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Modulation of liver X receptor signaling as novel therapy for prostate cancer

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Abstract

Liver X receptors (LXRs) are important regulators of cholesterol, fatty acid, and glucose homeostasis. LXR agonists are effective for treatment of murine models of atherosclerosis, diabetes, and Alzheimer's disease. Recently we observed that LXR agonists suppressed proliferation of prostate and breast cancer cells *in vitro* and treatment of mice with the LXR agonist T0901317 suppressed the growth of prostate tumor xenografts. LXR agonists appear to cause G1 cell cycle arrest in cells by reducing expression of Skp2 and inducing the accumulation of $p27^{Kip}$. T0901317 induced expression of ATP-binding cassette transporter A1 (ABCA1) and delayed the progression of androgen-dependent human prostate tumor xenografts towards androgen-independency in mice. Phytosterols, the plant equivalent of mammalian cholesterol, have recently been shown to be agonists for LXRs. β-Sitosterol and campesterol, the two most common phytosterols, suppressed proliferation of prostate and breast cancer cells. The anticancer activity of phytosterols may be due to LXR signaling. This review examines the potential use of LXR signaling as a therapeutic target in prostate and other cancers.

Introduction

Prostate cancer is a very common male-specific malignancy, the third leading cause of cancer death among males in the United States, and the leading cause of cancer death in men over 65 years old. It was estimated that there were around 232,000 new prostate cancer cases in the United States in 2005 [1]. In 1941, Charles Huggins reported that androgen ablation therapy causes regression of primary and metastatic androgen-dependent prostate cancer [2]. However, it is now known that 80–90% of prostate cancer patients develop androgen-independent tumors 12–33 months after androgen ablation therapy, leading to a median overall survival of 23–37 months from the time of initiation of androgen ablation therapy [3]. Since no therapy has been shown to substantially extend survival in patients with advanced recurrent prostate cancer, any approach that suppresses the growth of advanced prostate tumors or delays the progression of prostate cancer towards androgen-independency will benefit prostate cancer patients.

Compared to healthy prostate tissue, the production and secretion of cholesterol increases in prostate tumors [4]. Expression of fatty acid synthase (FAS), the enzyme responsible for converting acetate to fatty acids, is up-regulated in early stage prostate tumors and increases further in advanced tumors [5–8]. Liver X receptor (LXR)

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signaling regulates both cholesterol and fatty acid homeostasis [9–13]. Our recent observations suggest that LXR agonists can suppress the proliferation of prostate cancer cells both *in vitro* and *in vivo* [14]. Treatment with an LXR agonist also delays progression of prostate tumor xenografts towards androgen-independency in castrated athymic mice [8]. Dietary phytosterol, the plant equivalent of mammalian cholesterol, has long been known to exhibit anticancer activity [15]. The 4-desmethyl family of phytosterols and phytostanols have recently been shown to be effective LXR agonists [16], suggesting that anticancer activity of phytosterols may act through LXR signaling as well. We discuss this novel research area in this review and explore the possible use of LXR agonists as a treatment for prostate and other cancers.

Liver X receptor signaling

LXRα and LXRβ

Liver X receptors are ligand-activated transcriptional factors that belong to the nuclear receptor superfamily. Our lab is one of the first groups to clone LXR receptor, the UR/LXRβ. There are two LXR isoforms. LXRα isoform was identified by two groups and named RLD-1 [17] and LXR [18], whereas four groups identified the LXRβ isoform and named as UR [19], NER [20], OR-1 [21], and RIP-15 [22]. Although LXRα and LXRβ share high similarity in their DNA- and ligand-binding domains, expression of these proteins in various tissues differs. LXRα expression is restricted to liver, kidney, intestine, fat tissue, macrophages, lung, and spleen [11, 13, 22]. Expression of LXRα is highest in liver, hence the name liver X receptor α [18]. LXRβ is ubiquitously expressed, hence the early name UR (ubiquitous receptor) [19]. The human LXRα gene is located on chromosome 11p11.2, while the LXRβ gene is located on chromosome 19q13.3 [23, 24].

LXRα and LXRβ form heterodimers with the obligate partner 9-*cis* retinoic acid receptor (RXR) [17–22]. The LXR/RXR heterodimer can be activated with either an LXR agonist (oxysterols) or a RXR agonist (*cis*-retinoic acid). Oxysterols are oxygenated derivatives of cholesterol. Oxysterols, such as 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, and cholestenoic acid, are natural ligands for LXR [25–28]. A few synthetic LXR agonists have been developed, including non-steroidal LXR agonists T0901317 [29] and GW3965 [30], and steroidal LXR agonists hypocholamide [31] and YT-32 [32]. Auto-oxidized cholesterol sulfates, such as 5α, 6α-epoxycholesterol-3-sulfate and 7-ketocholesterol-3-sulfate, are antagonistic ligands of LXRs [10]. The structure of these LXR agonists and antagonists are shown in Figure 1. The ligand-bound LXR/RXR heterodimer binds an LXR response element (LXRE), usually a variant of the idealized sequence AGGTCAN_4AGGTCA, in the promoters of target genes [13, 19].

Role of LXR signaling in metabolism

LXRs are important regulators of cholesterol, fatty acid, and glucose homeostasis. Oral administration of an LXR agonist has an overall hypolipidemic effect in hypercholesterolemic rats, mice, and hamsters [31]. LXRα−/− mice are healthy when fed with a low-cholesterol diet. However, LXRα−/− mice develop enlarged fatty livers, hepatocellular degeneration, high hepatic cholesterol levels, and impaired liver function when fed a high-cholesterol diet [12, 13, 33, 34]. LXRβ−/− mice are unaffected by a high-cholesterol diet, suggesting that LXRα and LXRβ have separate roles.

LXRα and LXRβ regulate cholesterol transport. LXR induces expression of the cholesterol transporters ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) [13, 35, 36] as well as cholesterol acceptor apolipoprotein E (ApoE) [37]. Treatment with LXR agonists (hypocholamide, T0901317, or GW3965) lowers the cholesterol level in serum and liver and inhibits the development of atherosclerosis in murine disease models [10, 31, 38–40].

LXRs regulate fatty acid synthesis by modulating the expression of sterol regulatory element-binding protein-1c (SREBP-1c) [41, 42] and downstream lipogenic genes, including acetyl CoA carboxylase and FAS [43]. LXRs also regulate insulin signaling in liver [44, 45]. LXRα−/− LXRβ−/− double knockout mice lack insulin-mediated induction of an entire class of enzymes involved in both fatty acid and cholesterol metabolism [44]. Treatment with GW3965 suppresses

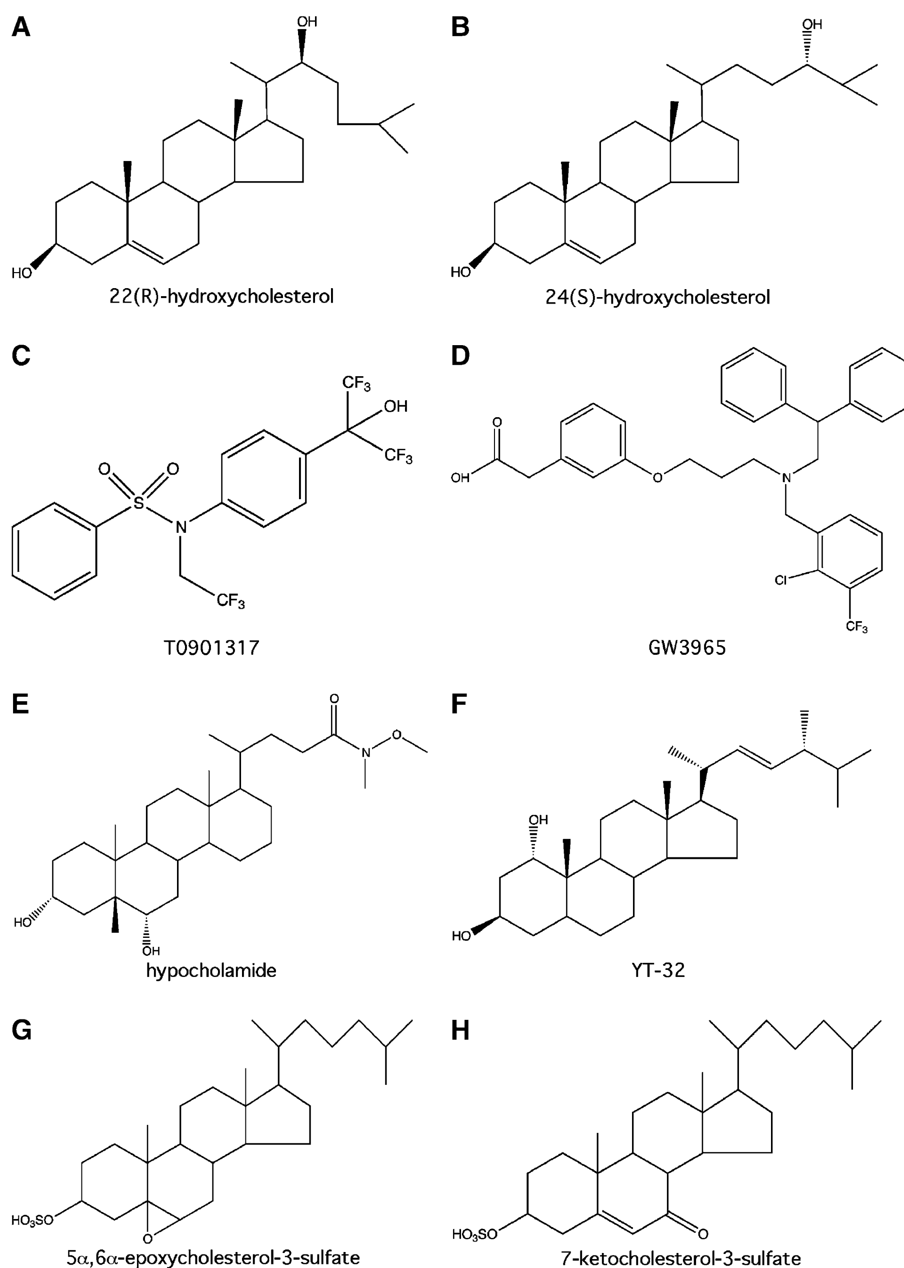

Figure 1. Structure of LXR agonists and antagonists. (A) 22(R)-hydroxycholesterol, (B) 24(S)-hydroxycholesterol, (C) T0901317, (D) GW3965, (E) hypocholamide, (F) YT-32, (G) 5α,6α-epoxycholesterol-3-sulfate, and (H) 7-ketocholesterol-3-sulfate.

gluconeogenesis and induces expression of glucokinase in liver in mice [46]. LXR activation also induces transcription of the insulin-sensitive glucose transporter GLUT4 in adipose tissue and promotes glucose uptake in adipocytes [46]. Treatment with T0901317 stimulates insulin secretion in pancreatic beta cells [47]. T0901317 treatment also reduces plasma glucose and improves glucose tolerance and insulin resistance in murine and rat obesity models [46, 48].

In response to bacterial infection or lipopolysaccharide (LPS) stimulation, macrophages exhibit inflammatory effects, including expression of nitric oxide synthase, cyclooxygenase-2 (COX-2) and

interleukin-6 (IL-6). Treatment of macrophages in culture with GW3965 inhibits the expression of inflammatory mediators [49]. GW3965 treatment also inhibits inflammation in mice [49].

LXR signaling is important for brain function as well [50]. LXRs regulate lipid homeostasis in the brain. LXRα−/− LXRβ−/− mice develop neurodegenerative changes in brain tissue [51]. Knockout of LXRβ results in adult-onset motor neuron degeneration in male mice [52]. Treatment with T0901317 decreases amyloidal beta production in an Alzheimer's disease mouse model [53].

Anticancer effects of LXR signaling

Antiproliferative effect

Based on our recent observations using several prostate cancer cell lines, we discovered that LXR agonists suppress proliferation of prostate cancer cells. LNCaP, PC-3, and DU-145 are commonly used prostate cancer cell lines. The LNCaP cancer cell line was established from a human lymph node metastatic lesion of prostatic adenocarcinoma [54]. PC-3 and DU-145 cells were established from human prostatic adenocarcinoma metastatic to bone [55] and brain [56], respectively. The proliferation of LNCaP cells is androgen-dependent while the proliferation of PC-3 and DU-145 cells is androgen-insensitive. LNCaP cells maintain the expression of androgen receptor (AR) but PC-3 and DU-145 cells express very little or no AR. Treatment of LNCaP, PC-3, and DU-145 cells with LXR agonists (22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, or T0901317) suppresses the proliferation of these cells [14].

Our studies revealed that the suppression of cell proliferation by LXR agonists is via induction of G1 cell cycle arrest [14]. T0901317 decreases the percentage of cells in S-phase and increases the percentage of cells in G1-phase. T0901317 suppresses the expression of S phase kinase-associated protein 2 (Skp2), a protein mediating the ubiquitination and degradation of the cell cycle inhibitor $p27^{Kip1}$, and causes the accumulation of $p27^{Kip1}$. Knockdown of $p27^{Kip1}$ in LNCaP cells increases the resistance of these cells to T0901317 treatment. Daily oral administration of T0901317 (10 mg/kg) suppresses growth of androgen-dependent LNCaP prostate tumors in athymic mice, resulting in a 2-fold difference in mean tumor volume between the control and the T0901317 treatment group [14] (Figure 2A).

Similar antiproliferative effect has been observed in vascular smooth muscle cells (VSMC) treated with GW3965 and T0901317 [40]. In VSMCs, T0901317 or GW3965 treatment inhibits retinoblastoma protein (Rb) phosphorylation and decreases the level of Skp2, cyclin D1, and cyclin A. T0901317 treatment inhibits expression of S phase-regulatory minichromosome maintenance protein 6 and stimulates the accumulation of $p27^{Kip1}$ in VSMC cells. Overexpression of Skp2 in VSMC cells completely prevents the inhibition of Rb phosphorylation and G1 cell cycle arrest caused by T0901317 [40].

T0901317 also suppresses the proliferation of two commonly used breast cancer cell lines, MCF-7 and MDA-MB435S in cell culture [14]. Expression of LXRα mRNA in LNCaP, PC-3, DU-145, MCF-7 and MDA-MB435S cells correlates with the cancer cells' sensitivity to T0901317 treatment. Among these cancer cell lines, MDA-MB435S cells express the least amount of LXRα mRNA and are most resistant to T0901317 treatment. Overexpressing LXRα in MDA-MB435S cells increased the sensitivity of MDA-MB435S cells to T0901317 treatment, suggesting that G1 cell cycle arrest induced by LXR agonists in cancer cells is partially mediated through LXRα gene regulation [14]. T0901317 treatment also suppressed the proliferation of several cancer cell lines, including H1299 (human non-small lung cancer cells), Saos-2 (human osteoblastic cells), A431 (human epidermoid carcinoma cells), SCC13 (human squamous carcinoma cells), HeLa (human cervical cancer cells), and HepG2 (human hepatoma cells) (Table 1). Compared to these cancer cell lines, HEK 293 (transformed human embryonic kidney cells) and Wi38 (human diploid fibroblast cells) were more resistant to T0901317 treatment, indicating the possibility of using LXR agonists as cancer chemotherapeutic agent (Table 1).

Inhibition of prostate cancer progression

To study the progression of prostate cancer cells from androgen-dependency to androgen-independency, we generated androgen-independent LNCaP sublines (104-R1, 104-R2, and CDXR)

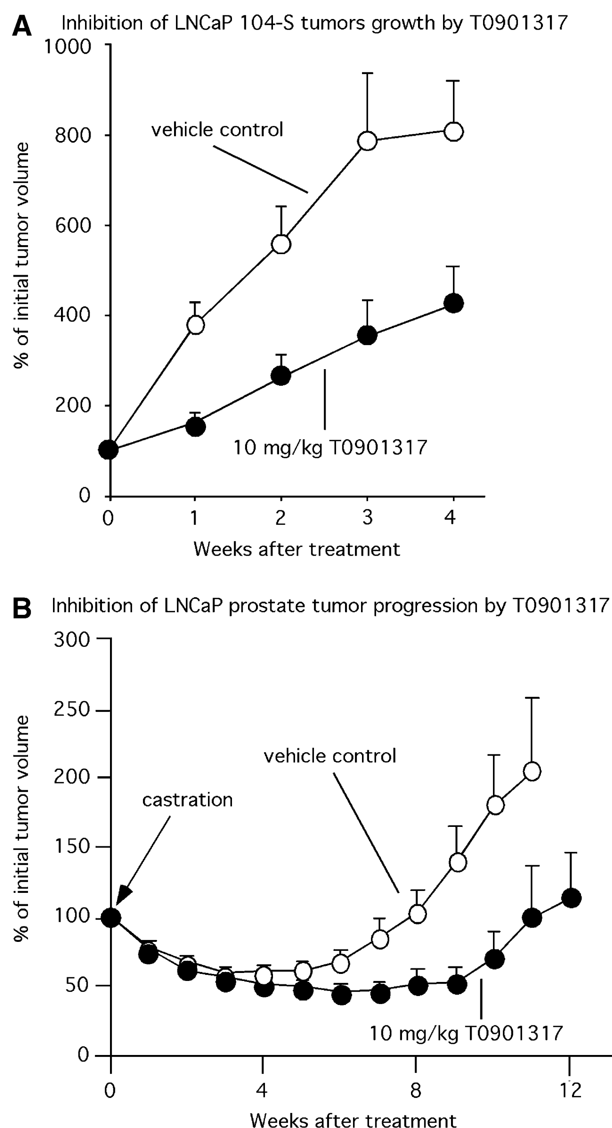

Figure 2. Inhibition of proliferation and progression of prostate cancer by the LXR agonists T0901317. (A) Inhibition of androgen-dependent LNCaP 104-S tumor growth in intact mice by T0901317 treatment. Mice were administered 10 mg/kg T0901317 (filled circle, 10 mice with 13 tumors) or vehicle alone (open circle, 10 mice with 15 tumors) by gavage once a day during the experiment period, resulting in a more than 2-fold difference in mean tumor volume between vehicle and T0901317-treated tumors after 4 weeks. Relative tumor volumes were expressed as mean ± SE. See Ref. [14] for details. (B) Inhibition of progression of androgen-dependent LNCaP 104-S tumors towards androgen-independency in castrated mice by T0901317 treatment. After castration, mice were administered 10 mg/kg T0901317 (filled circle, 9 mice with 15 tumors) or vehicle alone (open circles, 9 mice with 13 tumors) by gavage five times a week during the experiment period, resulting in a 4-week delay in time required for development of androgen-independent relapsed tumors between vehicle and T0901317-treated group. Relative tumor volumes were expressed as mean ± SE. See Ref. [8] for details.

from an androgen-dependent LNCaP subline 104-S after androgen deprivation [57–59]. These androgen-independent LNCaP cells have elevated AR expression and express prostate specific antigen (PSA), the most commonly used marker for detecting prostate tumor growth in patients, upon androgen treatment. Androgens paradoxically inhibit the proliferation of these cells, partially by down-regulating c-myc and inducing accumulation of $p27^{Kip1}$ [57–61]. Our LNCaP cell progression model mimics some clinical cases because increased AR expression is frequently observed in

Table 1. Inhibition of proliferation of multiple cancer cell lines by T0901317 treatment.

Cell line	Ec50	6 μM cell (%)
104-S	8.4	65
104-R1	6.4	53
104-R2	6.0	50
CDXR-3	6.1	51
R1Ad	9.0	62
R2Ad	4.9	42
IS-3	6.1	51
DU-145	10.0	66
PC-3	14.0	79
HepG2	2.9	41
MCF-7	4.7	45
MDA-435	7.8	58
SCC13	9.8	72
A431	14.2	80
H1299	13.2	87
HeLa	13.6	79
Saos-2	13.6	82
HEK 293	14.0	98
Wi38	16.2	95

Androgen-dependent AR-positive LNCaP human prostate cancer cells (104-S), androgen-independent AR-high LNCaP human prostate cancer cell lines (104-R1, 104-R2, CDXR-3), androgen-stimulated AR-low LNCaP human prostate cancer cell lines (R1Ad), androgen-insensitive AR-low LNCaP human prostate cancer cell lines (R2Ad, IS-3), androgen-insensitive AR-negative human prostate cancer cells (DU-145, PC-3), human hepatoma cells (HepG2), estrogen-responsive estrogen receptor (ER) positive human breast cancer cells (MCF-7), estrogen-insensitive melanoma MDA-MB-435, human squamous carcinoma cells (SCC13), human epidermoid carcinoma cells (A431), human non-small lung cancer cells (H1299), human cervical cancer cells (HeLa), human osteoblastic cells (Saos-2), human embryonic kidney cells (HEK) 293 and human diploid fibroblast cells (Wi38) were seeded in 96-well plates in Dulbecco's modified Eagle medium (DMEM) supplemented with 8% dextran-coated charcoal-stripped fetal bovine serum (CS-FBS). Cells were treated with different concentration of T0901317 (0, 1, 2, 4, 6, 8, 10, 12, 20 μM) for 5 days and assayed by measuring DNA content with the fluorescent dye Hoechst 33258 as described previously [14]. EC_{50} and percentage of surviving cell under 6 μM T0901317 treatment are listed in the table.

androgen-independent relapsed prostate tumors in patients [62, 63].

In our progression model, expression of LXRα and its target gene ABCA1 is higher in androgen-dependent LNCaP 104-S cells than in androgen-independent LNCaP 104-R1 and 104-R2 cells [64]. Expression of the LXRα, ABCA1, and sterol 27-hydroxylase (CYP27) genes decreases during prostate cancer progression towards androgen-independency in athymic mice [8]. 27-Hydroxycholesterol and cholestenoic acid, products of CYP27, are endogenous ligands for LXR [28, 65]. The change in expression of genes involved in LXR signaling suggests a potential role of LXR signaling during prostate cancer progression.

We found that suppression of ABCA1 expression by androgen coincided with increased proliferation of androgen-dependent LNCaP 104-S cells [64]. Thus, under androgen-depleted conditions, ABCA1 levels are high and proliferation of 104-S cells is inhibited. During progression, the surviving androgen-independent relapsed tumor cells appear to escape ABCA1 suppression by down-regulating expression of LXR target genes. T0901317 induces expression of the ABCA1 gene in 104-S tumors in athymic mice [14]. Compared to the control group, T0901317 treatment delays the development of androgen-independent relapsed tumors for 4 weeks in athymic mice bearing 104-S tumors after castration [8] (Figure 2B). This result indicates that treatment with LXR agonist can retard progression of prostate cancer *in vivo*.

Unlike other LXR signaling-related genes, the mRNA level of SREBP-1c increases during progression toward androgen-independence [8, 64]. A similar observation was reported by another group [66]. Expression of SREBP-1a, another isoform of SREBP-1, also increases during the progression of LNCaP cells to androgen-independency [8, 64, 66]. However, SREBP-1a is not a target gene of LXR. Androgen receptor signaling stimulates expression of FAS and other lipogenic genes in prostate cancer cells by activating the SREBP Cleavage Activating Protein (SCAP)/SREBP pathway [67]. Therefore, the different pattern of mRNA expression between SREBP-1c and other LXRs target genes may indicate that AR-signaling dominates the regulation of SREBP-1 expression during prostate cancer progression.

ABCA1 mediates cholesterol and phospholipid efflux from cells to apolipoprotein A-I [68]. Cholesterol is essential for formation of lipid rafts. Lipid rafts are cholesterol- and sphingolipid-rich components of the plasma membrane serving as platforms for signal transduction components mediating cell growth and survival [69]. There are two forms of lipid rafts, caveolar and flat lipid rafts. Caveolin proteins are the main component of caveolae. However, LNCaP cells do not express caveolins [69]. LNCaP cells express the

raft-resident protein flotillin-2 [70]. Up-regulation of flotillin-2 is associated with melanoma progression [71]. Flotillin is reported to complex with ABCA1 in macrophages [72]. Therefore, ABCA1 may regulate prostate cancer cell progression through interaction with flotillin.

LXR agonists suppress the activity of IL-6 [49]. IL-6 regulates growth and differentiation of various types of malignant tumors, including prostate cancer [73]. IL-6 activates AR-mediated gene expression through a signal transducer and activator of transcription 3 (STAT3)-dependent pathway in LNCaP prostate cancer cells [74]. IL-6/STAT3 signaling protects LNCaP cells from apoptosis induced by androgen deprivation and promotes androgen-independent proliferation of LNCaP cells [75, 76]. Caveolin-negative lipid rafts in LNCaP cells play an important role in the IL-6/STAT3 signaling pathway [70]. Caveolin-negative lipid rafts, IL-6 and STAT3 may therefore regulate the progression of prostate cancer in cooperation with LXR/RXR signaling. A summary of possible mechanism involved in the inhibition of cancer cell proliferation and progression by LXR agonists is illustrated in Figure 3.

Anticancer effects of phytosterol LXR agonist

Anticancer activity of phytosterols

Phytosterols, the plant equivalents of mammalian cholesterol, are essential components of all plants. Phytosterol are abundant in plant oil, seeds of legumes and nuts, vegetables, and fruits. The most common phytosterols include β-sitosterol, campesterol, and stigmasterol. Dietary phytosterols have long been known to be beneficial to health, and are reported to have anticancer activity [15]. Phytosterol-rich diets were reported to decrease the incidence of gastric [77], breast [78], lung [79], and prostate [80] cancer.

β-sitosterol inhibited the proliferation of LNCaP cells in cell culture [81]. Treatment of β-sitosterol and campesterol suppressed the proliferation of PC-3 cells in culture [82]. β-Sitosterol treatment also inhibited the growth and metastasis of PC-3 tumor xenografts [82]. Diets of β-sitosterol, a mixture of β-sitosterol and β-sitosterol glucoside, or a complex mixture of physoterols, suppressed the growth of estrogen receptor (ER)-positive MCF-7 breast tumors [83] and

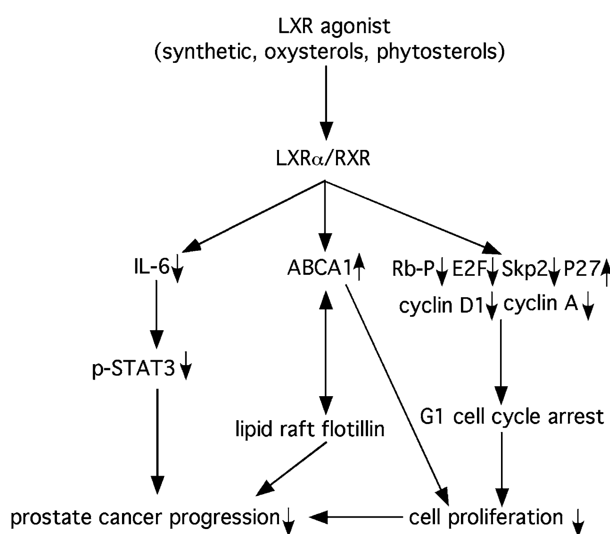

Figure 3. Inhibition of proliferation and progression of prostate cancer cells by LXR agonist treatment. LXR agonists inhibit phosphorylation of Rb, decrease expression of cell cycle related proteins (Skp2, cyclin A, cyclin D1, and E2F), and stimulate accumulation of $p27^{Kip}$. These events cause G1 cell cycle arrest and thus inhibit the proliferation of prostate cancer cells. Additionally, LXR agonists activate the LXRα/RXR heterodimer, which then activates the LXR target gene ABCA1. Up-regulation of ABCA1 inhibits proliferation of prostate cancer cells as well. LXR agonists suppress IL-6, resulting in a decrease in the level of phosphorylated STAT3. IL-6 and STAT3 are both important for promoting androgen-independent proliferation of prostate cancer cells. LXR agonist treatment inhibits IL-6/STAT3 signaling and therefore delays the progression of prostate cancer cell toward androgen-independency. Up-regulation of ABCA1 may also affect prostate cancer progression via interaction with flotillin in lipid raft as well.

ER-negative MDA-MB-231 breast tumors in mice [84]. However, the mechanism by which phytosterols suppress tumor growth is not clear because phytosterols are not readily absorbed into the blood stream.

Phytosterols and LXR signaling

Phytosterols and phytostanols of the 4-desmethyl family (e.g., sitosterol and sitostanol) effectively decrease low-density lipoprotein (LDL) cholesterol concentrations in serum, whereas 4,4-dimethylsterols (e.g., alpha-amyrin and lupeol) do not [16, 85, 86]. Dietary supplementation with phytosterols and phytostanols reduces intestinal cholesterol absorption and decreases plasma LDL cholesterol concentration in humans [87]. Since LXRs regulate homeostasis of cholesterol, it is believed that phytosterols and phytostanols affect cholesterol absorption and serum LDL cholesterol levels by regulating the LXR target genes ATP-binding cassette (ABC) transporters ABCG5 and ABCG8.

In support of this hypothesis, phytosterols and phytostanols from the 4-desmethylsterol family have been shown to activate LXRα and LXRβ with EC_{50} at 30–150 nM [16]. Agonistic activity of β-sitosterol with LXR is much stronger than campesterol or stigmasterol [16]. Accumulation of plant sterols profoundly reduced the cholesterol level in the adrenal gland of mice lacking ABCG5 and ABCG8 (G5G8−/− mice) [88]. A phytosterol-derived LXR agonist, YT-32, has been shown to induce intestinal ABCG5 and ABCG8 [32]. However, dietary phytosterols and phytostanols did not activate intestine ABCG5/8 gene in mice [87].

Since β-sitosterol and campesterol are effective LXR agonists and exhibit effective anticancer activity on several types of cancer, we suggest that phytosterols suppress growth and metastasis of tumors partially through activation of LXR signaling.

Conclusions

LXR agonists inhibit proliferation and progression of prostate cancer cells both *in vitro* and *in vivo* [8, 14]. LXR agonists also suppress the proliferation of several other cancer cell lines (our unpublished data). β-Sitosterol and campesterol, two of the most common phytosterols, inhibit proliferation and metastasis of several cancer cell lines. β-Sitosterol and campesterol have recently been identified as effective LXR agonists [16, 80–83]. LXR agonists may suppress the growth of prostate tumors and other carcinomas in patients.

The synthetic non-steroidal LXR agonists T0901317 and GW3965 are very potent agonists compared to natural oxysterols [29, 30]. Administration of T0901317 or GW3965 in mouse disease models was reported to be effective for treatment of atherosclerosis, diabetes, and Alzheimer's disease [11, 31, 38–40, 46, 48, 53]. However, both T0901317 and GW3965 have been reported to increase plasma and liver triglycerides in some mice models [29, 39]. Synthetic steroidal LXR agonist hypocholamide, a 6α-hydroxylated analog of bile acids, shows an overall hypolipidemic effect but does not increase the serum triglyceride level [31]. YT-32, a synthetic LXR agonist developed by modifying the phytosterol structure, selectively activated intestinal ABC transporters in mice without increasing plasma triglyceride levels [32]. It may be possible to develop other potent and effective LXR agonists without the undesirable side effects, such as hypertriglyceridemia. Although the exact mechanism responsible for inhibition of prostate cancer progression by LXR agonists requires further study, modulation of LXR signaling may be a novel and useful therapy for prostate and other cancers.

Acknowledgements

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