



Action mechanisms of Liver X Receptors



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ABSTRACT

The two Liver X Receptors, LXR α and LXR β , are nuclear receptors belonging to the superfamily of ligand-activated transcription factors. They share more than 78% homology in amino acid sequence, a common profile of oxysterol ligands and the same heterodimerization partner, Retinoid X Receptor. LXRs play crucial roles in several metabolic pathways: lipid metabolism, in particular in preventing cellular cholesterol accumulation; glucose homeostasis; inflammation; central nervous system functions and water transport. As with all nuclear receptors, the transcriptional activity of LXR is the result of an orchestration of numerous cellular factors including ligand bioavailability, presence of corepressors and coactivators and cellular context i.e., what other pathways are activated in the cell at the time the receptor recognizes its ligand. In this mini-review we summarize the factors regulating the transcriptional activity and the mechanisms of action of these two receptors.

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1. Introduction

Liver X Receptors (LXRs) are nuclear receptors belonging to the family of ligand-activated transcription factors [1]. There are two isoforms with 78% amino acid identity in their DNA-binding domain and ligand-binding domain. LXR α (NR1H3), first discovered by Magnus Pfahl and called RLD1 [2,3], and LXR β (NR1H2), also named RXR-interacting protein 15 (RIP15) [4], ubiquitously-expressed nuclear receptor (UNR) [5], steroid hormone-nuclear receptor (NER) [6] or orphan receptor-1 [7] because of its concomitant independent discovery by different laboratories. In humans, LXR α is located on chromosome 11p11.2 and LXR β on chromosome 19q13.3.

2. Tissue distribution of LXRs

In adult rodents, the profiles of protein expression of the two LXR isoforms are different, LXR α being highly expressed in the liver, adipose tissue, intestine, kidney, and macrophages, and LXR β expressed in epithelia active in transporting water such as pancreatic ductal epithelial cells [8], gallbladder and liver cholangiocytes [9,10] as well as in certain hypothalamic neurons [11], astrocytes and microglia [12].

During mouse development, starting from embryonic day 11.5, both LXR α and LXR β mRNA are detected in the liver. LXR α

maintains high expression throughout life while, hepatic LXR β decreases during later embryonic development [13]. Between mouse embryo ages days 11.5 and 16.5, LXR α mRNA appears to be detectable in brown adipose tissue, thyroid gland, and intestine while LXR β mRNA is strongly expressed in brain, retina, ganglia (vestibulocochlear, trigeminal, dorsal root), kidney, adrenal, thymus and thyroid gland [13]. In the brain, LXR β protein expression is detectable as early as embryo age day 14.5 in the neurons of the cortical plate [14].

3. Ligands

The first identified natural ligands that can activate LXRs at physiological concentration are oxysterols, in particular 24(S)-hydroxycholesterol, 22(R)-hydroxycholesterol, 24(S),25-epoxycholesterol, 27-hydroxycholesterol [15] and its metabolite, cholestenic acid [16]. Synthesis of 24(S)-hydroxycholesterol from cholesterol, the main mechanism of cholesterol removal from the brain [17], is catalyzed by cytochrome P450 46 A1 (CYP46A1). 22(R)-hydroxycholesterol is a naturally occurring oxysterol while 24(S),25-epoxycholesterol is made in cholesterol synthesis pathway from the cholesterol precursor squalene [18]. 27-Hydroxycholesterol is generated by a mitochondrial P450 enzyme, CYP27, involved in the alternative bile acid synthesis pathway [19]. The pattern of oxysterols that activates LXR, suggested that both anabolic and catabolic pathways in cholesterol metabolism may regulate LXR activity. Such a function of cholesterol metabolites was confirmed in *in vivo* experiments: (i). Knockout mice engineered to delete enzymes synthesizing 24(S)-HC, 25-HC and 27-HC are unable to induce LXR target genes in response to dietary cholesterol but remain responsive to a

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synthetic LXR agonist (T0901317) [20]; (ii). treatment of mice with inhibitors of cholesterol synthesis such as the archetypal statin, compactin, leads to a decrease in the synthesis of 24(S),25-epoxycholesterol, and to a decreased expression of LXR target genes [21,22]; (iii). bile duct ligation reduces hepatic oxysterol bioavailability and is associated with a down-regulation of LXR target genes, in rats [23]; (iv). adenovirus-mediated overexpression of cholesterol sulfotransferase, (SULT2A1) an enzyme capable of catabolizing oxysterols, prevents dietary induction of hepatic LXR target genes by dietary cholesterol but not by T0901317 [20].

In addition to oxysterols, D-glucose has been reported capable of binding to both LXR α and LXR β and inducing LXR transcriptional activity [24]. This role of LXRs as glucose sensors is not well understood since only the transcription factor carbohydrate-responsive element binding protein (ChREBP), and not LXRs, has been shown to induce glucose-regulated genes in the liver [25].

Two non-steroidal synthetic compounds, GW3965 and T0901317 (29, 30) as well the phytosterol, β -sitosterol (28) have been identified as LXR agonists capable of activating both LXR isoforms. However, T0901317 is not specific for LXR since it can also activate the Farnesoid X Receptor (FXR) [26] and the Pregnane X Receptor (PXR) [27]. Therefore, at present GW3965 appears to be the most selective synthetic LXR ligand.

Other activators of LXR include members of the Proton Pump Inhibitor (PPI) family, such as lansoprazole, pantoprazole and omeprazole [28]; a subset of natural bile acids which appear to be LXR-selective [16]; and N-acylthiadiazolines selective for LXR β but with low potency [29].

4. Mechanism of action

LXRs regulate gene transcription through two different mechanisms of action: direct activation (Fig. 1 upper panels) and transrepression (Fig. 1 lower panels). In the first mechanism, LXRs

form obligate heterodimers with the Retinoid X Receptor (RXR) [2] and bind to LXR-responsive elements (LXREs) consisting of a direct repeat of the core sequence 5'-AGGTCA-3' separated by 4 nucleotides (DR4) [30] in the DNA of target genes. Inverted repeat of the same sequence with no space region (IR-0) or with 1 bp spacer (IR-1) may also mediate LXR transactivation [31,32].

In the absence of ligands, LXRs are in a non-active state, binding to cognate LXREs in complex with corepressors such as the Nuclear Receptor Corepressor (NCoR) or the Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptor (SMRT) [33,34]. The binding of ligands induces a change in the conformation of LXRs that enables the release of corepressors, recruitment of coactivators [35] and in turn the direct activation of gene transcription. Several coactivators have been described for LXRs. These include: Peroxisome Proliferator Activated Receptor- γ (PPAR γ) coactivator-1 α (PGC-1 α) [36], the Steroid Receptor Coactivator-1 (SRC-1) [37] and the Activating Signal Cointegrator-2 (ASC-2) [38].

The second mechanism of action (Fig. 1), transrepression, plays a key role in regulating proinflammatory genes [39]. LXR β exerts a strong inhibition of the transcription of NF- κ B-regulated proinflammatory genes [40] that lack a direct binding site for LXRs. After ligand binding, LXR β undergoes a specific SUMOylation by SUMO-2/3 that promotes interaction with GPS2, a subunit of the N-CoR complex. In this setting the dissociation of the N-CoR complex from NF- κ B is prevented and in turn the transcription of proinflammatory genes is blocked [39].

5. Nuclear receptors influencing LXR activity

Two other nuclear receptors, PPAR γ and Small Heterodimer Partner (SHP) influence the cellular actions of LXR. Indeed PPAR γ , induces expression of LXR α in macrophages [41] while SHP, blocks the transcriptional activity of LXR α [42]. In the liver, SHP is one of the main effectors of the negative feedback regulation on CYP7A1,

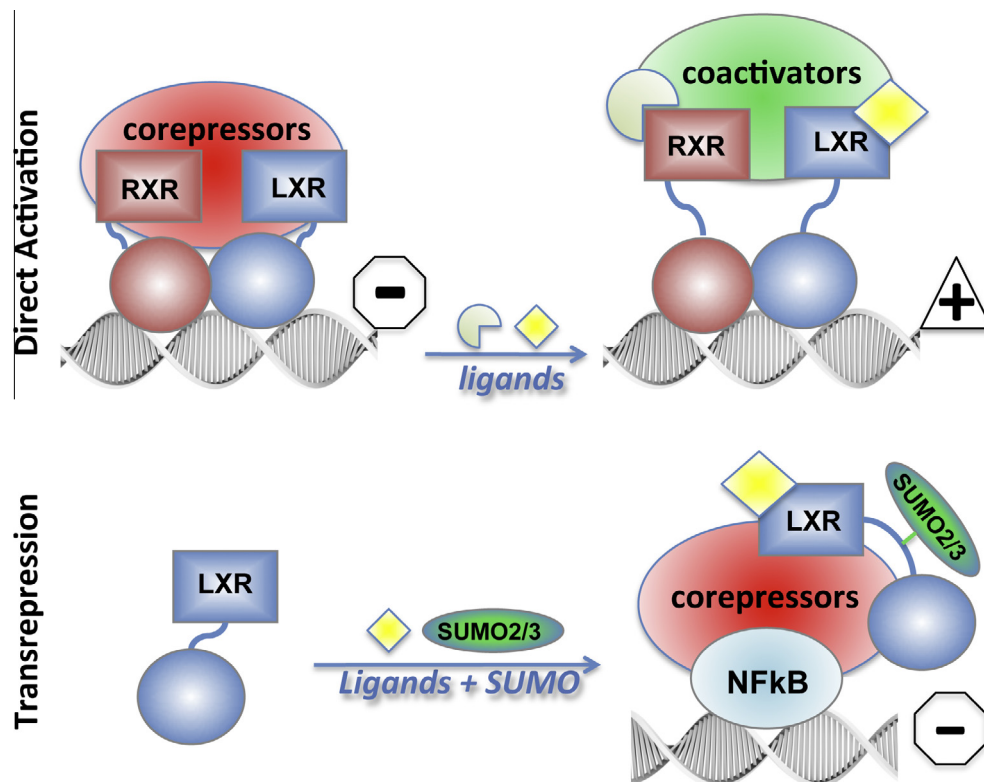


Fig. 1. LXRs influence gene expression by (i) directly promoting gene transcription after heterodimerization with RXR, binding with the ligands and interacting with coactivators and (ii) by transrepressing NF- κ B regulated genes after SUMOylation and interaction with corepressors.

the rate limiting enzyme in the bile acid synthesis pathway [43]. In adipose tissue, LXR α transcriptional activity appears to be estrogen-regulated and, in the LXR α promoter, a sequence that is negatively regulated by estrogens has been identified [44].

6. Future prospects for LXRs

Two of the most fascinating aspects of LXR signaling are the emerging roles of LXR β in neuroprotection [12] and water transport [8,11]. As they age, LXR β -/- mice develop motor neuron disease [45] and lose dopaminergic neurons from the substantia nigra [46]. Remarkably, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a Parkinson's disease-inducing chemical, causes more severe damage in the brains of LXR β -/- mice than in their WT littermates and treatment with the LXR agonist, GW3965, protects against loss of dopaminergic neurons in MPTP-treated WT mice [12]. LXR β is needed for the development of dopaminergic neurons in the midbrain [47,48] and, as research into this previously unknown regulator of dopaminergic neurons progresses, we can look forward to novel insights into the causes and progression of Parkinson's disease.

The discovery that LXR β regulates water channels [12,13] was a very unexpected finding since everyone was focused on the role of these receptors in lipid metabolism. The implications of the association between cholesterol and water homeostasis are still being evaluated but some of the detrimental effects of loss of LXR β in the central nervous system may be related to dysfunction in the end feet of astrocytes that make up the blood brain barrier as well as loss of regulation of the activity in microglia.

7. Conclusion

The biology of LXRs is a new and exciting area of medical research offering new insights into some of today's most mysterious and untreatable diseases such as motor neuron and Parkinson's disease. The implications of the functions of these receptors do not stop there, because understanding how LXRs regulate brain cholesterol homeostasis is likely to provide much needed information about the causes of Alzheimer's disease.

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