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Uncoupling Nuclear Receptor LXR and Cholesterol Metabolism in Cancer

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Liver X receptors (LXRs) are members of the nuclear receptor superfamily of DNA-binding transcription factors and act as sensors of cholesterol homeostasis. Under normal conditions, when intracellular cholesterol concentration increases, cells synthesize oxysterols and activate the LXR transcriptional network to drive cholesterol efflux and reduce cholesterol influx and synthesis. During normal and cancer cell proliferation, there is a net uncoupling between intracellular cholesterol increase and LXR activation resulting from the reduced intracellular oxysterol concentration. This review dissects the novel mechanisms of a previously unrecognized metabolic uncoupling, supporting the activation of the LXR axis as a bona fide therapeutic approach in cancer.

Introduction

Cholesterol is an essential component of mammalian cell membranes as well as a precursor of bile acids and steroid hormones (Simons and Ikonen, 2000). Because of its importance, cells have evolved complex mechanisms to closely regulate the abundance and distribution of sterols. The circulating levels of cholesterol are regulated by a balance among intracellular synthesis, dietary cholesterol absorption, and removal of the excess cholesterol from peripheral tissues. A large body of clinical and experimental evidence suggests the hypothesis that these finely tuned mechanisms become altered during cell division and membrane synthesis both in physiological hyperproliferative conditions and in carcinogenesis (Clendening et al., 2010; Dang, 2012). Epidemiological studies have shown that patients with cancer have high plasma levels of lipoprotein carriers of cholesterol and that cancer cells exhibit deregulated transcriptional levels of several genes implicated in cholesterol regulation and metabolism, such as low-density lipoprotein receptor (Ldlr), hydroxyl-methyl glutaryl-coenzyme A reductase (Hmgcr), and sterol regulatory element-binding proteins (Srebps) (Llaverias et al., 2011; Scheinman et al., 2013).

Cholesterol Synthesis

Cholesterol is synthesized via the mevalonate pathway, an enzymatic cascade in which the rate-limiting reduction of hydroxyl-methyl glutaryl-coenzyme A (HMG-CoA) to mevalonate is catalyzed by HMGCR. The expression of all of the enzymes acting in the cholesterol biosynthetic pathway is regulated by SREBP transcription factor family (Sato, 2010).

In sterol-rich conditions, SREBP resides in the endoplasmic reticulum as a high-molecular-weight precursor in a repressor complex represented by the SREBP-cleavage activation protein (SCAP), which acts as the cholesterol sensor changing conformation in a cholesterol-dependent fashion, and the anchor insulin-induced gene (INSIG). Moreover, the cholesterol is esterified by acyl-CoA cholesterol acyl transferase (ACAT) and stored in the cytosol in the form of lipid droplets (Figure 1). In sterol-poor conditions, SCAP escorts SREBP into the Golgi complex, where it is cleaved by a series of proteolytic steps (Goldstein et al., 2006). The released mature transcription factor migrates into the nucleus, where it recognizes its DNA binding sequence and activates gene target transcription (Figure 1). Studies performed on different types of tumors have revealed that cancer cells present deficient feedback control of HMGCR or increased HMGCR expression compared to untransformed cells, suggesting that the dysregulation of the mevalonate pathway may have the oncogenic power to drive malignant transformation (Clendening et al., 2010) and sustain tumor growth (Sorrentino et al., 2014). Moreover, mevalonate is a precursor of several major products regulating the cell cycle, including dolichol, geranyl-pyro-phosphate (GPP), and farnesyl-pyrophospate (FPP) (Goldstein and Brown, 1990). Dolichol has a stimulatory effect on DNA synthesis and is linked to several tumor cell proteins (Wejde et al., 1998). GPP and FPP cause isoprenylation of the intracellular G proteins Ras and Rho, which in turn regulate the signal transduction of several membrane receptors crucial for the transcription of genes involved in cell proliferation, differentiation, and apoptosis (Goldstein and Brown, 1990).

Cholesterol Influx

To sustain the whole-body cholesterol homeostatic state and reduce the high ATP consumption of cholesterol de novo biosynthesis, lipoproteins mediate the processing and delivery of dietary cholesterol to peripheral tissues via circulation. Low-density lipoprotein (LDL) particles deliver cholesterol to most peripheral tissues through a receptor-mediated mechanism (Brown and Goldstein, 1986). LDLRs are present on the plasma membrane of most cells and mediate the selective capture of LDL macromolecules, which are completely and rapidly degraded in the lysosome. The LDL-derived cholesterol acts in the cell at several levels, including suppression of transcription of the Hmgcr gene through the SREBP pathway and activation of ACAT to storage esterified cholesterol (Goldstein and Brown, 2009; Brown and Goldstein, 1986). By inhibiting the SREBP pathway, LDL also suppresses transcription of the Ldlr gene (Brown and Goldstein, 1999). Lipid profiles of cancer patients display reduction of plasma lipoprotein levels and their restoral to normal values upon successful remission, suggesting the importance of

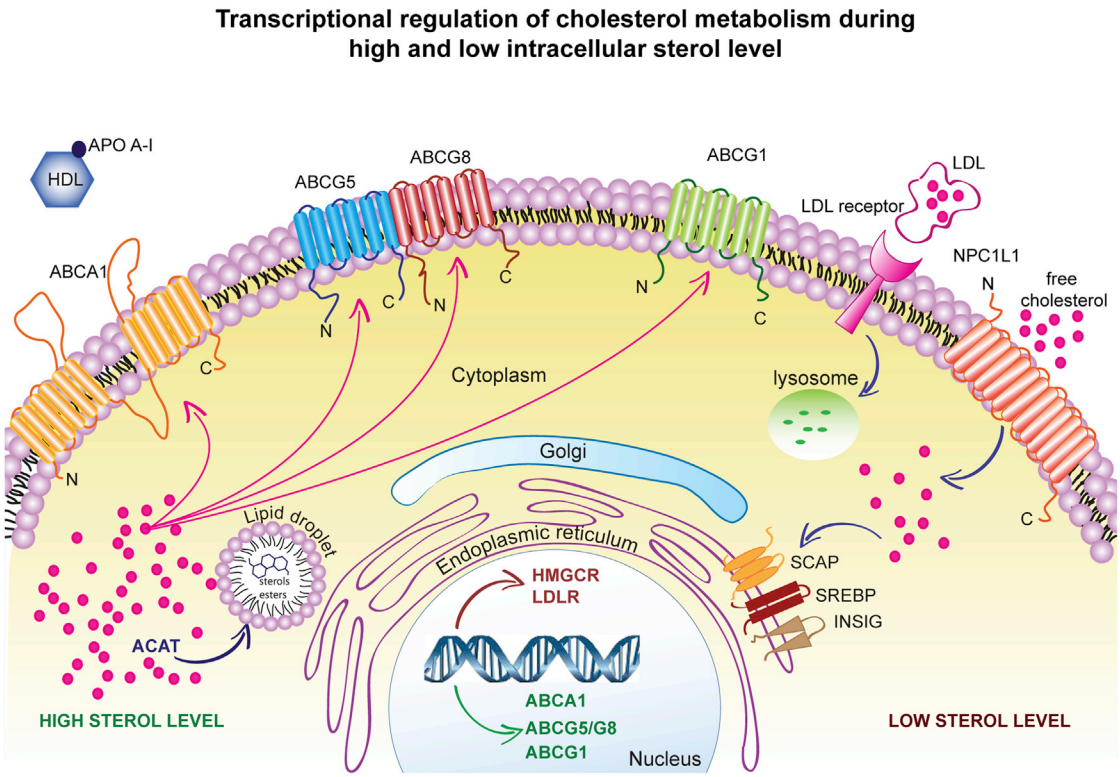

Figure 1. Transcriptional Regulation of Cholesterol Metabolism during High and Low Intracellular Sterol Levels
In low-sterol-level conditions, in order to satisfy the enhanced requirement of cholesterol, the complex SCAP-SREBP migrates in the Golgi apparatus to be cleaved and release the mature transcription factor SREBP, which activates cholesterol de novo synthesis via HMGCR and exogenous cholesterol capture via LDLR or NPC1L1, specifically in enterocytes. Conversely, in high-sterol-level states, LXRs activate the transcriptional expression of transporters such as ABCA1, ABCG5/G8, and ABCG1, which mediate cholesterol efflux from the cell. Moreover, sterol in excess is converted in esters and stocked within lipid droplets.

lipoprotein carriers in tumor growth and development, but this feature needs to be more investigated with the aim of considering lipoprotein levels a biomarker in cancer (Solomon and Freeman, 2011).

In addition, the intestinal epithelium is equipped with take-up dietary and biliary cholesterol mechanisms from the gut lumen into the enterocytes. Niemann Pick C1-like 1 (NPC1L1) is a protein localized at the brush border membrane of the enterocytes that mediates free cholesterol absorption into the enterocytes. In humans and nonhuman primates, it is also significantly expressed in the liver, where it transports newly secreted biliary cholesterol back into hepatocytes, preventing excessive loss of endogenous cholesterol. Ezetimibe, a drug that inhibits cholesterol absorption by blocking NPC1L1 gut transporters, was administered in a prostate cancer in vivo model showing antitumor effects (Solomon and Freeman, 2011).

Cholesterol Efflux

Cholesterol elimination from the cells is a key process necessary to prevent cholesterol retention and atherosclerotic lesion formation. ATP-binding cassette (ABC) transporters are key mediators of reverse cholesterol transport (RCT), a process by which excess cholesterol from peripheral tissues is returned to the liver by high-density lipoprotein (HDL) for ultimate excretion in bile. The early steps of cholesterol efflux are controlled by ABCA1, which is ubiquitously expressed and promotes net cholesterol efflux to lipid-poor apoA-I leading to discoid HDL formation (Vedhachalam et al., 2007). Moreover, ABCG1 cooperates with ABCA1 in macrophages by further adding cellular lipids to the nascent particle, which results in the maturation of HDL (Phillips, 2014). Additionally, the ABCG5/G8 transporters, localized on the basal membrane of hepatocytes and enterocytes, inhibit the absorption of cholesterol and plant sterols from the diet by mediating the efflux of these sterols from enterocytes back into the gut lumen and by promoting efficient secretion of cholesterol and plant sterols from hepatocytes into the bile (Wang et al., 2015).

Intracellular Cholesterol-Driven Regulation of Cell Cycle and Apoptosis

The cell membrane contains micro-domains known as lipid rafts, which are characterized by high cholesterol content and a strict compartmentalization from the rest of the membrane. These specific regions are associated with several proteins that regulate pro-oncogenic and apoptotic pathways and are involved in the initial stages of cancer development, tumor growth and the potential progression to a migratory and metastatic phenotype (Yang et al., 2014). Among key regulator proteins anchored to cholesterol rafts, death receptors are notably important because, once activated, they trigger apoptotic signal transduction. Avoiding apoptosis is one of the main strategies adopted by cancer cells to improve their rapid proliferative behavior, and they achieve this by modulating the cholesterol composition of lipid rafts, thus disrupting death receptor folding

and function. Two classes of death receptors, the Fas receptor and the TNF-related apoptosis-inducing ligand (TRAIL), have been extensively investigated, given their central role in apoptosis activation. Cholesterol depletion studies show a significant inhibition of the cell death program associated with Fas and TRAIL (Li et al., 2006; Song et al., 2007).

Several studies have focused on oncogenic signals transmitted by the Rac protein kinase (AKT) protein, a serine-threonine protein kinase that mediates cell survival and growth. Since AKT is closely associated with cholesterol rafts, many studies indicate that changes in membrane cholesterol content determine alterations in the proliferation rate of tumor cells through AKT signal modulation (Li et al., 2006). It was recently shown that in prostate tumor cells, reduction of membrane cholesterol levels causes the rearrangement of lipid rafts with strong effects on AKT. The cholesterol-driven disruption of lipid rafts is characterized by downregulation of the AKT signal and thus the renovation of the apoptotic impulse (Pommier et al., 2010). Furthermore, the cell cycle seems to be sensitive to intracellular cholesterol. Inhibition of cholesterol synthesis mediated by statin treatment results in breast cancer cell line arrest in the G1 phase of the cell cycle, given the SREBP pathway activation and then the increase of cyclin-dependent kinase inhibitors p21 and p27 (Rao et al., 1998). Moreover, the antitumoral effects of cholesterol biosynthesis inhibition in breast carcinoma cells have been associated with the suppression of the mitogen-activated protein kinase (MEK/Erk) pathway and the dramatic decreases in nuclear factor kappa-B (NF-κB) and activator protein-1 (AP-1) DNA binding activities (Campbell et al., 2006).

Nuclear Receptors LXR as Transcriptional Cholesterol Sensors

LXRs are members of the nuclear receptor (NR) superfamily of ligand-activated transcription factors and exist as two isoforms, LXRα and LXRβ. They act as whole-body cholesterol sensors, and their activation results in a net elimination of cholesterol from the body and amelioration of the plasma lipoprotein profile by mobilizing cholesterol from the periphery (Venkateswaran et al., 2000; Repa et al., 2000b), promoting its hepatic excretion and limiting its absorption (Repa et al., 2002; Yu et al., 2014; Duval et al., 2006), reducing its cellular uptake (Zelcer et al., 2009), and enhancing its conversion to bile acids in mice (Peet et al., 1998). The identification of 24S, 25, and 27 hydroxycholesterol as in vivo ligands of LXR endorsed these receptors as sterol sensors.

Identification, Cloning, and Expression Pattern

Originally considered orphan receptors, LXRs were identified between 1994 and 1996 as RXR heterodimers permissive to activation by both LXR and RXR ligands. LXRα and LXRβ bind to the LXR responsive element (LXRE), a specific DNA sequence represented by two 5′-AGGTCA-3′ hexameric half-sites separated by a four-nucleotide spacer (DR4 motif) (Willy et al., 1995; Apfel et al., 1994). Following ligand binding to LXR or RXR, corepressors are released and coactivators are recruited, resulting in gene transcription. Studies of sequence comparison revealed a 77% sequence homology in Lxrα and Lxrβ genes, while northern blot analysis showed a different tissue distribution, thus identifying LXRβ as the ubiquitous isoform (Apfel et al., 1994) and LXRα as selectively expressed in metabolically active tissues such as the liver, adipose tissue, adrenal glands, intestine, and macrophages (Chen et al., 2007).

Identification of Oxysterols as In Vivo Ligands

In 1996, Janowski and colleagues relocated LXRs as a specific class of nuclear receptor by discovering oxysterols, monooxygenated derivates of cholesterol, as LXR-specific ligands (Janowski et al., 1996). Similarly to the assay used for ligand identification of other receptors, concentrated lipid extracts from different tissues were screened in a high-throughput cotransfection assay, revealing a cholesterol-derived compound able to transactivate LXRα (Janowski et al., 1996). Structural-activity relationship studies (SAR) disclosed that the position of the hydroxyl group on the cholesterol backbone is determinant for LXR high-affinity binding and activation at concentrations occurring in vivo (Janowski et al., 1999). In general, biological oxysterols are classified in two main categories: those oxygenated on the sterol ring on the 7 position (e.g., 7α/β-hydroperoxycholesterol [7OOHC], 7-ketocholesterol [7KC], and 7α/β-hydroxy-cholesterol [7HC]) with a non-enzymatic origin, and those oxygenated on the side chain (e.g., 24S-hydroxycholesterol [24HC], 25-hydroxycholesterol [25HC] and 27-hydroxycholesterol [27HC]), generally produced in enzymatic reactions.

In physiological conditions, oxysterols act as regulators of cholesterol excess via both LXR activation and SREBP direct regulation in an LXR-independent way. In order to prevent SREBP maturation in Golgi apparatus and, consequently, suppress the expression of genes involved in sterol metabolism, oxysterols seem to bind to INSIG, retaining the SREBP-SCAP complex in the endoplasmic reticulum (Radhakrishnan et al., 2007; Janowski et al., 2001). Moreover, intracellular oxysterol storage triggers HMGCR binding to INSIG, inducing the recruitment of a membrane-associated ubiquitin ligase called GP78 and thus the ubiquitination and degradation of reductase by cytosolic 26S proteasomes (DeBose-Boyd, 2008). Finally, oxysterols and in particular 25HC are thought to activate intracellular cholesterol transport to the endoplasmic reticulum and thus intracellular storage in the form of esterified cholesterol (Du et al., 2004).

The LXR Transcriptome

Earlier evidence of the physiological role of LXR in lipid metabolism came from LXRα knock-out mice, which, after being fed a high-cholesterol diet, accumulated large amounts of cholesteryl ester in their livers (Peet et al., 1998) and showed an altered plasma lipoprotein profile, characterized by increased LDL and reduced HDL cholesterol levels (Janowski et al., 1996). Cytochrome P450 7A1 (CYP7A1), an enzyme that catalyzes the rate-limiting step in bile acid synthesis and promotes the excretion of fecal sterols (Peet et al., 1998), was identified as the first LXR target gene in the mouse. Subsequent studies of LXR activation in vivo revealed the unique role of LXR in promoting RCT by induction of ABCA1 (Venkateswaran et al., 2000; Repa et al., 2000b) and ABCG1 expression (Kennedy et al., 2005). In addition, LXRs modulate intestinal cholesterol excretion via the induction of ABCG5 and ABCG8 transporters (Repa et al., 2002) and regulate a cluster of lipoprotein genes as well as lipid-remodeling genes (Laffitte et al., 2003; Zhang et al., 2001), thus increasing fecal sterol excretion. Furthermore, the contribution of LXRs to the lowering of whole-body

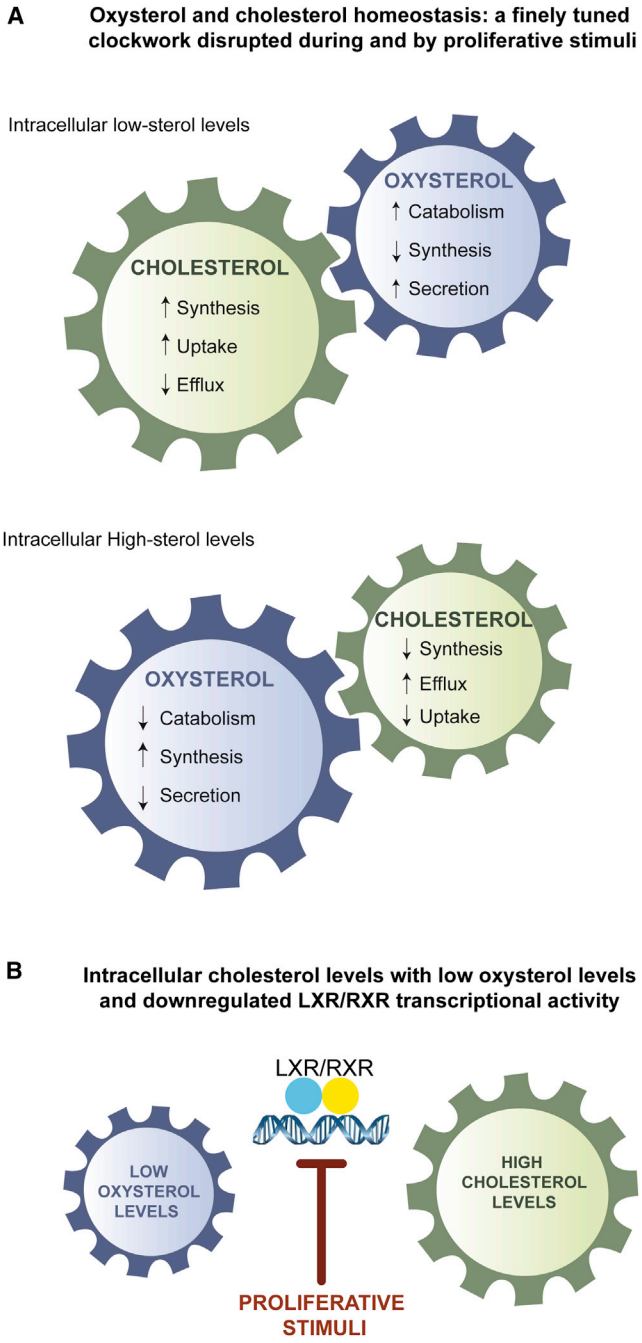

Figure 2. Oxysterol and Cholesterol Homeostasis: A Finely Tuned Clockwork Disrupted during and by Proliferative Stimuli

(A) Oxysterol hypothesis of cholesterol homeostasis. Upon intracellular low-sterol levels, cholesterol synthesis (HMGCR) and uptake (NPC1L1 and LDLR) are increased while cholesterol efflux via ABC transporters (A1, G1, and G5/G8) is inhibited. Moreover, oxysterol catabolism (via SULT2B1b) and secretion (ABCC1) are upregulated while oxysterol synthesis is repressed. On the other hand, in the presence of intracellular high sterol levels, both cholesterol and oxysterol metabolism are conversely regulated.

(B) Intracellular high cholesterol levels with low oxysterol levels and downregulated LXR/RXR transcriptional activity. Normal and cancer cells exposed to proliferative stimuli display the uncoupling of cholesterol and oxysterol homeostasis, via repression of LXR transcriptional activity, to increase intracellular cholesterol content.

cholesterol levels is given by their ability to shift acetyl-CoA units from cholesterol de novo biosynthesis to fatty acid synthesis. The lipogenic power of LXRs is mediated by direct upregulation of SREBP-1c, the main regulator of hepatic lipogenesis (Repa et al., 2000a), as well as direct induction of other lipogenic genes such as fatty acid synthase (Fas) (Joseph et al., 2002) and stearoyl-CoA desaturase 1 (Scd1) (Chu et al., 2006). Recent evidence also points to the ability of LXRs to reduce intestinal absorption of cholesterol, by the induction of the E3 ubiquitin ligase inducible degrader of LDLR (IDOL) and subsequent degradation of LDLR, which limits LDL cholesterol uptake in peripheral tissues (Zelcer et al., 2009). Moreover, LXR activation downregulates NPC1L1 expression in mice and human enterocytes, with a consequent reduction of cholesterol absorption (Duval et al., 2006).

In this scenario the intestine seems to be a key player in the control of cholesterol homeostasis. Recent studies of intestinal-specific LXR activation in vivo have provided evidence that upregulation of ABCG5/ABCG8 and ABCA1 transporters strongly reduces cholesterol absorption, increases pre-β HDL particles, and reverses cholesterol transport in vivo in the absence of hepatic steatosis (Lo Sasso et al., 2010b).

Uncoupling Cholesterol Homeostasis and LXR in Normal and Cancer Cell Proliferation

Forty years ago, Kandutsch and colleagues first elaborated the "oxysterol hypothesis of cholesterol homeostasis" based on the observation that oxysterols mediate feedback regulation of cholesterol biosynthesis rather than cholesterol (Kandutsch et al., 1978). Oxysterols are now recognized to potentially act at multiple LXR-driven points in cholesterol homeostasis, first by increasing cholesterol efflux gene expression, then by decreasing cholesterol synthesis and uptake at the transcriptional level via disruption of the SREBP pathway, and finally accelerating the degradation of HMGCR. In conditions of normal cell proliferation (Figure 2A) as well as in cancer cells (Figure 2B), the accumulation of intracellular cholesterol is required to sustain the high growth rate, and it is paradoxically coupled with a substantial reduction of oxysterol contents, thus downregulating the LXR transcriptome. This scenario is characterized by oxysterol synthetic pathway shutdown and a boost of catabolic and secretory cascades.

Oxysterol Metabolism: Synthesis, Catabolism, and Secretion

Oxysterols produced via enzymatic oxidation have been extensively studied, given their credibility as physiological regulators in vivo. 24HC, 27HC, and 25HC are the main LXR biological activators, synthesized by cytochrome P450 and non-heme iron-containing enzymes with different tissue distribution. Cholesterol 24-hydroxylase (CYP46A1) is a microsomal enzyme highly expressed in neurons, where it produces the so called "cerebrosterol" 24HC (Björkhem et al., 1998). Sterol 27-hydroxylase (CYP27A1) converts cholesterol in 27HC and is localized in the mitochondria of many tissues, especially the liver and macrophages. It also catalyzes the first step in the alternative pathway of bile acid synthesis (Russell, 2000). Cholesterol 25-hydroxylase is a small hydrophobic protein which belongs to the non-heme iron-containing protein family and is expressed at very low levels in most tissues. The product of its enzymatic

Table 1. In Vitro and In Vivo Evidence of Ability of LXR Activation to Inhibit Tumorigenesis

Tumor	In Vitro	In Vivo	Reference
Breast cancer	MCF7, T47D, ER-SK-BR3, MDA-MB231	MCF7 cell-derived breast xenograft, MMTV-PyMT mice	Gong et al., 2007; Nelson et al., 2013; Nguyen-Vu et al., 2013; Vedin et al., 2009
Colon cancer	HT29, LS174T, HCT116, Colo205	VP16LXRα/Apc^{min}; LXRαKO, LXRβKO, LXRα/βKO, HT29 cell-derived xenograft, AOM/DSS colitis-associated carcinogenesis	Lo Sasso et al., 2013; Vedin et al., 2009, 2013
Prostate cancer	LNCaP 104-S, LNCaP 104-R2, PC-3, DU-145	LNCaP 104-S cell-derived xenograft	Chuu et al., 2006, 2007; Fu et al., 2014; Fukuchi et al., 2004; Trasino et al., 2009
Glioblastoma	U87, U87-EGFRvIII, U87-EGFR, U87-EGFR-PTEN, LN299, T98	Human malignant glioma cells and human primary GBM39 cell-derived xenograft	Guo et al., 2011
Melanoma	B16F10, A-375, L10BIOBR	B16F10 cell-derived xenograft	Zhang et al., 2014; Pencheva et al., 2014
Ovarian carcinoma	CaOV3, HS-68, SKOV3, A278	–	Rough et al., 2010
Gallbladder cancer		LXRα$^{-/-}$, LXRβ$^{-/-}$, LXRα$^{-/-}$β$^{-/-}$ female mice	Gabbi et al., 2010

activity is represented by 25HC, which is able to suppress cholesterol de novo biosynthesis by inhibiting SREBP maturation and inducing the ubiquitination and degradation of HMGCR in a sterol-dependent manner (Goldstein et al., 2006).

The levels of ligand for nuclear receptors are regulated by catabolic enzymes. The expression of these enzymes protects from the action of circulating ligands, and often, with a feed-forward regulatory loop, it is the same ligand that controls its own catabolism. In low-sterol conditions, oxysterols are metabolized for inactivation and elimination from the body in order to abate the expression of LXR transcriptome and hold cholesterol back in the cell. One apparently widespread mechanism for the metabolism of oxysterols is sulfation of the 3β-hydroxyl group by SULT2B1b, a member of cholesterol sulfotransferase highly expressed in human liver and macrophages (Meloche and Falany, 2001). Chen and colleagues provided the first evidence about the ability of sulfation to inactivate oxysterols. When SULT2B1b was overexpressed in several cell lines and in cholesterol-fed mice, the attenuation of cholesterol capacity to induce LXR target genes such as Cyp7a1, Srebp1-c, Abcg5, and Abcg8 was observed (Chen et al., 2007). Besides inactivating oxysterols as LXR ligands, sulfation increases their polarity and thus facilitates their efflux from the cell by membrane transporters such as multidrug resistance-associated protein 1 (ABCC1). ABCC1 is a membrane-bound protein involved in the cellular defense mechanism, generally localized in the basolateral membrane of polarized cells (Evers et al., 1996) and expressed almost ubiquitously in many tissues (Flens et al., 1996). Like many other ABCC members, ABCC1 pumps anticancer drugs, xenobiotics, and metabolites like inactivated oxysterols into the extracellular space in order to prevent toxicity such as carcinogenesis (Kunická and Souček, 2014; Leslie et al., 2005). However, these transporters may also contribute to the multidrug resistance, which occurs in cancer treatment failure, rendering tumor cells protected by many drugs and chemotherapeutics (Kunická and Souček, 2014).

Antitumoral Effects of LXR Activation: Putative Mechanisms of Action

Many recent studies on pharmacological LXR activation in colon, breast, prostate, gallbladder, and skin cancer cells (Table 1) report the antiproliferative effect of LXR mediated by the alteration of tumor metabolism (Lo Sasso et al., 2013; Nelson et al., 2013) and microenvironment (El Roz et al., 2013; Pencheva et al., 2014; Wang et al., 2014), disruption of key growth pathways (Vedin et al., 2009, 2013; Uno et al., 2009), and activation of apoptotic processes (Zhang et al., 2014; Wente et al., 2007; Pommier et al., 2010).

Since LXR and tumor metabolism relationship is in-depth described in the next paragraph, the other putative LXR-mediated mechanisms of tumor growth inhibition are mentioned below. As observed in breast (Vedin et al., 2009), colon (Lo Sasso et al., 2013; Vedin et al., 2013), and prostate (Fukuchi et al., 2004) cancer studies, activated LXR leads to cell-cycle arrest via downregulation of SKP2 (S phase-associated kinase protein-2), a component of ubiquitin ligase that regulates the degradation of p27^{kip1}, one of the inhibitors of cell-cycle progression from G1 to S phase (Fukuchi et al., 2004). Studies performed on both estrogen receptor (ER)-positive and -negative breast cancer cell lines support the strong relationship between LXR and estrogen-signaling pathway, highlighting a more pronounced antiproliferative effect of LXR activation in ER-positive cells than ER-negative ones (Vedin et al., 2009). Further experiments performed by Nguyen-Vu and colleagues suggest the involvement of E2F2, a member of the E2F family of transcription factors. In ER-positive breast cancer cell lines, E2F2 seems to be downregulated after treatment with LXR agonists, thereby blocking proliferation (Nguyen-Vu et al., 2013). In prostate cancer cells, cholesterol depletion induced by LXR alters lipid raft composition, thus disrupting the correct folding and the activation state of proteins (i.e., AKT) that regulate downstream pathways crucial in cell survivors (Pommier et al., 2010). Candelaria and colleagues have studied LXR activation effects in pancreatic ductal adenocarcinoma cell lines (Candelaria et al., 2014) and have observed the downregulation of growth factor signaling, especially epidermal growth factor receptor (EGFR) activation, as previously described by Guo in glioblastoma cells (Guo et al., 2011). Furthermore, a new interesting mechanism of LXR modulating tumor microenvironment has been observed by Pencheva and colleagues in melanoma cancer models. They have observed LXR's ability in suppressing tumor invasion and angiogenesis by transcriptionally activating melanoma cell secretion of apolipoprotein E (APOE), a potent metastasis

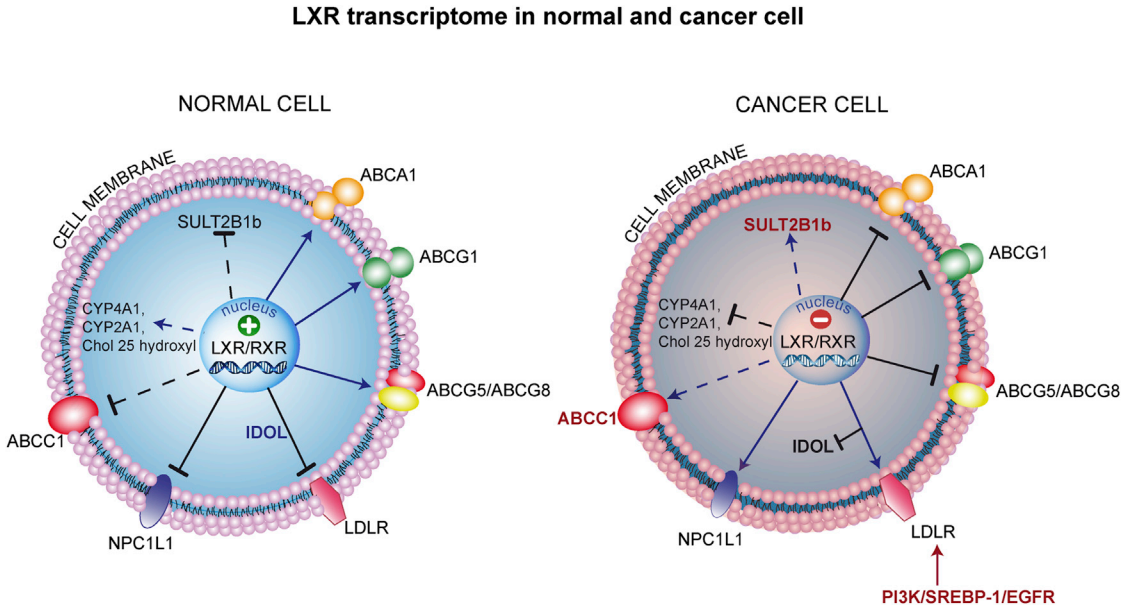

Figure 3. LXR Transcriptome in Normal and Cancer Cell

In normal cells, LXR transcriptional activity ensures an adequate intracellular sterol content via activation (ABCA1, ABCG1, ABCG5/ABCG8) or repression (NPC1L1 and LDLR) of its direct target genes. Of note, LXR activation is sustained by the prevalence of oxysterol anabolism (CYP4A1, CYP2A1, and Chol-25-hydroxylase) over catabolism (ABCC1 and SULT2B1b). In cancer cells, despite the intracellular cholesterol abundance, LXR transcriptome is downregulated as a result of the metabolic shift of oxysterol metabolism from anabolic toward catabolic pathways.

suppressor gene (Pencheva et al., 2014). This molecule, when released within the tumor microenvironment, acts by suppressing melanoma invasion ability by targeting LDLR-related protein 1 (LRP-1 receptor) on melanoma cells and endothelial recruitment and migration via LDLR-related protein 8 (LRP8) receptors (Pencheva et al., 2012). The tumor microenvironment is full of cytokines that play an important role in intercellular communication and in the inflammatory processes occurring in the tumor. Interferon-γ (IFN-γ) is one of the cytokines involved in antiproliferative, antiangiogenic, and proapoptotic effects against cancer cells (Zaidi and Merlino, 2011). IFN-γ especially targets macrophages, thus activating tumor cell cytotoxicity, antimicrobial activity, intracellular pathogen removal, and acquired immune responses (MacMicking et al., 1997). In a recent study, Wang and colleagues identified IFN-γ as a new putative molecular target of LXR activation. In in vivo experiments, administration of LXR ligand inoculated with lung tumor cells increases IFN-γ production, rates of survival, and tumor-free animals, an effect not observed in IFN-$\gamma^{-/-}$ mice, suggesting that the inhibition of tumor growth happens in an IFN-γ-dependent manner (Wang et al., 2014). Moreover, Blanc and colleagues have found that IFN is directly coupled with 25HC via an LXR-independent but SREBP-dependent subordinate mechanism, highlighting a previously unrecognized biological role for 25HC (Blanc et al., 2013). They have also observed that IFN-treated macrophages secrete more 25HC, suggesting a potential regulatory role of 25HC and LXR in IFN-based cancer treatment.

The Normal and Cancer Cholesterol-LXR Uncoupling Models

In vivo experiments provide evidence that tumor-unrelated cell proliferation, such as proliferating T cells (Bensinger et al., 2008) and liver regeneration after partial hepatectomy (Lo Sasso et al., 2010a), as well as tumor-related hyperproliferation status, are closely associated with enhanced cholesterol requirement, which is satisfied not only by upregulating de novo biosynthesis but also by increasing cholesterol uptake via LDLR. In most of these studies, it has been observed that LXR synthetic ligands are able to decrease cell growth and to induce significant tumor cell death in vivo, thanks to their ability to repress LDLR protein expression via IDOL activation, inhibit HMGCR activity, and induce cholesterol efflux via ABCA1, ABCG5, and ABCG8 (Figure 3) (Moschetta, 2011). Below are detailed hyperproliferative and cancer models of uncoupling cholesterol homeostasis with LXR activity.

Acquired Immune Response in T Cells

Adaptive immune responses are carried out by lymphocytes that, upon pathogen-associated immunostimulation, undergo rapid and extensive proliferation in order to defend the organism against infection. This characteristic of T cell function is sustained by lipid metabolism modulation and sterol intracellular availability, an unknown regulatory role that Bensinger and colleagues have elegantly revealed in a recent study (Bensinger et al., 2008). During T cell activation and proliferation, the transition from G1 to the S phase is realized by metabolic reprogramming, in which LXR-mediated downregulation of transporters ABCA1 and ABCG1 increases sterol concentration. Furthermore, LXR null mice show splenomegaly and lymphadenopathy typical of T and B cell expansion. Conversely, the ligand activation of LXR inhibits proliferative stimuli by decreasing sterol contents through ABCG1 upregulation. These data confirm the existence of an endogenous sterol signaling pathway regulating lymphocyte proliferation through LXR. The unexpected mechanism by which LXR transcriptome is downregulated during

lymphocyte proliferation is the cellular oxysterol deprivation by way of SULT2B1 overexpression (Chen et al., 2007; Fuda et al., 2007). The definition of a SULT2B1-LXR-ABCG1 axis that couples cellular cholesterol metabolism and proliferation has represented a milestone in understanding the basis of lipid role and regulation, with main relevance to rapid dividing cells.

Liver Regeneration after Partial Hepatectomy

The regenerating mouse liver is an effective model in vivo to study the modulation of cholesterol metabolism during cell proliferation. Since 1985, Field and collaborators have employed regenerating rat liver post-partial hepatectomy to investigate the effect of cell proliferation on plasma and liver metabolism, showing that during hepatic regeneration, triglyceride and cholesterol levels significantly decrease in plasma but increase in the liver (Field et al., 1985). Given LXR's ability to act as the sensor of cholesterol homeostasis, Lo Sasso and colleagues have investigated the mechanism by which LXR controls cholesterol hepatic contents in regenerating mouse liver (Lo Sasso et al., 2010a). They confirmed increased intrahepatic cholesterol levels, explained by the induction of cholesterol de novo biosynthesis and inhibition of catabolism into bile acids and biliary secretion. This event is characterized by the downregulation of the entire LXR transcriptome, including transporters ABCG5 and ABCG8, and the loss of its inhibitory effect on cholesterol intracellular accumulation. Moreover, significant changes in the expression of genes encoding for proteins and enzymes involved in oxysterol synthesis (CYP27A1 and CYP46A1), catabolism (SULT2B1b), and secretion (ABCC1) were measured in cholesterol-enriched proliferating hepatocytes. These data suggest that oxysterol deprivation determines the switch-off of LXR transcriptional activity. Accordingly, mice lacking LXRα and LXRβ (LXR$\alpha/\beta^{-/-}$) show no differences in their hepatic regenerative ability because the absence of LXRs mimics the ligand inactivation status of LXR transcriptome normally observed during liver regeneration.

In this scenario the loss of the feedback regulatory loop between cholesterol and oxysterol homeostasis drives hepatocyte regenerative capacity in order to feed cells with the cholesterol amount required to sustain liver regeneration (Lo Sasso et al., 2010a).

Cancer Models

The role of LXR in carcinogenesis (Chuu and Lin, 2010; Fukuchi et al., 2004) has been recently investigated in different tumor types such as glioblastoma, colon cancer, breast cancer, and prostate cancer.

Glioblastoma is the most common and lethal malignant brain tumor in adults. It is characterized by rapidly proliferating cancer cells with highly activated oncogenic pathways and signal transduction effectors that alter the metabolic status in order to satisfy the enhanced tumor cell requirement for cholesterol (Choe et al., 2003). A recent study by Guo and colleagues provided a new point of view on the molecular mechanisms by which cancer cells obtain cholesterol. The authors proved the existence of a phosphatidylinositide 3-kinase/SREBP-1 (PI3K/SREBP-1)-dependent tumor survival pathway, driven by a mutated and constitutively activated form of EGFR, which increases LDLR expression and provides cancer cells with the cholesterol required, bypassing cholesterol de novo biosynthesis. In this intriguing metabolic scenario, LXR is a key player in inducing, upon ligand activation, LDLR degradation via IDOL expression and determining glioblastoma cell growth arrest and cell death (Guo et al., 2011).

Tumor protective action of LXR has also been observed in a recent colon cancer study. Given the well-known role of cholesterol as a metabolic driver of cellular proliferation and the LXR role in regulating enterocyte cholesterol homeostasis, Lo Sasso and colleagues employed many in vitro and in vivo models of LXR activation in tumorigenesis. A xenograft study of colon cancer cells as well as Apc^{min} mice and familial adenomatous polyposis patients, which spontaneously develop intestinal tumors, showed protection against cancer cell growth upon LXR activation. Furthermore, microarray analysis points to LXR-driven cholesterol depletion at the basis of the antitumoral scenario (Lo Sasso et al., 2013).

Breast cancer is the most common feminine cancer and, despite the improvement in early diagnosis and treatment, is the second highest cause of cancer death. Evidence indicating hypercholesterolemia and metabolic syndrome as particularly important risk factors for breast cancer led investigators to study cholesterol involvement in breast tumorigenesis. The interesting discovery of these studies allocates oxysterols, particularly 27HC, as a selective ER modulator able to promote tumor growth. Moreover, the observation that elevated levels of CYP7B1, a cytochrome p450 monooxygenase responsible for the catabolism of 27HC, are associated with a better survival outcome supports the involvement of a regulatory role of oxysterol in breast tumorigenesis (Nelson et al., 2013). Conversely, Gong and colleagues endorse LXRs as metabolic deactivators of estrogen, given their ability to induce cholesterol conversion in bile acids and establish estrogen depletion status (Gong et al., 2007). However, this scenario is murine-specific since LXR does not activate CYP7A1 in the human liver. Similarly, cholesterol involvement in promoting prostate cancer development has been well established in recent years. The observation that LXR signaling is attenuated in prostate cancer progression from an androgen-dependent to an androgen-independent status and that the growth arrest occurred when LXR is re-activated by synthetic ligands endorse LXR signaling as a new target for controlling cancer cell proliferation (Chuu et al., 2006, 2007; Fukuchi et al., 2004).

The LXR Bona Fide Therapeutic Strategy in Cancer

Cellular cholesterol levels depend on integrated activities of synthesis, uptake, and efflux that are impaired in conditions of high-rate cell proliferation, especially tumor-related ones. Therefore, targeting cholesterol homeostasis could represent a valid therapeutic approach in cancer treatment, and several strategies that impact cell and blood cholesterol levels have been studied. A first approach in many in vitro and in vivo studies has been represented by the use of HMGCR inhibitors to block the mevalonate pathway and cholesterol de novo biosynthesis (Clendening et al., 2010; Clendening and Penn, 2012; Ginestier et al., 2012). Despite the promising results, statins seem to be ineffective as an antitumor drug because cancer cells gain selective proliferative power by enhancing LDLR-mediated uptake of exogenous cholesterol (Guo et al., 2011).

Studies on LXR activation in multiple tumor types (Table 1) have shown significant cell growth arrest and apoptotic pathway activation, endorsing the antitumoral power of LXR by modulating metabolism, microenvironment, and cell cycle.

LXR activation promotes cholesterol removal from cells by increasing the expression of membrane transporters ABCA1, ABCG5, ABCG8, and ABCG1 and inhibits exogenous uptake via LDLR by inducing IDOL-mediated LDLR degradation. Moreover, targeting LXRs may be a powerful and novel approach given their ability to regulate both cholesterol and fatty acid metabolism. Most studies in cancer biology have been focused on increased expression and activity of FAS, responsible for the de novo fatty acid synthesis. In physiological conditions, FAS activity is regulated by insulin and nutritional status via the induction of SREBP, which increases the expression of genes involved in lipid metabolism. In cancer cells, FAS seems to be sensitive to signal transduction pathways associated with a malignant phenotype (Van de Sande et al., 2005), and the hypothesis of its potential role as an oncogene (Baron et al., 2004) is supported by the need of cancer cells to supply the required energy by fatty acid oxidation (Liu, 2006). On the contrary, FAS inhibition is associated with the suppression of cell proliferation, adhesion, migration, and invasion, thus attenuating the malignant tumor phenotype (Yoshii et al., 2013). Although in cancer cells fatty acid synthesis is upregulated, and LXR activation could enhance the lipid feeding, in this review we provide evidence of LXR's ability to block cell proliferation, and we suggest the combined administration of LXR agonists and fatty acid synthase inhibitors as a potential therapeutic combination in cancer therapy. Moreover, LXR ligands could be a powerful approach in treating many tumors, given their action on both cell cycle and microenvironment. LXR activation has been reported to suppress cancer cell proliferation by disrupting key growth pathways, such as ER and EGFR, or downregulating tumor-promoting molecules such as SKP2, blocking cell-cycle progression from G1 to S phase (Candelaria et al., 2014; Vedin et al., 2009). Additionally, LXRs are able to modulate tumor microenvironment composition by enhancing the production of antimetastatic and antiangiogenic molecules such as APOE (Pencheva et al., 2014) and by activating the immune system via the action of IFN (Blanc et al., 2013; Wang et al., 2014). Future translational studies are urgently needed to identify eventual LXR-dependent side effects and to launch LXR agonism as a novel antitumor co-adjuvant strategy.

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