Targeting Liver X Receptors in Cancer Drug Discovery

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Abstract: Liver X receptors (LXRs) are members of the nuclear receptor superfamily of ligand-dependent transcription factors. LXRα is predominantly expressed in metabolic tissues, whereas LXRβ is ubiquitously expressed. Upon ligand binding, they regulate the expression of target genes involved in lipid metabolism, cholesterol homeostasis, and immune responses, including those which function in pathways that are commonly reprogrammed during carcinogenesis. Known LXR ligands include oxysterols and natural and synthetic agonists which upregulate LXR transcriptional activity and target gene expression. Synthetic inverse agonists have also been identified that inhibit LXR activity. While both types of ligands have been shown to inhibit cancer cells and tumor growth either directly or indirectly by modulating the activities of stromal cells within the tumor microenvironment, they appear to target different aspects of cancer metabolism and other cancer hallmarks, including immune evasion. This review summarizes the characterization of LXRs and their ligands and their mechanisms of action in cancer models and discusses the future directions for translating these discoveries into novel cancer therapeutics.

Keywords: liver X receptor (LXR); targeted therapy; lipid metabolism; inverse agonists; chemotherapy resistance

1. Introduction

Advancements in cancer drug discovery rely on the identification of novel target modalities specific to vulnerable components on oncogenic cascades that can be effectively targeted by small molecules, biologics, and other therapeutic strategies [1,2]. Nuclear receptors (NRs) are ligand-dependent transcription factors which have been considered highly “druggable” due to their ligand-binding domains which bind ligands that in turn modulate their activity [3,4]. Estrogen and androgen receptors are prominent examples of NRs which play key roles in cancer biology and targeted cancer treatments [5–7]. Liver X receptors (LXRs) are NRs which regulate the expression of target genes involved in metabolism and inflammation [3,4]. LXRs and their ligands have primarily been studied in metabolic diseases such as atherosclerosis and hepatic steatosis, but their roles in regulating cancer-related metabolic pathways and other oncogenic and tumor-promoting mechanisms have prompted efforts to determine their potential as targets and targeting agents in cancer therapeutics [8,9]. This review presents an overview and highlights recent advances in targeting LXRs in cancer drug discovery.

2. Discovery of LXRs and LXR Ligands

The NR superfamily includes 48 members in humans [10]. In the 1990s, two closely related NRs were discovered and initially called NER [11]. The first of these receptors was found to be expressed ubiquitously and was named ubiquitous receptor and OR-1 [12,13]. However, it was later renamed liver X receptor β (LXRβ) or NR1H2 following the adaptation of the nomenclature by the NR field [14]. LXRβ was independently discovered...
and named orphan receptor 1 (OR-1) by the Gustafsson group as a binding partner of retinoid X receptor (RXR), providing the first evidence that LXRs form heterodimers with RXR [15]. In contrast, the other related receptor shows limited expression, primarily in the liver, adipose tissue, and macrophages. It was initially named RLD-1 and liver X receptor (LXR) before being renamed LXRα and NR1H3 [16,17]. Structurally, LXRs contain an N-terminal activation function 1 (AF1) domain, a DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) and activation function 2 (AF2) domain [18,19]. The DBD of the heterodimer complex binds to liver X receptor response elements (LXREs) in promoter and enhancer regions of target genes [16]. The alignment of the human LXR isotypes reveals 64.45% identity in their overall amino acid sequences, with 77% identity within their LBDs.

Endogenous oxysterols, such as 22(R)-hydroxycholesterol, 24- and 27-hydroxycholesterol, were identified as activators of LXR transcriptional activity [20]. Subsequently, it was shown that oxysterols activate both LXRα and LXRβ. Structural variations between the receptors have been leveraged to develop isotype-specific ligand (5,6–24(S),25-diepoxycholesterol) which activates LXRα exclusively [21]. Apart from endogenous oxysterols, phytochemicals like β-sitosterol can act as natural LXR agonists [22]. Synthetic ligands have been developed to modulate LXR activity for clinical applications. T0901317 was the first LXR agonist to be synthesized. However, it was found to also bind and activate two other NRs, farnesoid X receptor (FXR) and pregnane X receptor (PXR) [8,23,24]. GW3965 was later developed and showed better LXR selectivity [25]. Although both are commonly used for mechanistic studies in preclinical settings, some associated adverse effects, such as elevated plasma triglyceride levels and hepatic steatosis in preclinical models, halted their progress [25,26]. To minimize potential side effects, synthetic agonists such as (22E)-ergost-22-ene-1α,3β-diol (YT-32), N, N-dimethyl-3β-hydroxycholenamide (DMHCA), and 2-(2-chloro-4-fluorobenzyl)-3-(4-fluorophenyl)-7-(trifluoromethyl)-2H-indazole (LXR-623) were developed and are being tested [9,27,28]. There have been reports of synthetic antagonists; however, their potential for therapeutic applications is unclear. Inverse agonists which bind the ligand-binding pocket but inhibit LXR activity have also been identified and utilized in cancer studies. These include SR9243, SR9238, GAC0001E5, and GAC0003A4 [29–32]. Future research and clinical applications involving the development of highly selective LXR modulators (SLiMs) will be facilitated by the growing body of knowledge regarding LXR ligands and their mechanisms of action [33]. The chemical structures of LXR ligands commonly used in functional studies are shown in Figure 1.

**Figure 1.** Structure of LXR ligands and canonical model of LXR transactivation (A–C). LXR ligands are grouped into endogenous, natural, and synthetic agonists and inverse agonists.
3. Molecular Mechanisms of LXRs and Ligands

In the canonical model, the ligand-activated LXR-RXR heterodimers drive the transcription of its target genes [15,34,35]. The silencing mediator of retinoic acid and thyroid hormone receptors (SMRT), also known as the NR corepressor (NCoR), forms a complex with heterodimers linked to DNA [36]. This arrangement allows for basal transcriptional activity but impedes full transcriptional activation in an unliganded state. In the presence of agonists, these corepressors are shed [37], and co-activators, such as members of the p160 family of NR co-regulators, are recruited. This promotes chromatin remodeling and histone alterations, ultimately resulting in target gene transactivation [38,39]. A newer class of LXR ligands, known as inverse agonists, inhibit the transcription of target genes. The binding of LXR inverse agonists enables the recruitment of corepressors to the LXR ligand-binding domain [32]. These different mechanisms of action are summarized in Figure 2. In addition, LXR inhibits the expression of certain inflammatory genes in an LXRE-independent manner. This mode of regulation was defined as trans-repression [40,41]. Furthermore, LXR interactions with membrane-bound and cytoplasmic proteins have been reported. Investigations on LXRβ in murine colon cancer cells revealed that it interacts with the membrane-bound channel protein pannexin 1 and is localized in the cytoplasm upon ligand treatment [42]. This suggests that LXRs may have non-genomic impacts on cellular physiology.
are summarized in Figure 2. In addition, LXR inhibits the expression of certain genes in an LXR independent manner. This mode of regulation was designated as trans-repression [40,41]. Furthermore, LXR interactions with membrane-bound channel proteins have been reported. Investigations on LXR function in cancer cells revealed that it interacts with the membrane-bound channel protein pannexin 1 and is localized in the cytoplasm upon ligand treatment [42]. This suggests that LXRs and the prevention of metastasis [61]. In breast cancer, the knockdown of LXRα reversed the inhibition of cell proliferation [60]. Further, the depletion of LXRα and β promoted

![Figure 2](image_url)  
**Figure 2.** LXR-RXR heterodimer sits on the LXR response element and is bound by corepressor complexes. In the presence of an LXR agonist, corepressor complexes are shed, and co-activator complexes are recruited, which increases the transcription of target genes. Upon inverse agonist binding, corepressors are further recruited and thereby inhibit LXR transcriptional activity.

**4. LXR in Normal Physiology and Diseases**

Genetic studies utilizing Lxrα knockout (KO) mice exhibited abnormalities in the metabolism of cholesterol in the liver and a consequent rise in plasma cholesterol levels when the animals were fed a high-cholesterol diet [43]. KO mice studies revealed their inability to catabolize excess cholesterol due to the downregulation of cholesterol 7α-hydroxylase (Cyp7a1), the enzyme that limits the synthesis of bile acids. LXR activation in macrophages and the intestine altered cholesterol transport and lipoprotein levels [44]. First-generation LXR agonists (GW3965, T0901317) caused unfavorable increases in liver and plasma triglyceride levels but successfully decreased atherosclerotic plaques in animal models [8]. Notably, the discovery of LXR roles in lipogenesis was made possible using treatments with these synthetic agonists [3]. Elevated plasma triglyceride and phospholipid levels were observed due to an increase in the expression of fatty acid synthesis genes in mice and hamsters when treated with LXR agonists [25,26]. Lxrβ KO mice were found to have additional metabolic activities, as they demonstrated reduced weight gain in an obesity model and increased energy expenditure in brown adipose tissues [45–47]. Along with developmental and metabolic phenotypes in the central nervous system, these animals showed signs of chronic pancreatitis caused by water transport abnormalities [48–50]. Furthermore, LXRs are expressed in a variety of immune cells. LXRs have immunomodulatory functions that affect autoimmunity and response to pathogens and tumor growth [41,51–56].

**5. LXR and LXR Ligands in Cancers**

A 1942 study observed the hyperaccumulation of epoxy cholesterol in enlarged prostate tissue and linked that to prostate cancer tumorigenesis [57]. More recent work described the importance of sterol regulatory element-binding proteins SREBP1, SREBP2, and LXR target genes in prostate tumors [58,59]. These findings encouraged Fukuchi, Liao, and colleagues to use the synthetic LXR agonist T0901317 in prostate and breast cancer to investigate potential antitumor effects [60]. T0901317 lowered the percentage of LNCaP cells in the S-phase of prostate cancer and blocked cell proliferation in xenograft models. In mouse models for pulmonary carcinoma, T0901317 treatment resulted in inhibitory effects and the prevention of metastasis [61]. In breast cancer, the knockdown of LXRα reversed the inhibition of cell proliferation [60]. Further, the depletion of LXRα and β promoted
cancerous lesions in mouse peripheral squamous cell lung cancer [62]. Following these promising observations, many studies have been conducted in different cancer models to uncover the importance of LXRs and their ligands (see Table 1).

Table 1. Cancer models responsive to LXR ligand treatments.

<table>
<thead>
<tr>
<th>Primary Site</th>
<th>Type of Cancer</th>
<th>Expressed LXR</th>
<th>LXR Ligand</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>ALL, AML, CLL, Multiple Myeloma</td>
<td>LXRα, LXRβ</td>
<td>GW3965, T0901317</td>
<td>[63–66]</td>
</tr>
<tr>
<td>Brain</td>
<td>Glioblastoma</td>
<td>LXRβ</td>
<td>GW3965, LXR-623</td>
<td>[67–70]</td>
</tr>
<tr>
<td>Breast</td>
<td>Ductal carcinoma, Adenocarcinoma</td>
<td>LXRα, LXRβ</td>
<td>GW3965, T0901317, SR9243, GAC0001E5, LXR-623</td>
<td>[71–80]</td>
</tr>
<tr>
<td>Colon</td>
<td>Adenocarcinoma, Carcinoma</td>
<td>LXRα, LXRβ</td>
<td>GW3965, T0901317, SR9243</td>
<td>[29,81–83]</td>
</tr>
<tr>
<td>Kidney</td>
<td>Carcinoma</td>
<td>LXRα, LXRβ</td>
<td>SR9243, LXR-623</td>
<td>[84]</td>
</tr>
<tr>
<td>Liver</td>
<td>Carcinoma</td>
<td>LXRα, LXRβ</td>
<td>GW3965, T0901317</td>
<td>[85–91]</td>
</tr>
<tr>
<td>Lung</td>
<td>Adenocarcinoma</td>
<td>LXRα, LXRβ</td>
<td>T0901317, SR9243</td>
<td>[29,92–95]</td>
</tr>
<tr>
<td>Ovaries</td>
<td>Carcinoma</td>
<td>LXRα, LXRβ</td>
<td>T0901317</td>
<td>[96]</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Adenocarcinoma</td>
<td>LXRβ</td>
<td>GW3965, T0901317, SR9243, GAC0001E5, GAC0003A4</td>
<td>[29,32,97–100]</td>
</tr>
<tr>
<td>Prostate</td>
<td>Carcinoma</td>
<td>LXRα, LXRβ</td>
<td>GW3965, T0901317, SR9243</td>
<td>[29,60,101–103]</td>
</tr>
<tr>
<td>Stomach</td>
<td>Adenocarcinoma</td>
<td>LXRβ</td>
<td>GW3965, T0901317</td>
<td>[104,105]</td>
</tr>
<tr>
<td>Skin</td>
<td>Melanoma</td>
<td>LXRβ</td>
<td>GW3965, T0901317</td>
<td>[106,107]</td>
</tr>
</tbody>
</table>

ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, CLL: Chronic Lymphocytic Leukemia, LXR: Liver X Receptor.

5.1. LXR-Modulated Cell Cycle Mechanisms and Signaling Pathways

The original study investigating the effects of LXR ligands in prostate cancer revealed inhibited cell proliferation and cell cycle progression. LXR agonist T0901317 treatment elevated the p27 protein independent of mRNA levels, a cyclin-dependent kinase (CDK) inhibitor [60]. Moreover, ligand treatment lowered the expression levels of S-phase kinase-associated protein 2 (SKP2), which is known to target protein p27 and promote its degradation. Thus, this study provided a potential understanding of the mechanism of inhibition [60]. In ovarian cancer, p27 and p21 protein levels were upregulated with T0901317 treatment, and apoptosis was observed. However, LXR knockdown did not affect cell growth and survival. This suggests potential LXR crosstalk with FXR (farnesoid X receptor) mediated by agonist T0901317 in ovarian cancer [96]. The mechanisms of action related to cell cycle arrest can differ in breast cancer. Ligand treatment disrupted SKP2 transcript and protein levels, but no changes were observed in p21 and p27 [72]. LXR agonist T0901317 inhibited beta-catenin expression in prostate cancer, resulting in lower levels of vital oncoproteins, cyclin D1 and c-MYC [102]. In cytokine-stimulated T-lymphoblasts, T0901317 reduced the levels of phosphorylated Rb, which blocked the entry into the S phase of the cell cycle, but p27 protein levels remained unaffected, like in ovarian cancer [63]. In hepatocellular carcinoma (HCC), T0901713 was shown to disrupt cyclin D1 and increase p21 and p27 via SOCS3. The suppressor of the cytokine signaling 3 (SOCS3) gene is essential for cellular inflammation responses. Treatments with LXR agonist GW3965 have been shown to upregulate SOCS3 expression and apoptosis with decreased cell proliferation, migration, and invasion [66,108]. SOCS3 upregulation in prostate cancer highlights a possible association between LXR functions in lipogenesis and inflammation. Opposite responses were observed when the SOCS3 expression was knocked down. Incidentally, in prostate cancer, upregulated SOCS3 levels were observed during disease progression, and it was suspected to be an oncogene [109]. Overall, the role of SOCS3 in modulating anti-proliferative and anti-metastatic functions remains unclear. Microarray analysis in breast cancer cells with an LXR agonist, GW3965, showed downregulation in E2F2, a transcription factor involved in cell cycle regulation [73]. Inhibited cell proliferation was also observed in colon cancer cells with GW3965, where SKP2 transcript
and protein levels were downregulated in addition to CDKs and the proto-oncoprotein MYC [81,110]. Similar patterns were reported in pancreatic cancer [97]. Lipid raft formation plays a major role in compartmentalizing receptors and inducing downstream signaling. With the treatment of T0901317, rafts marker flotillin-2 (FLOT2) was downregulated in breast cancer [111]. Interestingly, GW3965-treated pancreatic cancer cells showed lowered epidermal growth factor receptor EGFR expression, suggesting a crosstalk between LXR and growth factor signaling mechanisms [97]. In prostate cancer, it has been demonstrated that the EGFR/AKT/FOXO3A pathway can regulate LXRα [103]. In gastric cancer cells, the downregulation of LXRα led to the suppression of the PI3K/AKT/NF-κB pathway and reduced invasion and EMT [112]. Clonogenic growth was inhibited in multiple myeloma cells using treatments with GW3965 and T0901317; however, overall cell proliferation and apoptosis were unchanged. These results were found to be conflicting due to a lack of evidence related to the mechanisms of action [64,106,107]. It has also been observed that targeting with LXR agonists can inhibit Bcl-xL and elevate pro-apoptotic signaling [113].

5.2. LXR-Associated Metabolic Genes in Cancers

LXR is a transcription factor that regulates metabolism through LXR target genes such as ATP-binding cassette sub-family A member 1 (ABCA1) which encodes a membrane-bound cholesterol transporter. The downregulation of ABCA1 expression was observed in prostate tumorigenesis, and knockdown was shown to induce prostate cancer cell proliferation [71]. The activation of LXR with synthetic agonists led to increases in ABCA1 expression in prostate cancer, providing a possible mechanism for their anti-proliferative effects [71]. In a related study, ligand treatments in prostate cancer increased the expression of a related gene, ABCG1, altered lipid raft signaling via the protein kinase AKT1, and induced apoptosis [114]. Similarly, ligand treatments in breast cancer were also shown to upregulate cholesterol efflux through ABCG1, resulting in increased apoptosis and cell proliferation disruption [74]. LXR agonist GW3965 has been shown to upregulate sterol regulatory element binding transcription factor 1 (SREBF1) and ABCG1 expression in basal-like breast cancer in vitro, and combinatory treatments with platinum-based chemotherapeutic drugs were shown to inhibit tumor proliferation in vivo [77]. It has also been demonstrated that LXR inactivation through SREBP-2 upregulation might be necessary for the estrogen receptor-independent activity of selective estrogen receptor modulator (SERM) treatments [115]. When LXR agonists GW3965 and T0901317 were introduced to some cancer cell lines, de novo lipogenesis (DNL) related genes sterol regulatory element binding transcription factor 1 (SREBF1) and fatty acid synthase (FASN) were shown to be associated with resulting anti-proliferative effects and an increased accumulation of triglycerides [116]. In addition, FASN knockdown in prostate cancer cell lines has shown a partial inhibition of the anti-proliferative effects. In contrast, GW3965-mediated inhibitory effects were not disrupted upon the knockdown of the SREBF1 expression in breast cancer, and the knockdown effects of FASN on ligand response were unclear [72]. In advanced pancreatic cancer, a vital DNA repair enzyme, polynucleotide kinase/phosphatase (PNKP), was reportedly regulated by LXR-SREBF1. LXRα knockdown or inhibition using triptonide was shown to downregulate PNKP, leading to apoptotic cell death [117]. The degradation of the low-density lipoprotein receptor (LDLR) has been shown to be enhanced by LXR agonist GW3965 in glioblastoma cells, leading to apoptosis in cells carrying EGFR mutations [67].

Apolipoprotein E (APOE) is a LXR target gene that regulates lipid transport. In melanoma cells, APOE expression was shown to be upregulated by GW3965 and T0901317 treatments, lowering invasion and metastasis in both cell lines and murine study models [107,118]. Most importantly, metastasis suppression was demonstrated to be mediated by elevated APOE binding with low-density lipoprotein receptor-related protein 1 (LRP1) in melanoma cells [118]. Another LXR target gene, apolipoprotein A1 (APOA1), was shown to be suppressed in a transcriptomic study of surgically resected gallbladder cancer tissue samples. Furthermore, LXR/RXR signaling was a significantly dysregulated pathway as compared to gallbladder mucosal controls [119]. Bexarotene is an RXR ligand approved to
treat advanced lymphoma malignancies, including cutaneous T cell lymphomas (CTCL) and has also been investigated in several other types of cancer [120–122]. The mechanism of action of bexarotene involves the upregulation of APOE levels. The RXR ligand treatment alone was inadequate for upregulating cellular differentiation. On the other hand, introducing T0901317 or GW3965 combined with bexarotene potentiated the effect [65]. These results propose that the ligands responsible for LXR-RXR interaction can play a vital role in promoting antitumor effects directly or indirectly. The peroxisome proliferator-activated receptor gamma (PPARG, NR1C3) is a prime regulator of energy homeostasis. Treatments with LXR ligand GW3965 and PPARG ligand rosiglitazone lowered cellular glutathione levels, upregulating oxidative stress and cell death [82]. Additionally, combinations of T0901317 with glutathione peroxidase 4 (GPX4) inhibitors were shown to facilitate ferroptosis, a lipid oxidation-driven cell death pathway in lung cancer [123].

5.3. LXRs and Hormone Signaling

Approximately 40% of cancers are hormone-dependent, and a lack of target options and acquired resistance are key concerns. Delineating the potential interaction between hormone signaling mechanisms and LXR signaling could facilitate the development of novel therapies. LXR agonist T0901317 has been shown to compete with androgen in binding to androgen receptors; subsequently, this ligand was described as a weak anti-androgen [101]. Treatments with agonists T0901317 and GW3965 dysregulated two critical enzymes related to androgenic activity. Sulftotransferase 2A1 (SULT2A1/ST2A1) sulfurylates androgen and converts it to an inactive form, and its expression is elevated upon LXR agonist treatments. In contrast, steroid sulfatase expression levels were reported to be downregulated; this enzyme converts the inactivated form back to active androgen [124]. Similarly, decreased estrogen sulfotransferase (EST) expression was observed in the liver, disrupting estrogenic activity in target tissues [75]. Additionally, GW3965-treated mice mirrored the same observations on estrogen production but failed to clarify the direct effects on breast cancer cells. Furthermore, treatments with GW3965 were shown to lower ESR1 transcript and protein levels; the primary molecular driver in ER-positive breast cancer and cell proliferative and survival pathways were inhibited [72,73]. In comparison, AR stability was not altered in LXR agonist-treated prostate cancer, suggesting LXR agonist-driven hormone receptor disruption could be breast cancer-specific. Interestingly, LXR agonists can also exert antitumor activity independent of hormone receptors. For example, GW3965 showed anti-proliferative effects in breast cancer cells that lack hormone receptors [72,73].

6. LXRs, Tumor Immunity, and Tumor Microenvironment

LXR ligands can potentially influence tumor cells and cancer development due to the ubiquitous expression and pleiotropic activities of LXRs. Such an effect can occur systemically or within the tumor microenvironment. In particular, the expression and roles of LXRs in immune cells point to possible ligand effects on tumor immunology and immune surveillance.

LXR-dependent sterol homeostasis determines the proliferation activation of different T-cell types. T-cell activation induces the simultaneous suppression of cholesterol transport and cholesterol biosynthesis. Sterol metabolizing enzyme SULT2B1 regulates these coordinated events. SULT2B1 blocks LXR signaling, activating T-cell proliferation [125]. In this study, similar observations were recorded upon loss of LXRβ. In addition, mice lacking LXRβ exhibited lymphoid hyperplasia and a superior response to antigenic challenge. Glycosphingolipids and cholesterol strongly influence T-cell function by regulating membrane homeostasis. The glycosphingolipid biosynthesis enzyme glucosylceramide synthase is an LXR target gene that exerts this function. LXR regulates metabolic pathways in human CD4+ T-cells by controlling the membrane lipids. LXR activation alters membrane stability, reducing proinflammatory T-cell function and the rapid activation of proximal T-cell signaling molecules. In contrast, Tregs have a lower membrane order and behave differently upon LXR activation when compared to effector T cells [126]. The inactivation of LXR also
improves immunotherapy via the activation of CD8+ T cells. In a triple-negative breast cancer (TNBC) model, LXR inverse agonist SR9243 promotes the infiltration of CD8+ T cells via enhanced mitochondrial activity. Furthermore, LXR inverse agonism promotes dendritic cell (DC) migration and suppresses myeloid-derived suppressor cells (MDSCs) and Treg populations [78].

The LXR activation of tumor-associated macrophages (TAMs) exerts a protective effect on prostate cancer cells and blocks ferroptosis. LXRα drives the transcription of miR-181a-5p in prostate cancer cells, which induces M2 polarization and suppresses ferroptosis [127]. In a murine model of lung carcinoma, the activation of LXR reduced immune suppressive T-regulatory (Treg) cells by suppressing chemokine Ccl17 expression in tumor associated macrophages (TAMs). This enhanced antitumor immune response and inhibited lung cancer in an LXR-dependent manner [128]. LXR agonism improves response to immunotherapy by reducing the abundance of myeloid-derived suppressor cells (MDSCs) which are generally immunosuppressive. ApoE binds to LRP8 receptors on MDSCs and inhibits their proliferation, in turn improving antitumor immune response [129].

In a transgenic mouse model of breast cancer, an LXR pan-agonist, N, N-dimethyl-3-β-hydroxycholenamide (DMHCA), reduced tumor progression by decreasing the abundance of MDSCs and increasing CD4+ and CD8+ effector T cells [130]. LXR forms an intricate feedback loop with type I interferons and STAT1. Upon the activation of Toll-like receptors (TLRs), IFNβ activates STAT1, which induces LXR expression. LXR, in turn, inhibits STAT1, reduces the expression of type 1 interferon-stimulated genes (ISGs), and reduces inflammation [131]. Both LXR isotypes are essential for functioning of natural killer T (NKT) cells in mice. Lxr KO mice displayed a marked reduction in hepatic invariant NKT cells and increased liver metastasis. Cytokine production, antitumor activity, and NKT cell development were drastically affected in the Lxr KO mice [132].

The expression of C-C chemokine receptor type 7 (CCR7) in dendritic cells (DC) enables DC migration to lymph nodes and the activation of T cells. LXR activation in the stroma by tumor-derived factors reduces CCR7 expression and prevents the cells from migrating to lymph nodes. Such inhibition dampens the antitumor immune response. The expression of LXRα appears necessary for this ligand-driven mode of immune surveillance escape. The migration of dendritic cells in Lxr knockout mice was higher than that of wild-type in tumor-bearing animals [133]. On the other hand, treatments with agonist T0901317 enhanced the lifespan of animals injected with murine lung cancer cells and stimulated the expression of interferon γ (IFNγ) in macrophages and T cells. Additionally, it may stimulate tumor immunosurveillance [92].

Cells in the tumor microenvironment mediate some of the antitumor effects of the LXR agonists. LXR activation in endothelial cells decreased angiogenesis and limited tumor growth. This effect is regulated by a reduction in cholesterol-dependent vascular endothelial growth factor receptor-2 (VEGFR2) signaling. The metabolism and transport of cholesterol are linked to these anti-angiogenic mechanisms that have been reported. Ligand treatment indirectly inhibited tumor growth of murine cancer cells, which are insensitive to LXR agonists GW3965 and T0901317 treatments in vitro, by reducing angiogenesis [134]. Stromal cells mediate the actions of LXR-regulated ApoE in tumor cells, as well as tumor growth and metastasis. The antitumor effects of LXR agonist therapies in melanoma models were mainly attributed to ApoE production by stromal and tumor cells, which inhibited tumor growth, angiogenesis, and metastasis [107].

It is unclear if these mechanisms and consequences are unique to the model systems used in the published work. It is also unclear if the responses vary based on the tumor cells’ particular mutational landscape and the tissue of origin. However, to prevent any confounding or undesirable treatment effects, the effects of LXR ligands on this and other elements of tumor immune surveillance and escape should be thoroughly defined and thoughtfully considered in the development of therapeutic applications in the future.
7. Targeting LXR with Inverse Agonists

The vast majority of mechanistic and functional studies on LXR and LXR ligands have been conducted with LXR agonists T0901317 and GW3965. These ligands were initially developed as treatments for atherosclerosis but were shelved due to hypertriglyceridemia in preclinical models. LXR-623, a lipid-neutral agonist, was developed to address this, but clinical trials were terminated due to reported adverse effects on the central nervous system (CNS) [9]. Relatedly, CNS phenotypes were observed in Lxrα/β knockout mice, although it is not clear whether this is due to developmental defects or the disruption of LXR signaling [50]. Nonetheless, the findings from the agonist studies suggest that inverse agonists which should exert opposite actions may mitigate the adverse effects on triglyceride levels, if they are similarly effective in targeting tumor cells. Flaveny and colleagues were the first to demonstrate the efficacy of an inverse agonist, SR9243, in targeting cancer cells [29]. Treatments with SR9243 inhibited cell proliferation and disrupted colony formation in pancreatic, colorectal, and lung cancer cells, while no significant changes were observed in non-malignant cells. However, LXR inverse agonist SR9243 inhibited tumor growth and downregulated lipogenic (SREBF1, FASN, and SCD1) and Warburg effect (GCK1, PFK1, and PFK2) gene expression, with no adverse effects such as elevated triglyceride and cholesterol levels, acute weight loss, hepatotoxicity, and inflammation in vivo. In a follow-up study in triple-negative breast cancer models, SR9243 treatments promoted CD4+ Th1 polarization, reduced Treg viability, and increased CD8+ T-cell activation [78]. Moreover, inverse agonist treatments activated immune-mediated tumor clearance. These findings together point to both direct and indirect antitumor mechanisms of LXR inverse agonists in a disease-specific manner.

To identify novel LXR ligands with antitumor activities, our group screened a focused library of putative LXR ligands in multiple pancreatic cancer cell lines and discovered two small molecules, GAC0001E5 (1E5) and GAC0003A4 (3A4), with potent inhibitory activities. Both molecules were shown to function as LXR inverse agonists by recruiting corepressors and downregulating the transcription of LXR target genes. Furthermore, 1E5 and 3A4 also function as the first, to our knowledge, LXR “degraders”, by downregulating LXRβ protein levels following prolonged treatments [32]. Subsequent mechanistic studies of 1E5 in pancreatic and breast cancer cells indicated the targeting of glutamine metabolism, a commonly reprogrammed metabolic pathway involved in cellular energetics, amino acid and nucleotide metabolism, and antioxidant production in transformed cells [76,98]. Recent studies, specifically in HER2-positive breast cancer cells, revealed that, in addition to the previously described effects on glutaminolysis and lipogenesis, 1E5 treatment significantly reduced HER2 transcript and protein expression [135]. These results point to the potential crosstalk between metabolic homeostasis and the expression of receptors involved in mediating the effects of growth signals in cancer cells. In cancer cells, 3A4 appears to disrupt fatty acid and cholesterol metabolism and activate ceramide metabolism [99]. Treatments with 3A4 induced both the apoptosis and necroptosis of pancreatic cancer cells. It should be noted, however, that the disruption of LXR activity and expression may promote tumorigenesis in specific tissues and cell types, particularly in aged animals. The double-knockout of both LXR isotypes led to spontaneous peripheral squamous cell lung carcinoma in 14-months-old mice [62]. Recently, it was reported that 18-months-old double-knockout mice developed TTF-1/P63-positive non-small-cell lung carcinoma [136]. The tumor promoting effects in these animal models may be due to the indirect effects of LXR knockout which appears to disrupt the expression of estrogen receptor β (ERβ), a well-established tumor suppressor gene [137]. Taken together, these findings provide key insights and indicate the mechanistic diversity of LXR inverse agonists and the possibility of their application in targeting multiple vulnerabilities found in tumor cells and their microenvironment. These mechanisms are arranged according to major cancer hallmarks in Figure 3. A detailed description highlighting downstream LXR target genes and associated cancer types is added in Tables 2 and 3.
Figure 3. The antitumor mechanisms of LXR (A) activation by agonists and (B) inhibition by inverse agonists that targets different hallmarks of cancer (MDSC-myeloid derived suppressor cells, CTL-cytotoxic T lymphocyte).
Table 2. Mechanisms of action of LXR agonists.

<table>
<thead>
<tr>
<th>Cancer Hallmark</th>
<th>Activity</th>
<th>Cancer Type</th>
<th>Downstream Genes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activating Invasion and Metastasis</td>
<td>Inhibition of Metastasis</td>
<td>Prostate, Murine melanoma, Murine breast</td>
<td>APOE, LRP1, LRP8, CDKN1B, CDKN1A, CDH1, CDH2</td>
<td>[61,103,118]</td>
</tr>
<tr>
<td>Evading Growth Suppressors</td>
<td>Inhibition of Cell Proliferation</td>
<td>Breast, Cervical, Epidermoid carcinoma, Glioblastoma, Hepatoma, Lung, Melanoma, Multiple myeloma, Osteosarcoma, Pancreas, Prostate, Squamous carcinoma</td>
<td>ABCA1, ABCG1, SREBP1-c, IDOL, LDLR</td>
<td>[60,64,67,72,73,101,138]</td>
</tr>
<tr>
<td>Immune Escape</td>
<td>Increase in CTL Activity</td>
<td>Murine melanoma</td>
<td>Unknown</td>
<td>[129]</td>
</tr>
<tr>
<td>Inducing Angiogenesis</td>
<td>Suppression of Vessel Formation</td>
<td>Endothelial cells, Murine melanoma</td>
<td>ABCA1, ABCG1, CETP, SREBP1-c, IDOL, LDLR, APOE, LRP8</td>
<td>[118,134]</td>
</tr>
<tr>
<td>Resisting Cell Death</td>
<td>Induction of Apoptosis</td>
<td>Breast, Gastric, Lung, Prostate, Murine melanoma</td>
<td>APOE, LRP8</td>
<td>[129]</td>
</tr>
</tbody>
</table>

Table 3. Mechanisms of action of LXR inverse agonists.

<table>
<thead>
<tr>
<th>Cancer Hallmark</th>
<th>Activity</th>
<th>Cancer Type</th>
<th>Downstream Genes</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deregulating Cellular Energetics</td>
<td>Inhibition of Cholesterol Metabolism</td>
<td>Pancreas</td>
<td>ABCA1, ABCG1</td>
<td>[99]</td>
</tr>
<tr>
<td></td>
<td>Inhibition of Lipogenesis</td>
<td>Breast, Lung, Prostate</td>
<td>SREBP1c, FASN, ACC, SCID</td>
<td>[14,29]</td>
</tr>
<tr>
<td></td>
<td>Disruption of Glutamine Metabolism</td>
<td>Breast, Pancreas</td>
<td>GOT1, GOT2, GLUD1, GLS1, SLC7A11</td>
<td>[14,76,98]</td>
</tr>
<tr>
<td></td>
<td>Inhibition of Glycolysis</td>
<td>Colon, Lung, Prostate</td>
<td>GCK1, PFK1, PFK2</td>
<td>[29]</td>
</tr>
<tr>
<td>Evading Growth Suppressors</td>
<td>Inhibition of Cell Proliferation</td>
<td>Breast, Pancreas</td>
<td>SREBP1c, FASN, ACC, SCID, ABCA1, ABCG1</td>
<td>[32,76]</td>
</tr>
<tr>
<td>Immune Escape</td>
<td>Increase CD8+ T-cell Activity</td>
<td>Triple negative breast cancer (C57BL6) Mouse CD4+ and CD8+ T cells</td>
<td>Unknown</td>
<td>[78]</td>
</tr>
<tr>
<td>Resisting Cell Death</td>
<td>Induction of Apoptosis and Necroptosis</td>
<td>Colon, Lung, Pancreas, Prostate</td>
<td>Unknown</td>
<td>[29,99]</td>
</tr>
</tbody>
</table>

8. Conclusions and Future Directions

Notable progress has been made in the development of novel cancer-specific LXR ligands. However, there are several questions that remain to be addressed. Given that LXR isotypes possess nearly 70% sequence homology, LXRα and LXRβ-specific ligands should be explored. Cancers are highly heterogeneous in nature, with a great degree of dissimilarity. LXRα and LXRβ are differentially expressed in different tissue types. An expanded understanding of these micro-patterns can help to administer LXR isotype-specific agonist or inverse agonist treatment, thereby reducing potential off-target effects. Utilizing tumor-specific LXR expression profiles as a prognostic marker for predicting LXR ligand response could be a feasible approach for LXR-based therapeutics. For example, breast, melanoma, and pancreatic cancer show increased LXRβ expression in both clinical and experimental settings [97]. In these cases, using LXRβ-specific and tissue-specific ligands is ideal for enhancing efficacy and ensuring minimal adverse effects. For example,
intestine-specific LXR ligand GW6340 may be successful in treating small intestine and colorectal cancer with no toxicity on the liver or CNS [140]. Furthermore, the concept of photopharmacology can enable localized ligand treatment in a tissue-specific manner so as to bypass adverse effects. Pioneering studies utilizing light-dependent LXR agonists based on the T0901317 scaffold may pave the way for more such approaches [141].

Additionally, the role of LXR splice variants is still not well understood. One recent study in TNBCs has demonstrated that the abundance of full-length LXRα is associated with poor prognosis relative to other LXR splice variants [142]. Thus, transcriptomic and proteomic analysis would be beneficial to develop cancer-specific LXR splice variant profiles and their mechanism of action. This can help better understand the heterogeneity of LXRs in different tumor models.

A prominent avenue in therapeutics is the use of combinatorial therapy. By targeting multiple cancer-promoting pathways, the overall efficacy of treatment can be enhanced. LXR ligands can be utilized to improve the sensitivity of available front-line therapies. For example, radiotherapy (RT) plays a vital role in treating locally advanced tumors. LXR agonist GW3965 has been demonstrated to resensitize RT-resistant NSCLC [143]. Novel LXR inverse agonist GAC0001E5 (1E5), in combination with chemotherapeutic agents like gemcitabine in PDAC, have been shown to increase efficacy in vitro [98]. It was also found that T0901317 potentiates the antitumor effect of first-line therapy sorafenib in hepatocellular carcinoma cells, expressing a high LXRβ/α ratio [86]. Additional studies with GW3965 showed similar results [88]. In the context of acquired resistance, tyrosine kinase inhibitors (TKIs) like gefitinib in combination with LXR agonist T0901317 or GW3965 have been demonstrated to be effective against TKI resistance in non-small cell lung cancer [93,139,144–147]. It has also been demonstrated that inverse agonist GAC0001E5 has anti-proliferative effects in tamoxifen-resistant breast cancer [76]. The additive effects of combining LXR ligands with current first-line therapies could also improve treatment efficacy by reducing effective concentrations of individual treatment modules. In turn, this could reduce the probability of developing acquired resistance and treatment-associated adverse effects.

The employment of high-throughput screening and AI-based modeling will further accelerate the development of additional cancer-specific LXR ligands. The small molecules thus obtained need to be tested for their pharmacokinetic and pharmacodynamic properties to prepare a complete pharmacological profile, highlighting all potential side effects. Furthermore, 3D cell culture models, like spheroids and organoids, can be utilized to streamline the drug screening process. Taken together, these methods can rapidly expand available LXR-based therapies for potential use in the clinical context.

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References


13. Teboul, M.; Enmark, E.; Li, Q.; Wikström, A.C.; Pelto-Huikko, M.; Gustafsson, J.A. OR-1, a Member of the Nuclear Receptor Superfamily That Interacts with the 9-Cis-Retinoic Acid Receptor. Proc. Natl. Acad. Sci. USA 1995, 92, 2096–2100. [CrossRef] [PubMed]


44. Hong, C.; Walczak, R.; Dhamko, H.; Bradley, M.N.; Marathe, C.; Boyadjian, R.; Salazar, J.V.; Tontonoz, P. Constitutive Activation of LXR in Macrophages Regulates Metabolic and Inflammatory Gene Expression: Identification of ARL7 as a Direct Target. J. Lipid Res. 2011, 52, 531–539. [CrossRef] [PubMed]


100. Chen, Z.; Lai, X.; Ding, H.; Zhang, A.; Sun, Y.; Ling, J.; Chiao, P.J.; Chen, Z.; Xia, X. ATF4/TXNIP/REDD1/mTOR Signaling Mediates the Antitumor Activities of Liver X Receptor in Pancreatic Cancers. *Cancer Inov. 2022, 1, 55–69. [CrossRef]


115. Li, D.; Rashid, A.; Chen, Z.; Xia, X. ATF4/TXNIP/REDD1/mTOR Signaling Mediates the Antitumor Activities of Liver X Receptor in Pancreatic Cancers. *Cancer Inov. 2022, 1, 55–69. [CrossRef]


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