RESEARCH HIGHLIGHT

Transcriptional and non-transcriptional roles of LXRs in cancer cells

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Liver X Receptors (LXRs) were proposed to have anticancer properties. LXRs affect cancer cell proliferation and cell death through mechanisms that seems mostly to rely on their transcriptional activities. We recently identified a new non-genomic role of LXRβ in colon cancer cells. Under LXR agonist treatment, LXRβ induces an atypical cell death called pyroptosis *in vitro* and *in vivo*. Together with other reports, we raise the importance of targeting LXRs in cancer treatment.

Keywords: LXR; Cancer; Proliferation; Cell death

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Liver X Receptor (LXR) α (NRH1H3) and β (NR1H2) belong to the nuclear receptor family. Their activation by natural ligands, such as oxysterols, or by synthetic agonists, such as T0901317 or GW3965, increases the expression of target genes implicated in lipid metabolism and notably in cholesterol efflux (e.g. *ABCA1* (ATP-binding cassette transporter A1) and *ABCG1* (ATP-binding cassette transporter G1)) or fatty acid synthesis (e. g. *FAS* (fatty acid synthase) or *SREBF1* (sterol regulatory element-binding transcription factor 1)) [1].

Despite these roles, LXRs were also described to be implicated in cancer incidence. Thus, LXRβ deficient mice develop preneoplastic lesions of the gallbladder which evolved to cancer in old animals, and high-cholesterol fed LXRα/β deficient mice develop prostatic intra-epithelial neoplasia [2, 3]. Moreover, it has been shown that LXRs are expressed in different cancer cell types, e. g. prostate, breast, ovarian, colon, liver, glioblastoma, melanoma, myeloma, osteosarcoma and pancreatic cancers. Their activation under agonist treatment allows the control of cancer cell proliferation and to a lesser extent induces cell death, *in vitro* and *in vivo* (Table 1).

All these reports described mechanisms that seem to involve only the transcriptional activity of LXRs. However even if LXRs activation leads frequently to the modulation of the expression of cell cycle regulators (e.g. SKP2 (S-phase Kinase-associated Protein 2), p21, p27 or cyclins), the direct transcription/repression activity of LXRs was not always clearly described [4-7]. The influence of LXRs on SKP2 expression was effectively depicted by Blaschke et al. by showing the ability of T0901317 and GW3965 to decrease PDGF (platelet-derived growth factor) activation of SKP2 promoter [8]. Then, the decreased expression of SKP2 can account for p27 and p21 stabilization, regulation of cyclin expression and cell cycle disruption [9]. Moreover the direct control of LXRα on the FOXO1 (Forkhead box O1) expression can also explain the ability of LXR agonists to control p27 and p21 expression, as FOXO1 has been

Table 1. Mechanisms involved in LXR ligand effects on proliferation and cell death

Organ	Cell lines	LXR isoform	Mechanism	Transcription (T) /Repression (R)	Proliferation (P) / Cell Death (CD)	Ref.
prostate	LnCap	ND	↘ Socs3	ND	P	[24]
	LnCap	LXRα	↘ skp2, ↔ p27, G1 arrest	R	P	[5]
	LnCap	ND	AR antagonism	R	P	[25]
	LnCap	LXRα and β	↗ abcg1, lipid raft rupture and ↘ Akt, caspase-3 activation	T	CD	[14]
Ductal adeno.	BxPC-3, MiaPaca-2, PANC-1	LXRβ	↘ skp2, pErk	R	P	[4]
Breast	MCF-7, T47D	ND	↘ skp2, cyclin A2 and D1 and ↗ p53	T / R	P	[6]
	MCF-7, T47D	ND	↘ pRb, E2F2	ND	P	[26]
	MCF-7	ND	↘ bcl-2, ↗ Bax, abca1, cholesterol efflux	T	P	[27]
Ovary	SKOV3, CAOV3, OVCAR3	ND	↘ cardiotropin-1, reverse LDLox promitogen effects	ND	P	[28]
GB	U87	ND	↗ IDOL, LDLR degradation	T	CD	[13]
Myeloma	KMS12, NCI-H929, U206	ND	Inhibition of Hedgehog pathway	R	P	[29]
Liver	HepG2, Hep3B	LXRα	↘ FOXM1	R	P	[11]
Osteo sarcoma	U2OS, Saos-2	LXRα	↔ p27, p21, ↗ FOXO1	T	P	[30]
Melanoma	B16F10, MeWo, HT144, SK-Mel-2	LXRβ	↗ApoE	T	P	[31]
	B16F10	LXRβ	Caspase-3 activation	ND	P / CD	[16]
Colon	HCT116	LXRα and β	Suppression of β-catenin signaling (MMP7, Myc)	R	P	[12]
	HCT116, Colo205	LXRβ	↘ cyclin B1 and E, ↘ pRb	ND	P	[17]
	HT29, Caco-2, HCT116, LS174T	LXRα and β	↘ skp2, cyclin D1 and ↗ p21	T	P / CD	[7]
	HCT116, HT29, HCT8, SW480	LXRβ	Interaction of LXRβ with pannexin 1, activation of purinergic pathway and caspase-1	No effect	CD	[15]

described to transcriptionnaly regulate the expression of these cell cycle regulators [10]. The induction of p53 expression and activation was also proposed to be responsible for the effects of LXRs on cell cycle [6]. Finally LXRs can also control cellular oncogene pathways such as FOXM1 (Forkhead box M1) by a direct repression of its expression or such as β-catenin, by disrupting the expression of its target genes (MMP7 (matrix metallopeptidase 7) or Myc) [11,12].

On the other hand, few reports described that LXRs can also mediate cancer cell death. These effects can be due to a

transcriptional activation of LXR target genes implicated in lipid metabolism. For example the induction of ABCG1 expression will entail membrane lipid raft disruption, inhibition of serine/threonine protein kinase Akt activity and caspase activation in prostate cancer cells and the induction of IDOL (Inducible degrader of the LDLR (Low Density Lipoprotein Receptor)) expression will drive LDLR degradation in glioblastoma cells [13,14]. In our study, we clearly demonstrate that LXR agonists can induce colon cancer cell death independently of any transcriptional activity. In particular the first mechanistic events that induce cell death occur within the first minutes of treatment while LXR target gene (i.e. *ABCA1* or *SREBF1*) expression was increased only from 24 hours in these cells. In addition the transcription inhibitor actinomycin D had no effect on LXR-dependent cell death [15].

How to explain these discrepancies between the effects of LXR ligands on proliferation vs cell death and on the type of cell death induced, i.e. apoptosis versus pyroptosis?

First, the cell death pathway engaged or not by LXR agonists can be due to the agonist (GW3965 or T0901317), the time and/or the concentration used in the experiments. The studies that described LXR-mediated cell death used a range of LXR agonist (T0901317 or GW3965) from 0 to 10µM. The experiments performed on glioblastoma, prostate or colon cancer cells, described DNA degradation (TUNEL (Terminal deoxynucleotidyltransferase dUTP Nick End Labeling) positive cells and/or cells in subG1) associated either with caspase activation or membrane permeabilization, which respectively account for apoptosis or necrosis [7, 13, 14]. On the other hand T0901317 was shown to induce apoptosis of melanoma cells as assessed by annexin V staining and caspase-3 activation [16]. In our experiments, we worked with 20µM of T0901317 or 30µM of GW3965. We have shown that LXR agonist-induced features are characteristics of pyroptosis, i.e. ATP release, caspase-1 activation (and to a lesser extent late caspase-7 activation), chromatin fragmentation, cell swelling until becoming a balloon-shaped vesicle around the nucleus and membrane permeabilization. Moreover we did not observe any caspase-3, -8 or -9 activation in our settings [15]. Maybe the higher concentrations of LXR ligands used, can explain some of the differences observed with other studies. However, we also observed pyroptosis stigmata *in vivo*, suggesting that even if our *in vitro* concentrations can be estimated to be too high, despite everything we have demonstrated that those effects can also be retrieved *in vivo*. We noticed some discrepancies between our results and other studies, published on colon cancer cells. These works mainly focused on the effects of LXR agonists on cancer cell proliferation and showed an effect on the β-catenin pathway or on cell cycle regulator expression (cyclins, SKP2, p21) [7, 12, 17]. Even if these effects on cell cycle regulators are observed in two independent studies, the first one described the involvement of only LXRβ, whereas the second one showed the necessity of both LXRα and LXRβ [7, 17]. To our part, we never looked at the effects on cell proliferation. The only study that dealt with the effects of LXR ligands on colon cancer cell death, showed an effect on caspase-dependent apoptosis. However most of the experiments were performed on HCT116 infected with AdVP16LXRα [7]. We also noticed little effects of LXR agonists on apoptosis, but in our hands the majority of cell death induced by these ligands, involve a cell membrane permeabilization and caspase-1 activation [15].

Second, the cell type and the expression/localization of LXRα or LXRβ can dictate the incidence of LXR agonist treatment. LXRs were previously described to be localized in the nucleus of HEK293 cells overexpressing fluorescent-tagged LXRα or LXRβ, in an NLS (Nuclear Localization Signals)-dependent manner [18]. To our knowledge, we were among the first ones to show that LXRβ can have a non-nuclear localization in cancer cells. Recently, Candelaria *et al*., showed that LXRβ is localized both in the cytosol and the nuclei of neoplastic cells from patients with pancreatic adenocarcinoma [4]. In our study, we showed that LXRβ has no nuclear localization in colon cancer cells but rather a cytoplasmic and to a lesser extent a plasma membrane localization. Under agonist treatment, LXRβ associates with pannexin 1 (within the first minutes) and more particularly with the intracellular C-terminal domain of this membrane channel to induce ATP release [15].

Non-genomic roles of LXRβ were previously described in non-tumoral cells. First, LXRβ interaction with Syk (Spleen tyrosine kinase), PLC (Phospholipase C)-γ2 and PPAR (Peroxisome Proliferator-Activated Receptor)-γ, regulate platelet functions [19]. Secondly, LXRβ is associated with ABCA1 and ABCA12 (ATP-Binding Cassette transporter A12) in myeloid cells, at the plasma membrane level. Under LXR ligand treatment, LXRβ 1) dissociates from ABCA1-ABCA12 complex to raise cholesterol efflux and 2) translocates to the nucleus to play its transcriptional role [20, 21]. Finally, in endothelial cells, LXRβ was also shown to interact with ERα in lipid raft domains to promote cell migration under agonist treatment [22]. In our setting, the cytoplasmic localization of LXRβ can explain its capacity to interact with the intra-cellular domain of pannexin 1 to allow ATP release. Extracellular ATP activates the P2RX7 (purinergic receptor 2X, ligand-gated ion channel, 7) pathway, leading to NLRP3 (Nod-Like-Receptor Pyrin domain containing 3) /ASC (Apoptosis associated Speck-like protein containing a Caspase activation recruitment domain) -dependent caspase-1 activation and pyroptosis of colon

cancer cells [15].

We have shown *in vivo* that an LXR agonist can reduce tumor growth by inducing caspase-1 activation, specifically in tumor cells in an LXRβ, pannexin 1 and NLRP3-dependent manner. Pyroptotic cells can release “find-me” and “eat-me” signals, such as ATP that will recruit macrophages and ameliorate the phagocytosis of dead cells and probably the priming of adaptive immune cells. Moreover caspase-1 activation can also lead to the maturation of inflammatory cytokines such as IL-1β or IL-18 [23]. Further investigations will be necessary to explore the interaction between pyroptosis of colon cancer cells and the tumor microenvironment such as immune cells, stromal cells or endothelial cells. The understanding of these relationships will help to develop tools to ameliorate LXR ligand anti-cancer activities.

Conflicts of interest

The authors declare that there is no conflict of interest.

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