Review Article

LXR, prostate cancer and cholesterol: the Good, the Bad and the Ugly

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Abstract: Cholesterol is a fundamental molecule for life. Located in the cell membrane, this sterol participates to the cell signaling of growth factors. Inside the cell it can be converted in hormones such as androgens or modulate the immune response. Such important functions could not be solely dependent of external supply by diet hence de novo synthesis could occur from acetate in almost all mammalian cells. If a deficiency in cholesterol sourcing leads to development troubles, overstocking has been associated to various diseases such as atherosclerosis and cancers. Cholesterol homeostasis should thus be tightly regulated at the uptake, de novo synthesis, storage and export processes. Various transcription factors have been described these last years as important to regulate cholesterol levels. Besides, synthetic molecules have been developed for many years to modulate cholesterol synthesis, such as statins. Many articles have associated prostate cancer, whose incidence is constantly increasing, to cholesterol disequilibrium. Targeting cholesterol could thus be a new pharmacological hit to counteract the initiation, development and/or progression of prostate cancer. Among the transcription factors regulating cholesterol homeostasis, the nuclear receptors Liver X Receptors (LXRs) control cholesterol uptake and export. Targeting the LXRs offers a new field of investigation to treat cancer. This review highlights the molecular relationships among LXRs, prostate cancer and cholesterol, more than a “Ménage à trois”, The Good, the Bad and the Ugly.

Keywords: LXR, cholesterol, prostate cancer, lipid raft, pharmacological modulation

Introduction

Prostate cancer is one of the most common malignancy [1], mainly affecting elders. Various risk factors have been involved including aging, ethnic origins, hormonal status and energy balance. Among the lipids, cholesterol has a particular position. This fundamental molecule is part of the cell membrane and thus plays an architectural role in its organization by maintaining the fluidity or by securing important proteins in the membrane when located in the so-called “lipid rafts”. Cholesterol is also involved in “ligand-type” signaling: as the precursor of androgen synthesis as well as in the production of oxysterols, which activate the nuclear receptors LXR\textsubscript{α} and LXR\textsubscript{β}. Maintaining a tight regulation of cholesterol homeostasis is thus of primary importance since it could affect cell signaling and the proliferation/apoptosis balance. Reducing de novo cholesterol synthesis and/or uptake, or increasing reverse transport by exporting cholesterol from the cell could represent an efficient way to control prostate epithelial proliferation. This review is focused on the deleterious effect of a higher cholesterol (The Ugly) concentration on prostate cancer (the Bad) and the role of LXRs (The Good) in maintaining cholesterol homeostasis to avoid progression of prostate cancer (Figure 1). The Saga started in 1909 and is still going on.

LXRs and cholesterol: when the Good controls the Ugly

The liver X receptors

Liver X Receptors (LXRs) are transcription factors initially isolated in the liver [2, 3], and acti-
vated by cholesterol derivatives, the oxysterols [4]. LXRα (NR1H3) and LXRβ (NR1H2) share 80% identity both in their DNA- and ligand-binding domains. Their structure is characteristic of the nuclear receptor superfamily, which possesses three functionally independent domains [5, 6]. The N-terminal modulator domain contains an activating function of the transcription (AF1) independent from the presence of the ligand. This domain presents several putative sites of phosphorylation potentially important for LXR activity modulations [7, 8]. The DNA-binding domain recognizes the LXR response elements (LXRE) characterized by two direct repeats of the hexanucleotide motif AGGTCA usually separated by four nucleotides. Part of this domain is also involved in the heterodimerization with the Retinoid X Receptor RXR (NR2B1-3), which binds 9-cis retinoic acid, the requisite LXR partner [3]. The carboxy-terminal region is responsible for the ligand-binding and contains the AF2 region necessary for the transcriptional initiation of target genes [4]. This domain is masked by co-repressors in absence of ligand. For a review on LXR-functioning, see Viennois et al. 2011 [9].

LXRα and LXRβ are differentially expressed in tissues. While LXRβ expression is accepted to be rather ubiquitous, LXRα is more restricted and mainly found in liver, intestine, fat tissue, macrophages, kidney and gonads, suggesting their important function in the control of cholesterol homeostasis (for a view on LXR expression see www.nursa.org). The fundamental role of LXRs in lipid homeostasis is highlighted by the highly conserved function of these receptors among species [10], and has been continuously demonstrated since the first observation of a link between LXRα and cholesterol homeostasis by Peet et al. [11]. They observed that mice lacking LXRα and fed a high cholesterol diet rapidly accumulate large amount of cholesterol ester in the liver inducing a liver steatosis. Actually these mice are unable to sense and respond to dietary cholesterol and develop an impaired bile acid metabolism due to a default in the transcription of the cholesterol 7α-hydroxylase (Cyp7a1), encoding an enzyme essential in bile acid synthesis [11].

The discovery of the natural ligands of LXRs by Janowski et al. [4, 12], largely improved our comprehension of the unique role of LXRs in controlling cholesterol homeostasis. In these studies, oxysterols, the natural derivatives of cholesterol, activated LXR at physiological concentrations. Following this finding the development of synthetic ligands of LXRs (e.g. T0901317 [13] and GW3965 [14]) and the generation of a mouse model lacking Lrα and/or β, greatly contributed to the comprehension of the oxysterol/LXR dependent pathways in cells, and gave the opportunity to identify several target genes and therefore functions of the LXRs [9]. Thus, it has been admitted that LXR activities are associated with four schematic functions: 1) lipid metabolism, including cholesterol and fatty acids homeostasis; 2) steroidogenesis; 3) glucose homeostasis; 4) inflammation and immunity. Since in this review we will focus more specifically on the role of LXRs on cholesterol homeostasis, we will not develop further their other physiological functions. For more information about them, refer to Viennois et al. 2011 [9].

LXRs: two sensors of cholesterol homeostasis

Cholesterol is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. In addition cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. Besides, this molecule is also part of the membrane signaling pathway by its specific distribution in lipid rafts (see above). Furthermore, cholesterol also functions in intracellular transport, cell signaling and nerve conduction. Hence, although cholesterol is important and necessary for human health, its intra- and extra-cellular concentrations have to be strictly controlled as high levels of cholesterol in the blood have been linked to damages to arteries and cardiovascular diseases.

Modulation of de novo synthesis and uptake of cholesterol: LXRs act at various levels to control the intracellular pool of cholesterol. The first possible source of cholesterol results from the enzymatic reaction leading to the transformation of Acetyl-CoA in mevalonate by the HMG-CoA reductase [15]. That reaction ultimately leads to the formation of de novo cholesterol. In mice lacking Lxr, higher expression of Srebp2, Hmgcoa and Squalene synthase has been observed [16], while the oral treatment of wild type mice with T0901317 led to a decrease in Hmgcoa synthase and Squalene synthase.
gene expression [17], suggesting a role of LXRs in the negative modulation of de novo cholesterol synthesis.

A second way to modulate the pool of intracellular cholesterol regards its cellular import via the LDL-receptor (LDLR). Even though a correlation was repeatedly observed between LXR activation and LDLR protein reduction, the mechanism has been described only recently. LXRs activate the expression of the E3 ubiquitin ligase Idol (Inducible Degradation of the LDLR), ultimately leading to the targeted degradation of LDLR, thus resulting in the reduction of the intracellular pool of cholesterol [18].

**Induction of bile acid synthesis:** Cyp7a1 is the first and rate limiting enzyme that catalyzes the initial step of bile acid biosynthesis from cholesterol. Although it is not the primary function of bile acid synthesis, this reaction also allows the liver to reduce in rodent the excess of cholesterol in cells. Interestingly, while in wild type mice fed a high cholesterol diet Cyp7a1 expression increases, this induction is not observable in Lxrα-deficient mice fed similarly [11]. Additionally, in these mice the diet induces a hepatic steatosis due to an accumulation of cholesteryl esters in the liver [11, 16].

**Induction of reverse cholesterol transport:** The last way LXRs use to control cholesterol levels is by exporting it outside the cells. Indeed, several ATP-binding cassettes encoding genes such as ABCA1 [19-21] and ABCG1 [22] are LXR bona fide targets. These ABC transporters actively efflux cholesterol to the extracellular acceptor HDL and increase the reverse cholesterol transport. In addition, LXRs have also been shown to modulate Apolipoprotein E level, an essential component of the VLDL particles [23]. Furthermore, LXRs modulate the expression of the genes encoding ABCG5 and ABCG8 that export sterols from the inner compartment of hepatocytes to the bile duct [24, 25] and from the enterocytes into the gut lumen [26].
 Altogether LXRs demonstrate a critical role in controlling the amount of intracellular cholesterol and in its processing outside the cells.

Steroid synthesis: We and others have shown that LXRs could regulate the rate of cholesterol transformation into steroids in various tissues such as testis [27]. A decrease in the amount of circulating testosterone can be detected after LXR activation by the synthetic agonist T0901317 [28]. That well identified mechanism is dependent on the activation by LXRs of Sulfotransferase 2a1 that deactivates androgens, and the inhibition by LXRs of the steroid-sulfatase that activates androgens [28]. Interestingly, those hormones have a key role in prostate cancer development. LXRs might thus have also a role to play in this part of the anti-cancer journey.

Cholesterol and prostate cancer: when the Ugly plays with the Bad

Due to its different roles, cholesterol is hence linked to cell proliferation (see above). Indeed, its synthesis increases in tissue with high proliferation rate such as in cancer. On the other side, inhibition of HMGCoA-reductase blocks cell growth [29].

Prostate cancer: the Bad at a glance

Prostate cancer (PCa) is the second most diagnosed cancer and a leading cause of cancer related death [1]. The incidence of PCa is constantly increasing due in part to new methods of diagnostic, and also to the increase in life expectancy. Indeed, this cancer has a slow evolution and about 85% of diagnosed PCa are in patients older than 65 years old [30]. Interestingly, it is accepted that more men die with PCa than from it. Indeed, an American study performed after autopsy determined that 50% of the men of 50 years old have latent PCa [31]. However the development and the cause of the disease is still poorly understood, and various factors such as genetic/ethnic origin, diet, life style and environmental factors have been suggested to play a role on it [32].

As already stated, great differences in the incidence of PCa are observed depending on the ethnical origin or the country of the patients. A Caucasian American has 30% less risk to develop a PCa compared to an African American [30], but at the same time Asians develop twice less PCa than Americans. These differences are in part due to the ethnical factors, and thus to the genetic background and the lifestyle of the individuals. However it could also show disparities in the accessibility of the diagnostic tests and treatments.

Yet, the genetic background cannot explain everything since the first generation of Asian migrants living in the US have a more important risk of PCa than those leaving in Asia [33]. This unexpected observation is credited to be due to factors acting on PCa development rather than on PCa initiation, and presumably on the higher lipid consumption in the USA [34]. Additionally a comparable observation has been done with increased incidence to develop a PCa for Japanese population that moved to America [35]. In this study the authors also compared the migrants according to their age at arrival, and did not find any correlation with the risk to develop PCa. They therefore concluded that PCa risk may be increased by later rather than early life style event [35]. These two studies are therefore suggesting a potential lifestyle/diet parameter that can greatly influence the development of PCa.

Role of cholesterol in prostate cancer: the Ugly goes with the Bad!

Cholesterol accumulation in tumors is not a recent observation. White demonstrated in 1909 an accumulation of crystals of lipid nature in tumors [36]. Later Swyer and his coworkers showed for the first time an increase of cholesterol content in zone of the prostate affected by a mild hypertrophy [37] compared to healthy tissues. Afterward, similar observations were obtained on other types of cancer [38-40]. Two mechanisms are generally put forward to explain this intracellular cholesterol accumulation: a higher circulating cholesterol uptake, and the increase in the accumulation of the enzymes of the mevalonate pathway [41, 42].

Moreover, increased uptake of LDL particles and therefore exogenous cholesterol attributable to a loss of modulation in the LDL receptor expression, and a higher de novo cholesterol synthesis due to the upregulation of the HMG-CoA reductase, have been suggested as key components of that accumulation [17, 43]. The
final result of that process could potentially give sufficient bricks for the membrane to expand and to the tumor to grow and develop [44].

Diet, cholesterol and PCa

Since the late 90’s, multiple lines of evidence have been highlighting the potential influence of diet on PCa appearance. First, intake of products from animal origin is correlated to a higher risk of developing metastatic PCa, but not on the initial development of PCa [45] as shown by the identical prevalence between vegetarians or meat eaters [46]. Second, the presence of dietary fat in the diet was shown to be a risk factor of PCa, although the exact contribution of fat was not clearly established [47]. Third, an increase in PCa incidence, angiogenesis and metastasis was observed in the TRAMP mouse model of PCa fed a western-type diet [48]. Finally aggressiveness of PCa was increased in elders having important dietary fat intake [49]. So far data linking excessive consumption of cholesterol, rates of circulating cholesterol and risk of PCa have been controversial [50], even though studies suggest an impact of cholesterol in the development of high grade PCa [51-53]. Inversely, Platz et al. pointed out that a “weak” level of circulating cholesterol (< 200mg/dL) was associated with a reduction of the risk of developing a prostate cancer of high grade [54]. Finally, circulating cholesterol increases tumor size of LNCaP xenografts in a mouse model, as well as intratumoral synthesis of androgens [55]. This suggests that the androgen dependent tumor growth could be under a deep association with circulating cholesterol. Likewise, high serum HDL is inversely correlated with PCa [53, 56]. Since HDL formation is dependent on the export of cholesterol via the ABCA1 and ABCG1 transporters that are under the positive modulation of LXRs, it could be suggested a possible beneficial role to over activating LXRs in PCa, even though this needs to be demonstrated.

Modulation of circulating cholesterol and PCa: when reasoning the Ugly can block the Bad

Altogether the presented data raise the question of the molecular mechanisms by which the cholesterol can favor tumor progression. Some observations on cancer development after treatment with statin, a cholesterol-lowering drug that specifically inhibits the HMG-CoA reductase and therefore the formation of de novo cholesterol, partially answer that question. Indeed early investigations suggested a potent growth inhibitory effect as well as an anticancer potential of statins in vitro and in vivo [57, 58], partially explained by their ability of inducing apoptosis via the activation of the Caspase-7 [59]. Moreover in the PC3 prostate cancer cell line, statins also prevent the cell migration potential therefore reducing the formation of metastatic prostate colonies [60]. Then it seems that these cholesterol-lowering agents can act at different level on PCa progression. The potential use of statins to prevent PCa is currently under active investigation mostly on prospective studies. Until now, numbers of studies have been published and extensively reviewed [61]. Statin treatments do not seem to have any beneficial effect on the rate of appearance of prostate cancer conversely to the incidence of advanced PCa [62-64]. Interestingly this effect even increases when statins are used for more than five years [65].

Androgen synthesis is dependent on the amount of circulating cholesterol; besides, PCa is linked to androgen synthesis; moreover statins are cholesterol-lowering drugs. Altogether what could be the potential impact of statins on the hormonal status in prostate cancer? Actually, statins do not seem to decrease the circulating androgen [66], even though a decreased synthesis of androgens cannot be excluded since statins users show a decline in serum PSA levels, an androgen regulated gene in prostate [67].

Cholesterol is not only used as a precursor of steroid synthesis. Indeed, it can be found enriched in cell membranes in regions called rafts essential for the activation of the kinase cascade Akt and consequently for tumor survival [68]. Zhuang et al. showed that simvastatin decreases the cholesterol content of lipid rafts, leading to a decrease in Akt phosphorylation and activation, and subsequently to an increase of LNCaP cells apoptosis [69]. These results improve our comprehension of the mechanism of statins in cancer progression, and also suggest lipid rafts as new players in PCa development. In accordance with that suggestion, the essential component of lipid rafts caveolin 1 is associated with the aggressive-
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ness of the PCa tumor and therefore considered as a marker of poor prognosis in PCa [70, 71]. Accordingly, the use of an antibody targeting the caveolin 1 can block the metastatic process in PCa [72]. Both observations then confirm the important role of lipid rafts in PCa progression.

**LXRs and prostate cancer: a benefic effect of the Good over the Bad?**

**LXR activation leads to cell cycle arrest in prostate cancer cell lines**

Since LXRs control cholesterol homeostasis, these nuclear receptors have been considered as putative pharmacological targets in prostate cancer. Hence, activation of LXRs by natural (22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol) or synthetic (T0901317) agonists led to cycle arrest of LNCaP cells via the lack of degradation of p27Kip1, an essential inhibitor of the cell cycle. Moreover, and as expected, treatment with LXR agonists also induced the protein accumulation of ABCA1, thus activating cholesterol efflux [73]. Conversely, targeted disruption of ABCA1 increases the proliferation rate of LNCaP cells [74]. Moreover, Chuu et al. observed that LXR-target genes were down-modulated during the tumor progression in mouse, while activation of LXRs by T0901317 delayed the progression of PCa [75]. Altogether, these studies are clearly in favor of an important protective role of LXRs in prostate cancer progression, even if no data are available in human yet.

**How could LXRs be so good?**

As presented above, activating LXRs will lead to the modulation of cholesterol concentration by their action on the various pathway involved.

**LXRs antagonize the development of prostate tumor by interacting with the androgen pathway:** Prostate cancer development is tightly associated with androgens. Indeed, it is frequent to treat PCa patients with anti-androgens in order to block the androgen response, and therefore the early development of PCa [76]. Interestingly, the androgen receptor (AR) modulates the expression of HMG-CoA synthase and reductase, and SREBP2, whose product controls genes involved in cholesterol homeostasis such as the LDL-Receptor (LDLR) [77]. The consequences of these modulations are: 1) an increase in intracellular cholesterol due to a higher de novo production and uptake via LDLR; 2) an increase in androgen synthesis from cholesterol. This may give an alternative explanation to the prostatic tumor growth dependence to cholesterol. Additionally, AR reduces LXR activation in prostate cancer, by competing for their coactivators [78].

There is also a mirror effect as LXRs reduce the proliferation of androgen-dependent cells via androgen deprivation [28], and inhibit tumor growth and slow down the passage of androgen dependent to androgen independent prostate cancer [75]. Furthermore T0901317 has also been suggested to act as an AR antagonist, even though the Kd found is highly questionable [79].

**LXRs block cancer development through their transcriptional activity:** Controlling the expression of key genes of cholesterol homeostasis is of primary importance to block cancer progression. Modulation of IDOL by LXRs [18] should decrease the amount of LDLR on the cell surface and then LDLR uptake. Moreover as described previously, LXRs also modulate ABCA1 and ABCG1 two transporters responsible for the export of endogenous cholesterol. Associated to the crucial role of cholesterol on prostate cancer development, LXR activation thus reduces the potential pathogenicity of over accumulation of cholesterol, and therefore may limit the development of PCa.

**LXRs induce apoptosis of prostate cancer cell line through lipid raft signaling:** Activation of LXRs is associated with an important decrease of phosphorylation of Akt, a key player in the mechanism of cell survival, at the level of lipid rafts [68]. When LXRs are liganded, the pool of cholesterol is decreased in prostate cancer cells in parallel with lipid raft size and number. The consequences are a decrease in activated Akt and the induction of cells apoptosis [80, 81]. Then the effect of LXR activation on lipid rafts and PCa cells is very similar to that observed after statin treatment, thus highlighting LXRs as a potential therapeutic target in PCa.

**LXRs down-modulate inflammatory molecules correlated with cancer:** Cancers are often associated with increased inflammation inside and
surrounding the tumor [82]. This phenomenon is also characteristic of PCa, since a strong iNOS accumulation is found in tumor compared to peripheral tissue in PCa [83, 84] and is associated with high Gleason score [83, 85]. Associated to iNOS, COX-2, another pro-inflammatory enzyme, is highly expressed in tumor associated macrophages [86]. In the mouse model of prostate cancer TRAMP, Cox-2 expression increases with progression of carcinogenesis and the use of a COX-2 inhibitor increases the survival of mice with prostate cancer [87]. Additionally, IL-6, which promotes tumor growth [88], and activates the PI-3K/Akt signal transduction pathway [89], is highly expressed and associated with morbidity in PCa [90-92]. These observations are of particular interest since LXRs are known to down-modulate the accumulation of inflammatory molecules as iNOS, COX-2 and IL-6 [93], another argument making them good targets in PCa.

Pharmacologically targeting LXRs in PCa: Are the Good always trustable?

Considering the various beneficial effect of LXR activation on PCa ex vivo and in mouse models, using LXR modulators to treat patients seems very promising [9, 94]. Unfortunately, most of the LXR agonists also have a consistent deleterious effect since they lead to transient hypertriglyceridemia ([95] and reviewed in [96]). The screening of new LXR-ligands is currently under active investigations to limit this inconvenience [97]. The first tissue specific LXR ligand identified has been the (22E)-ergost-22-ene-1α,2βdiol (YT-32). In mice, oral gavage with YT-32 decreased the amount of cholesterol present in the plasma and led to the intestinal accumulation of the LXR-target genes ABCA1, ABCG5 and ABCG8 without any modification of the expression of these genes in the liver [98]. More recently another intestine specific ligand of LXRs, GW6340, leads to LXR-target gene accumulation in the small intestine without increasing neither the liver triglycerides content or the hepatic LXR target genes expression [99]. Besides this side effect due to a hepatic activity of LXRs on the triglyceride synthesis, the fact that LXRs are also highly expressed in many tissues has to be taken into account. Hence, LXR-623 agonist was tested in healthy volunteers [100]. The authors showed a higher accumulation of the expression of ABCA1 and ABCG1 in blood cells in parallel with lowering the LDL and cholesterol levels in the serum [101]. Unfortunately, an important adverse effect on the central nervous system was observed, which ended the trial [100]. As for the other nuclear receptors targeted in breast (ER) and prostate (AR) cancers, the development of selective Liver X modulators (SLiM) [96] could be a very promising treatment option in numbers of different pathology where cholesterol is involved, including in cancer. Considering the potential important role of LXRs and cholesterol in prostate cancer, the use of SLiM may slow down the evolution into high grade PCa, although more investigations will be necessary.

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Declaration of conflicts of interest

The authors have nothing to declare.

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