoma (OAC) revealed that LRH-1 promotes cancer cell survival by increasing cellular levels of Bcl-xL and GATA-binding factor 6 (GATA6).[103] Similar to LRH-1, GATA6 is a transcription factor with important functions in cellular differentiation and proliferation, and has thus also been implicated in different cancer types.[152–154] These findings suggest that affecting the expression of an antiapoptotic Bcl-2 family member and another potentially oncogenic transcription factor are two mechanisms of how LRH-1 regulates proliferation of OAC and likely also other cancer types. In 2018, Wang et al. could establish a link between LRH-1, Bax, and p53 protein expression, and caspase activity. Studying the role of microRNA-381 in osteosarcoma cells, they were able to show that the LRH-1/WNT/beta-catenin signaling axis controls proliferation, but also prevents apoptosis in this type of cancer by inhibition of caspase 3 and 9 activities, while simultaneously positively regulating Bcl-2 expression. Additionally, upregulation of the LRH-1/WNT/beta-catenin pathway reduced p53 and Bax expression further contributing to cellular viability.[64] Interestingly, an inverse correlation between microRNA-381 and LRH-1 expression was also observed in HCC. Also in this context, targeting LRH-1 by microRNA-381 reduced LRH-1-mediated WNT/beta-catenin signaling.[62] Decreased microRNA-381 expression in different cancer types thus might contribute to enhanced LRH-1 levels and increased LRH-1/WNT/beta-catenin signaling.

In summary, LRH-1 supports resistance to environmental threats, cytotoxic stimuli as well as increasing intrinsic stress that cancer cells are commonly confronted with by regulating the expression of factors involved in general stress responses, but also by preventing mitochondrial apoptotic signaling through two different mechanisms: (a) induction of antiapoptotic proteins, such as Bcl-2 family members, but probably also by (b) inhibition of caspase activity and transcriptional repression of the pro-apoptotic proteins such as Bax and p53.

4 | POTENTIAL OF LRH-1 TARGETING APPROACHES IN CANCER THERAPY

The previous chapters of this review established that high LRH-1 expression and activity positively contributes to

an increased viability of cancer cells. Next to resolution of intrinsic stress and prevention of antitumor immune surveillance, LRH-1 regulates proliferation, stemness, and mitochondrial energy metabolism, while counteracting mitochondrial apoptosis signaling pathways. Affecting several mitochondrial processes, the NR LRH-1 essentially protects survival of cancer cells and can therefore be considered as a potential proto-oncogene. LRH-1 might thus be a promising novel target in the treatment of various types of tumors in which its expression and/or activity is pathologically enhanced.[155] Accordingly, pharmacological LRH-1 antagonists are likely suitable tools to inhibit cancer cell proliferation, and at the same time also sensitize cancer cells to given apoptosis triggers, or even directly induce their cell death. It must, however, be taken into account that LRH-1 is vital for the function of several organs of the adult organism, such as the liver and pancreas. Especially considering the essential implication of LRH-1 in metabolic processes, it is perfectly conceivable that long-term application of LRH-1 inhibitors might cause undesired adverse effects, including the development of diabetes and/or liver damage.

Analysis of mice with heterozygous or tissue-specific LRH-1 deletion, however, substantially lowers these concerns. While homozygous deletion of LRH-1 results in embryonic lethality, in the past many studies were conducted using mice with a heterozygous LRH-1 deletion (LRH-$1^{+/-}$). LRH-$1^{+/-}$ animals are viable, have a normal life span even though intestinal, pancreatic and hepatic LRH-1 mRNA levels are reduced up to 50%.[22,156] Despite having slightly shortened intestinal crypts, LRH-$1^{+/-}$ mice do not show any severe changes in organ morphology when compared to wild type or control mice.[22] And even though LRH-$1^{+/-}$ animals are slightly more susceptible to high-fat diet-induced obesity, the only mildly enhanced body weight gain was not accompanied by any changes in glucose and lipid metabolism. Especially the comparable response of $LRH^{+/-}$ and wild-type mice in glucose and insulin tolerance tests argues against the concern that LRH-1 inhibitors might cause diabetic conditions.[156]

Further, Lee et al. generated and characterized mice with a tissue-specific LRH-1 deficiency in either hepatocytes or intestinal epithelial cells. These animals are also viable and exhibit a relatively normal phenotype as well as organ histology. Only some mild changes in bile acid composition and plasma cholesterol levels, due to the reduced expression of different metabolic LRH-1 target genes, were observed in liver- and intestine-specific LRH-1 knockout mice.[157] Even though it could be shown that LRH-1 is involved in many different physiological processes, these findings suggest that LRH-1 is not solely responsible for their smooth regulation, and loss or inhibition of LRH-1 might be compensated by other factors. LRH-1 is therefore not absolutely essential for the tissue-specific physiological processes in the adult organism, which opens a therapeutic window of opportunity to especially target cancer cells that are highly dependent on LRH-1 activity. For this reason, there has been growing interest in the identification of synthetic, small molecules that selectively inhibit the transcriptional activity of LRH-1. Such antagonists are not only extremely useful to further elucidate the role of LRH-1 in different malignancies but might also be interesting therapeutic tools in cancer treatment.

In recent years, two different inverse agonists had been discovered that block the constitutive activity level of LRH-1 very effectively via different modes of action. In 2013, Benod et al. discovered the first pharmacological LRH-1 antagonist, named compound 3 (Cpd3, 1-(3′-{1-[2-(4-morpholinyl)ethyl]-1H-pyrazol-3-yl}-3-biphenylyl) ethanone), in a structure-based approach that included high-throughput molecular docking screening and subsequent experimental verification of the predicted antagonists.[158] They were able to show that this small molecule binds directly into the hormone-binding site of human LRH-1. This destabilizes the receptor conformation, presumably due to the replacement of helix H12 from the LBD core as predicted by virtual molecular docking studies. Without affecting the mRNA levels of LRH-1, Cpd3 potently inhibits the transcriptional activity of LRH-1 with an IC_{50} value of 5 ± 1 μM. As a consequence, the expression of LRH-1 target genes, such as SHP or cyclin E1, is significantly reduced upon treatment with this small molecule inhibitor. The study further convincingly confirms that Cpd3 at the same time does not interfere with the transactivation of the close LRH-1 homolog SF-1, as well as several more distantly related NRs, such as ERα.[158] Additionally, also a structural analog of Cpd3, compound 3d2 (Cpd3d2, 4-(3-{1-[2-(dimethylamino)ethyl]-1H-pyrazol-3-yl}phenyl)-N,N-5,6-tetramethyl-2-pyrimidinamine) binds to and destabilizes the LBD of LRH-1. Comparably to the original compound, 3d2 potently antagonizes the transcriptional activity of LRH-1, but not other NRs, including SF-1.[158]

The second type and even more potent non-phospholipid small molecule repressor of LRH-1, SR1848, was first discovered and mentioned in a probe report by Busby et al. in 2010.[159] In an approach mainly based on promoter reporter assays, they screened for synthetic compounds inhibiting the LRH-1-mediated transactivation of the aromatase and StAR promoter, respectively. This led to the identification of two structurally similar antagonists of LRH-1, SR1848 (ML180, 6-[4-(3-Chlorophenyl)-1-piperazinyl]-3-cyclohexyl-1H-pyrimidine-2,4-dione) and ML179 (3-Cyclohexyl-

6-[4-[3-(trifluoromethyl)phenyl]-1-piperazinyl]-1H-pyrimidine-2,4-dione) that slightly differ in potency and maximum efficacy.[159]

Corzo et al. further confirmed that SR1848 inhibits the transcriptional activity LRH-1, and dose-dependently diminishes the expression of LRH-1 targets, such as aromatase and SHP. Furthermore, SR1848 also reduced the relative abundance of LRH-1 mRNA in Huh7 and HepG2, two human HCC lines that express high levels of LRH-1.[160] This is in line with the finding that LRH-1 can induce its own expression.[80] A reduction of LRH-1 target gene expression in response to SR1848 might thus at least be partially attributed to a decrease in LRH-1 levels. In contrast to Cpd3 and Cpd3d2, SR1848 is, however, not specific for LRH-1 as it also antagonizes SF-1 in a dose-dependent manner.[159,160] Particularly noteworthy is the rapid onset of SR1848-mediated effects. Already within 2 hr, a significant reduction of mRNA transcript levels of LRH-1 and several of its downstream target genes could be observed.[160] The mechanism of action of SR1848 was investigated using chromatin immunoprecipitation and immunohistochemistry assays. In marked contrast to Cpd3 and Cpd3d2, SR1848 induces the rapid nuclear export of LRH-1 to the cytoplasm of cells. This naturally interferes with the chromatin-binding capacity and induction of LRH-1 target gene transcription.[160] As no direct binding of SR1848 to the LBD of LRH-1 could be demonstrated, Corzo et al. hypothesized that SR1848 might interact with regions outside of the LBD or rather indirectly interfere with the localization of LRH-1.

Even though displaying an entirely different mode of action, Cpd3/3d2 and SR1848 have a similar inhibitory effect on cell growth and proliferation of different cancer cell lines. The antiproliferative activities of both antagonists are LRH-1-dependent and most probably caused by the concomitant reduced expression of oncogenes that promote cancer cell proliferation.[158,160] Cpd3 and Cpd3d2 inhibit the proliferation of cancer cells dose-dependently without showing any general cytotoxicity. Notably, both antagonists inhibit the proliferation potential of AsPC-1 cells, a pancreatic ductal adenocarcinoma (PDAC) cell line with high LRH-1 levels, whereas proliferation of an LRH-1-negative PDAC line (L3.3) remained unaffected.[8,158] Corroborating these results, Benod et al. demonstrated that both antagonists also compromise the proliferation rates of LRH-1 expressing human colon as well as breast cancer cells.[73,74,98] Similarly, already the initial probe report of Busby et al. demonstrated that SR1848 has a potent inhibitory activity in different human cancer cell lines. It decreases the viability of breast cancer cells by up to 90% when used at low micromolar concentrations.[159] Furthermore, SR1848 also strongly diminished the proliferation capacity of the human HCC line Huh7 that under steady-state conditions exhibits high levels of nuclear LRH-1. The lack of cytostatic effects in an ovarian adenocarcinoma cell line with comparably low LRH-1 expression (SK-OV-3) suggests that the antiproliferative activity of SR1848 is also LRH-1-mediated. Concordantly, the expression of pro-proliferative LRH-1 targets, namely cyclin D1 and E1, was only significantly inhibited in SR1848-treated Huh7, but not SK-OV-3 cells.[160]

Despite or perhaps exactly because of their different mode of action Cpd3/3d2 and SR1848 are highly useful tools to study the diverse functions of LRH-1. Thus, these small molecule LRH-1 antagonists have been successfully used in various studies to investigate LRH-1-regulated processes. For example, Cpd3 was used to identify Calreticulin as a novel LRH-1 target gene, thus establishing a role for LRH-1 during renal fibrosis.[161] The first in vivo application of Cpd3 in zebrafish embryos demonstrated that LRH-1 is essentially involved in multiple stages of liver and pancreas organogenesis.[162] Confirming a vital role for LRH-1 in cell proliferation, SR1848 treatment was shown to suppress the cell division activity of primary murine granulosa cells ex vivo. As observed in other cell types, the abundance of pro-proliferative LRH-1 target gene transcripts, including cyclin D1 and E1, was simultaneously markedly reduced, which recapitulated the phenotype of LRH-1 knockout cells.[163] In our own studies investigating the role of LRH-1 in the hematopoietic system, we were able to confirm the antagonist effect of both inhibitors in macrophage and T cells in vitro and in vivo.[9,11]

While investigating the role of LRH-1 in cells of the immune system, we recently observed that LRH-1 inhibitors also have anticancer effects in leukemic T and B cells. Unpublished data indicate that Cpd3d2 as well as SR1848 strongly suppress the proliferation capacity of acute lymphoblastic leukemia (ALL) cells in vitro. Similar as in other cancer cells, this seems to be accompanied by a reduction of pro-proliferative LRH-1 target gene expression and resembles the effect of siRNA-mediated LRH-1 knockdown. Remarkably enough we observed that both antagonists further have the capacity to induce apoptotic cell death in different T- as well as B-ALL cell lines. Even more interesting is, however, the observation that simultaneous treatment with certain other anticancer drugs leads to a massive increase in dead cells and synergistic cytotoxicity (Michalek, Brunner, unpublished). Small molecule LRH-1 inhibitors thus seem to enhance the sensitivity of leukemic cells toward other drugs, many of them currently being used in the standard therapeutic regimen for the treatment of T-ALL. In addition to different types of solid cancers, LRH-1 hence might also be implicated in certain hematopoietic malignancies, as it seems to be a critical

regulator of leukemia cell proliferation. LRH-1 somehow also contributes to the survival of malignant T and B cells, even though further research is required to elucidate the exact underlying molecular mechanisms.

Only recently, in vitro application of SR1848 contributed to the discovery that LRH-1 is also implicated and a possible therapeutic target in castration-resistant prostate cancer (CRPC).[164] This type of cancer is typically associated with persistent androgen receptor (AR) signaling caused by the constant biosynthesis of intratumoral androgens and thus commonly treated with steroidal inhibitors.[165] Xiao et al. were able to show that LRH-1 is not only upregulated in CRPC tissue, but also promoting growth and androgen production of prostate cancer cells in vivo. LRH-1 transactivates the expression of several mitochondrial steroidogenic enzymes, including StAR and CYP17A1, contributing to the increased de novo androgen synthesis in CRPC. Mimicking the effect of siRNA-mediated LRH-1 knockdown, treatment of the CRPC cell line VCaP with SR1848 significantly decreases the expression of these steroidogenic enzymes in vitro. Even more importantly, however, is that the SR1848-mediated pharmacological inhibition of LRH-1 dose-dependently sensitizes VCaP cells to androgen deprivation.[164] These findings suggest that the pharmacological suppression of LRH-1 activity using antagonists, like SR1848, might be a useful therapeutic strategy for the inhibition of persistent AR signaling and proliferation in CRPC.

One remaining problem, however, is that so far very little is known about the in vivo efficacy as well as unwanted side effects of the described LRH-1 antagonists in mammals. Even though there is limited availability of published research regarding these topics, there is some evidence that SR1848 and Cpd3/3d2 can indeed repress LRH-1 signaling in vivo. Ten-day intraperitoneal SR1848 administration significantly decreased LRH-1 and SHP mRNA levels in the pancreas and adrenal gland of SR1848-treated mice in comparison to controls. At the same time, blood analysis revealed that important physiologic analytes remained unaffected after SR1848 treatment.[160] Similarly promising, we could show that also injection of a single dose of Cpd3d2 into mice can have therapeutic effects on immune cell-mediated hepatitis without showing any general toxicity.[9,11] Even though hepatocytes express high levels of LRH-1 and various metabolic processes in the liver are dependent on LRH-1, no obvious liver damage or hepatocyte apoptosis was observed after pharmacological inhibition of LRH-1 alone.[11] Thus, it is feasible to believe that residual LRH-1 activity after Cpd3d2 treatment is sufficient to maintain normal physiological functions of the liver, and likely also other tissues and organs expressing high levels of LRH-1. As immune cells and transformed cells appear to have less spare capacity of residual LRH-1 activity, this opens a therapeutic window of opportunity for the treatment of inflammatory disorders and cancer.

5 | CONCLUSIONS

In summary, the NR LRH-1 is a transcription factor with diverse physiologic functions. Their deregulation is often associated with cancer (Figure 1). Overexpression as well as increased LRH-1 activity had been implicated in a wide variety of human malignancies.[54–58,103–105] Affecting multiple distinct signaling pathways, uncontrolled LRH-1 activity stimulates cell proliferation and increases cell viability, and thereby contributes to growth, progression, and aggressiveness of tumors. Several mechanisms possibly contribute to aberrant LRH-1 activity in malignant cells. Apart from overexpression or activating genetic lesions in the LRH-1 gene, also an imbalance in the relative abundance of interaction partners with coactivator or corepressors function could result in uncontrolled LRH-1 signaling. Additionally, aberrant extracellular mitogenic stimuli and activation of ERK 1/2 might cause a constitutively phosphorylated and thus active status of LRH-1.[39,40] The consequent increased expression of pro-proliferative factors, including cyclin D1, E1 and c-Myc, confers a selective proliferative advantage of cancer cells (Figure 2). Simultaneously, upregulating factors involved in general stress responses LRH-1 enables the protection from environmental threats as well as increasing intrinsic stress that highly proliferating cancer cells are commonly facing.[100,123,148,151]

Most of the LRH-1-regulated processes promoting the survival of tumor cells are associated with mitochondrial functions. To begin with, increasing evidence suggests that LRH-1 induces the expression of antiapoptotic proteins, such as Bcl-xL and Bcl-2.[64,103] Concurrently, it seems to inhibit caspase activity and transcriptionally repress pro-apoptotic factors, such as Bax and p53.[64] LRH-1 thus likely contributes to stress, respectively, cell death resistance and survival of malignant cells by directly counteracting mitochondrial apoptosis signaling.

Regulating the expression of key mitochondrial steroidogenic enzymes, LRH-1 further promotes local GC production. Cancer cell-derived immunosuppressive GCs are known to dampen immune responses, shape the tumor microenvironment, and thus allow escape from tumor immune surveillance.[19,166] Similarly, cancer cells might utilize LRH-1-mediated FasL expression to attack and induce apoptosis in infiltrating immune cells.[9,81] In a cell nonautonomous manner, aberrant LRH-1 signaling thus probably contributes to tumor immune escape by at

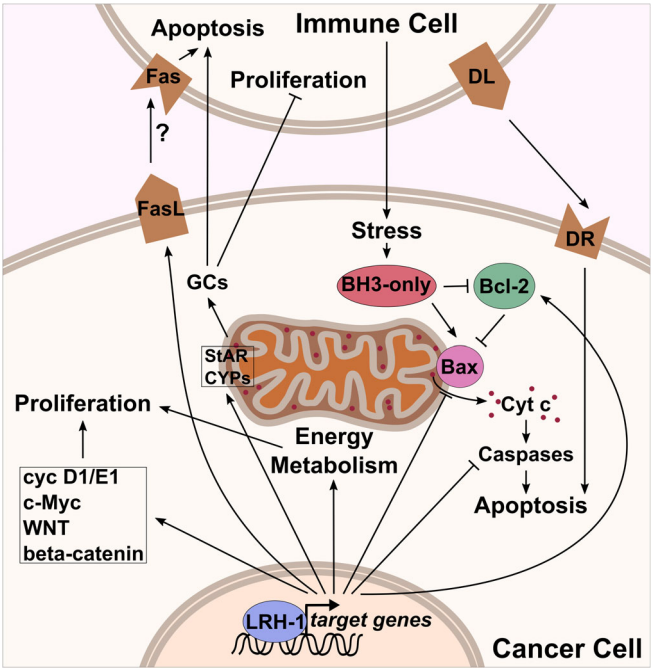

FIGURE 2 LRH-1 as a regulator of cancer cell proliferation and survival. Overexpression as well as increased LRH-1 activity directly promotes cancer cell proliferation due to enhanced expression of cyclin D1, E1, and c-Myc as well as stimulation of WNT and beta-catenin signaling. LRH-1-promoted survival of tumor cells is mainly associated with mitochondrial functions. Directly counteracting mitochondrial apoptosis signaling, LRH-1 induces the expression of antiapoptotic Bcl-2 family members, while transcriptionally repressing Bax and inhibiting caspase activity. Regulating the expression of mitochondrial steroidogenic cytochrome P450 (CYP) enzymes and StAR, LRH-1 further promotes production of immunosuppressive GCs. Tumor-derived GCs inhibit proliferation and promote death of immune cells that would otherwise initiate the DL-mediated cytotoxicity or stress-mediated apoptosis of cancer cells. Contributing to tumor immune escape, cancer cells might conversely utilize LRH-1-mediated FasL expression, to attack and induce apoptosis in infiltrating immune cells. By increasing mitochondrial mass and induction of mitochondrial metabolic target genes LRH-1 further stimulates mitochondrial energy metabolism. LRH-1 thereby might essentially contribute to the fulfillment of the increased energy demand concomitant with the synthesis of proteins important for the rapid proliferation and survival of cancer cells. Cyc, Cyclin; CYPs, cytochrome P450 enzymes; Cyt c, cytochrome *c*; DL, death ligand; DR, death receptor; FasL, Fas Ligand; GCs, glucocorticoids; StAR, steroidogenic acute regulatory protein; WNT, Wingless and INT-1

least two different mechanisms that could clearly favor progression of certain cancer types.

Most importantly, LRH-1 stimulates mitochondrial energy metabolism by increasing mitochondrial mass and induction of mitochondrial metabolic target genes that promote increased lipid, amino acid, and glucose utilization.[56,121,130,135] LRH-1 thereby likely contributes to an indispensable metabolic reprogramming that ensures fulfillment of the increased energy demand concomitant with the synthesis of proteins that are vital for their rapid proliferation and survival of tumor cells. Elevated LRH-1 activity promoting diverse, but mainly mitochondrial processes, is clearly beneficial for the viability of malignant cells. Specifically targeting LRH-1-driven proliferation and cell death resistance in tumor cells using LRH-1 antagonists might thus be a promising approach in cancer therapy.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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