LXRβ subcellular localization: A new tool to investigate cancer cell response to LXR ligand-induced cytotoxicity?

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Liver X Receptors (LXRs) and their ligands are known for their potential anticancer properties. Recently, our team underlined for the first time that these ligands induce colon cancer cell death through the activation of the inflammasome pathway in an LXRβ-dependent manner. Moreover, a truncated form of the Retinoid X Receptor α (RXRα), t-RXRα, produced only in cancer cells and not in normal colon epithelial cells, interacts with LXRβ to maintain it in the cytoplasm. This specific localization of LXRβ in colon cancer cells dictates their sensitivity towards LXR ligand cytotoxicity whereas its nuclear localization in normal colon epithelial cells prevents it. Our results highlight LXRβ subcellular localization as a promising marker for LXR ligand efficacy in colon cancer treatment.

Keywords: LXRβ; RXRα; colon cancer; epithelial cells; subcellular localization


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The nuclear receptor superfamily is composed of transcription factors that modulate the expression of target genes through transactivation and transrepression mechanisms. However they have been described to have genomic and non-genomic functions [1]. For example, Nur77 (or NR4A1) has been described to modulate several pathways in a transcriptional-independent manner, especially in cancer cells. While in normal cells, Nur77 disrupts β-catenin activity, its phosphorylated form avoids its inhibition on the β-catenin pathway in most clinical colorectal cancers [2]. Moreover RXR (Retinoid X Receptor) is able to translocate Nur77 into the cytoplasm under RXR ligands or apoptotic stimuli leading to apoptosis [3].

We made a similar observation for LXRs and more particularly for LXRβ [4]. In our colon cancer cell line models, cell death was induced without any LXRβ transcriptional activity. Indeed, LXR agonist treatment led to an ATP release within the first minutes through pannexin 1 channel. Then, locally secreted ATP molecules bind to their receptor P2RX7 and trigger the NLRP3 (Nod-Like-Receptor Pyrin domain containing 3) inflammasome-mediated caspase-1 activation and subsequent pyroptotic cell death [4]. All these events were achievable thanks to the cytoplasmic and sub-membrane localization of LXRβ in colon cancer cells. This enables the interaction of LXRβ with the intra-cellular C-terminal domain of pannexin 1 and the
opening of this channel to release ATP in the extracellular space \[4, 5\]. Like for Nur77, LXRβ is maintained in the cytoplasm of colon cancer cells through RXR. More precisely, we showed that the cytoplasmic sequestration of LXRβ was mediated by the truncated form of RXRα, i.e. t-RXRα (Figure 1) \[6\]. Previous works identified this calpain II-dependent cleaved form of RXRα for being responsible for cancer cell proliferation in vitro as well as in vivo through Akt phosphorylation pathway \[7, 8\]. In accordance with Zhou et al. who described the presence of t-RXRα in breast and liver tumors but not in the surrounding normal tissues \[9\], we showed that t-RXRα is expressed only in colon cancer samples from patients relative to the healthy epithelial cells counterpart. Cancer cells with high expression of t-RXRα present a highly cytoplasmic LXRβ localization and a better response to LXR ligand-induced cell death. On the contrary, human healthy epithelial colon cells, without t-RXRα were resistant to LXR ligands (Figure 1) \[6\].

In overexpressing tagged LXRα or LXRβ models, previous studies reported that LXRα is prone to be localized in the nucleus in an NLS (Nuclear Localization Signal)-dependent manner \[9, 10\]. However other reports described a cytoplasmic localization for LXRα. This cytoplasmic localization of LXRβ accounts for a non-genomic role of this receptor. This was strengthened by the observation that LXRβ interacts with Syk (Spleen tyrosine kinase), PLC (Phospholipase C)-γ2 and PPAR ( Peroxisome Proliferator-Activated Receptor)-γ and regulates platelet functions, a cell type lacking nuclei \[11\].

In nucleated cells, this localization can be induced by the microenvironment of the cells and/or depends on the cell type itself.

First the microenvironment can be composed of different amounts of LXR ligands such as glucose or oxysterols. Glucose modulates LXRα localization in pancreatic β cells. In low glucose (4,2 mM) concentration culturing conditions, LXRα is cytoplasmic while its localization is nuclear in higher glucose (8mM) concentration conditions \[12\]. In mammals, oxysterols are available either exogenously by daily food intake or endogenously by enzymatic synthesis \[13\]. Several studies showed an increase in oxysterols in cancer tissue. This can be due to a decrease in SULT2B1B, which is the enzyme responsible for oxysterol elimination by sulfurylation, in prostate cancer cells compared to healthy adjacent tissue \[14\]. Moreover, in patients suffering from
breast cancer, oxysterols were shown to be highly produced compared to normal breast tissue \[^{15}\]. Similar results were obtained \textit{in vitro} on colon cancer cell lines compared to fibroblasts \[^{16}\]. In these studies, the subcellular localization of LXR\(\beta\) was not investigated and we cannot know whether endogenous oxysterol levels in tumor cells were correlated with a particular LXR\(\beta\) localization.

Secondly, without excluding a role of the microenvironment, different subcellular localizations of LXR\(\beta\) were described in different cell types. Thus, LXR\(\beta\) was shown to be nuclear in progenitor endothelial cells whereas it is localized in plasma membrane lipid raft microdomains in endothelial cells \[^{17, 18}\]. The cytoplasmic localization of LXR\(\beta\) in endothelial cells may be explained by the expression of ALK-1 (Activin receptor-Like Kinase-1, a TGF-\(\beta\) receptor essential for endothelial cell function), specifically in these cells. When interacting together, ALK-1 phosphorylates LXR\(\beta\) and then in turn LXR\(\beta\) inhibits the transcriptional response of ALK-1 \[^{19}\]. However, under agonist treatment, LXR\(\beta\) interacts with ER\(\alpha\) in lipid raft microdomains to promote cell migration \[^{17}\]. Another example is the cytoplasmic localization of LXRs (without distinction between the two isoforms) in all layers of the epidermis. In this study, an LXR agonist inhibits keratinocyte and sebocyte cell proliferation \[^{20}\]. LXR\(\beta\) also interacts with ABCA1 and ABCA12 (ATP-Binding Cassette transporter A12) in myeloid cells at the plasma membrane. Upon LXR agonist stimulation, LXR\(\beta\) is released from ABC transporters complex to enhance cholesterol efflux and moves to the nucleus to act as its well characterized role, a transcription factor \[^{21-23}\]. In cancer cells, other studies described a cytoplasmic localization of LXR\(\beta\). In thyroid malignant tissues, LXR\(\beta\) is highly expressed and localized in the cytoplasm. However, there is no explanation about the importance of this high cytoplasmic expression in this context \[^{24}\]. Another cancer model, where LXR\(\beta\) is localized in the cytoplasm is pancreatic ductal adenocarcinoma. In human samples a nuclear staining of LXR\(\beta\) was shown in normal pancreatic ducts whereas a nucleo/cytoplasmic staining was observed in cancerous samples. In pancreatic cancer cell lines, LXR agonists exerted anti-proliferative effects through LXR\(\beta\)-dependent pathway \[^{25}\]. However in these examples of cytoplasmic localizations of LXR\(\beta\), no explanation of the mechanism responsible for this localization was addressed. In our study on colon cancer cells, we investigated some leads to explain this localization. First, genetic degradation of master genes including cell proliferation, cell death or metastasis are classical hallmarks of cancer pathogenesis. A recent study showed that two LXR\(\beta\) genotypes (rs2695121 - TC/CC) and (rs35463555 - GA/AA) were correlated with a higher risk of gallbladder cancer development in human \[^{26}\]. These DNA modifications are not located within LXR\(\beta\) NLS contained in the transactivation domain and no one knows whether it could impact on the localization of LXR\(\beta\). LXR\(\beta\) mRNA sequencing was performed in our hands in HCT116 and NCM460 cells which displayed an opposite LXR\(\beta\) localization. Even though we identified a mutation on mRNA, this one was silent and no difference on protein sequence was subsequently observed \[^{26}\]. Second, in colon cancer cells the truncated part of RXR\(\alpha\), t-RXR\(\alpha\), is bound to LXR\(\beta\) and keep it localized in the cytoplasm, allowing ligand cytotoxicity. On the contrary, t-RXR\(\alpha\) is not or weakly expressed in normal colon cells leading to the nuclear localization of LXR\(\beta\) and a ligand toxicity tolerance (Figure 1) \[^{6}\]. However, the study reporting the cytoplasmic localization of t-RXR\(\alpha\) failed in finding correct explanations for this specific localization even though the NLS of t-RXR\(\alpha\) is kept \[^{8}\]. One could think that another partner could be involved in this sequestration of the LXR\(\beta\)/t-RXR\(\alpha\) complex.

To conclude, the interest to use LXR ligands in the treatment of cancer has been proved by many studies \[^{27, 28}\]. The importance of LXRs was studied with the use of KO mice, si/shRNA and the importance of their transcriptional activity was investigated with reporter gene experiments or PCR to evaluate target gene expression. Our studies highlight a non-transcriptional role of LXR\(\beta\) in colon cancer cells which can precede the transcription of target genes under agonist treatment. Thus, the sub-cellular localization of LXR\(\beta\) can be of importance to predict the response to LXR ligand treatment. One can speculate that it can also be a companion marker for the evolution of cancer or the response of patients to conventional treatment, as it was already shown for other nuclear receptors such as Nurrl (NR4A2) \[^{29, 30}\].

**Conflicting interests**

The authors have declared that no conflict of interests exist.

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**Abbreviations**


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