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Title:

Nuclear receptors and lipid sensing

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1. Abstract

Fluctuations in concentration of diverse lipid classes occur in response to diet and metabolism. These changes are managed and mediated by a cell network of enzymes, pumps, and carriers under the control of the lipid responsive nuclear receptors. The understanding of how dysregulation of lipid metabolism are causes and indicators of disease beyond the

cardiovascular system has developed in the last decade. A particular emphasis on the role of lipids and lipid-sensing nuclear receptors has emerged in the fields of cancer and the immune system's interaction with cancer. The range of known lipid-based ligands has also expanded. Lipids are not just signalling molecules, but also play structural roles in cells and tissues, for example as major constituents of the lipid bilayer – positioning them as integrators and mediators of signaling. This chapter will discuss the major groups of lipid-sensing nuclear receptors focusing on the liver x receptors, farnesoid x receptor, and the peroxisome proliferator-activated receptors. Initially the reader is presented with information on how these receptors behave and function at the molecular biology level, the range of selective modulation of function by endogenous ligands, and examples of how activity is fine-tuned by mechanisms such as miRNA regulation and post-translational modification of the proteins. We then explore the advances in understanding that have positioned these receptors as therapeutic targets in cancer and immuno-oncology. Finally, the chapter explains the gaps in understanding and experimental challenges that should be prioritized in the coming decade.

2. The molecular biology of lipid-sensing nuclear receptors

Nuclear receptors (NR) sense lipids from broad subclasses that include fatty acids, phospholipids, sphingolipids, and sterols. Cholesterol, integral to the plasma membrane's barrier function, is the precursor for an array of ligands including hormones, seco-steroids, oxysterols, and bile acids, which drive NR activity. Cholesterol constitutes around 40% of the mammalian cell membrane and mediates signal transduction pathways that originate from liquid ordered nanodomains within this barrier to the external milieu. Fatty acids and phospholipids also influence plasma membrane fluidity and function and act as ligands for the peroxisome proliferation activated receptors (NR1C1-3/PPARs) and liver receptor homologue (NR5A2/LRH1) respectively. Lipid-NR pathways therefore link the cell's external barrier and its metabolic state to transcriptional activity and cell fate processes.

2.1 Liver x receptors (NR1H2, NR1H3)

Cellular and tissue regulation of cholesterol is controlled by liver x receptors alpha (LXRα) and beta (LXRβ). LXRα and LXRβ are expressed in the liver and a variety of extra-hepatic tissues including brain, reproductive organs, gut-axis, bone, and vital organs. The two paralogues target a battery of genes involved in flux (e.g. ATP binding cassette transporters), metabolism (e.g. CYP450s, hydroxylases), and transport (apolipoproteins) of sterols. The LXRs therefore underpin cholesterol's physiological roles in the liver, integrity of the blood brain barrier,

neuronal function, amyloid pathology, cellular proliferation and migration, inflammation and immune cell differentiation and function, xenobiotic efflux, and autophagy.

2.1.1 Structure of the LXRs

The LXRs are classically considered to be bound to gene regulatory regions that contain a direct repeat with a four-nucleotide spacer (DR4: AGGTCA). LXRs are activated by hydroxylated cholesterol metabolites that dock to the broad affinity ligand binding domain (LBD). This binding event induces allosteric change in the protein structure and co-repressors are exchanged for co-activators. The presence of ligand increases the amount of LXR at binding sites (Feldmann et al., 2013), either via auto-regulated induction of expression or stabilization of the transcription factor-DNA interaction. The LXRs contain an activation function domain (AF1) at the N-terminus and a flexible hinge region that links the LBD and DBD. With the C-terminal region of the LBD is a second activation function 2 domain (AF2) (Svensson et al., 2003). The hinge region of LXR is subject to post-translational modifications that modifies the response to ligand and co-factor exchange (Becares et al., 2017; Becares et al., 2019; Wu et al., 2015). Alternative splicing and alternative promoter usage lead to differential expression of these protein domains patterns for LXRα **(Fig1A)** and to a lesser extend LXRβ **(Fig1B)** as well. Five LXRα splice variants have been experimentally validated to date (Chen et al., 2005; Endo-Umeda et al., 2012; Lianto et al., 2021) but as many as 62 differentially spliced transcripts are predicted to exist (Annalora et al., 2020). LXRα2 and LXRα5 have shortened ligand binding domains and LXRα4 is generated from an alternative transcriptional start site altering the length of the AF1 domain. Contrarily, only a single LXRβ splice variant appears to have been reported as expressed at the protein level to date (LXRβ4) and contains a shortened LBD (Lianto et al., 2021). The function of this variant has not been established, and is expressed in whole breast tumour tissues, but not epithelial cell lines, so may be expressed under some pathological conditions or in non-tumour cells of the cancer microenvironment.

2.1.2 Endogenous selective modulators of LXR

Oxysterols were discovered as ligands for the LXRs in the 1990s (Janowski et al., 1996) and can be synthesized by a range of enzymes or through auto-oxidative routes **(Fig2)**. As a diverse class of cholesterol derived lipids, over the last decade the range of known oxysterol based LXR ligands has increased, as has our understanding of the role that the oxysterol:LXR axis plays in health and disease (Griffiths and Wang, 2021; Lizard et al., 2021). Although historically considered as intermediates in the metabolism of cholesterol to bile acids or steroid

hormones, oxysterols are now established as potent signaling molecules in their own right, with an array of cell biology functions elucidated.

The diversity of oxysterols allows categorization based on the chemical moiety(s) that distinguishes them from cholesterol. Hydroxy-, epoxy-, or keto- modifications to the cholesterol backbone leads to distinct oxysterol classes with different transactivation potential for LXR. In physiological systems a pool of these LXR ligands would typically be present, which differ between tissues. The oxysterol constituents of this pool are not present at equimolar concentrations and have varying EC_{50} values in their capacity to activate LXR. For example, when comparing the side-chain hydroxycholesterols (scOHC): 22-OHC, 24-OHC, 25-OHC, 27-OHC and the epoxycholesterol 24,25-EC, there are distinct differences in both circulating concentrations (Stiles et al., 2014) and LXR transactivation potential (Hutchinson et al., 2019a; Hutchinson et al., 2019b; Janowski et al., 1999). For example, 27-OHC is by far the most abundant of these oxysterols in the circulation (by >50-fold when compared with 22-OHC or 24,25EC) and in some tumours such as breast (Solheim et al., 2019) but is a significantly weaker LXR agonist (Hutchinson et al., 2019b; Janowski et al., 1999). In different tissues other oxysterols are the majority species. In the brain 27OHC is rare and 24S-OHC (cerebrosterol) is the dominant OHC. These differences in the concentration and ability to transactivate the LXRs provide tissue specific modulation of the receptors at a level beyond expression of the NR protein. Selective modulation of LXR has also been found in epoxy class of oxysterols mediated by conjugation to histamine or via metabolism by 11βHSD2, and generates cross-talk between LXR and other NRs. When 5,6β-EC is metabolized by 11βHSD2 to 6-oxo-cholestan-3β,5α-diol (OCDO) it binds to GR (KD=10μM) and LXRβ (KD=12.5μM) with similar affinity, and promotes proliferation in a GR dependent manner (Voisin et al., 2017). However, if the stereoisomer 5,6α-EC is instead conjugated to histamine it generates dendrogenin A, a compound that is reduced in tumour tissue and that can drive lethal autophagy in cancer via LXR activation (Segala et al., 2017). OCDO is considered a competitive inhibitor of cortisol given that it decreases cortisol affinity to GR at 1μM (Voisin et al., 2017). The synthesis of OCDO and dendrogenin A and their roles as LXR ligands are comprehensively reviewed (Poirot and Silvente-Poirot, 2013; Poirot and Silvente-Poirot, 2018).

The plethora of endogenous LXR ligands with their differing ability to transactivate LXR that is typically found in human serum and tissue therefore add nuance and adaptability to the oxysterol:LXR axis. Selective modulation of LXR activity by OHCs is a function of the local oxysterol pool; although a relatively simple ligand-receptor interaction at the molecular level, extensive specialization is conferred to the pathway at the tissue level. Genome-wide analyses have been reported for LXR knockdown in mouse (Bideyan et al., 2022; Boergesen et al.,

2012), the synthetic ligand T0901317 (Feldmann et al., 2013; Pehkonen et al., 2012) and GW3965 (Oishi et al., 2017), but formal genome wide-comparisons of multiple endogenous ligands at the RNA- or ChIP-Seq level are yet to be reported. Such comparisons would add valuable information regarding selective modulation by ligands.

2.1.3 Fine-tuning of LXR signaling

As for most NR, LXR activity is fine-tuned at the cell and tissue level by several mechanisms. Differences in expression of splice variants, ligand concentration, and co-factors act to alter the transcriptional output from LXR. Interestingly, LXRβ is predicted to bind far more co-repressors than LXRα, yet LXRα is predicted to bind to a wider range of co-activators (Broekema et al., 2018). Experimental validation of such predictions is challenging and are currently not yet evidenced in the literature. Given the changes to protein structure generated by alternative splicing, it is plausible that co-factors may form distinct complexes depending on the LXR variant to which they are binding. Such a variety of overlapping but diverging transcriptional complexes allows for subtle fine-tuning for the response of LXR to ligand. Another important factor that alters LXR's activity is post-translational modification of the Hinge region. Phosphorylation of S198 confers specificity to LXRα transcriptional activity, with expression of some but not all target genes differentially modulated in the presence of this post-translational modification (Torra et al., 2008). An adjacent modification on LXRα (pS196) has been linked to severity of liver disease and to activation of a subset of LXR target genes (Becares et al., 2019). Activation of LXR target genes is also modified by interactions with miRNAs. LXR induces ABCA1 through promoter binding, but also down-regulates expression of miR-26, a miRNA that binds and degrades the *ABCA1* transcript (Sun et al., 2012). A coherent type-IV feed forward loop that simultaneously activates expression of the target gene while transrepressing it's miRNA inhibitor is therefore established, which allows for rapid and massive induction of *ABCA1* transcript.

2.2 Farnesoid x receptor alpha (NR1H4)

FXR function is linked to bile acid (BA) metabolism and cholesterol bioavailability for BA synthesis. Although the initial steps of cholesterol metabolism towards the BA route are initiated by the LXRs. In non-primate mammals FXRβ is activated by the cholesterol precursor lanosterol, so expression of this gene results in an alternative pathway for cholesterol metabolism in all other mammals (Otte et al., 2003). Several excellent reviews are available regarding FXR's gene/protein structure and ligand binding repertoire (Jiang et al., 2021), and function (Gadaleta et al., 2015). Aberrant FXR activity influences the pathogenesis of obesity, diabetes and dyslipidemia (Fang et al., 2015; Pathak et al., 2018; Sun et al., 2018), liver

disease (Chiang and Ferrell, 2020), inflammatory bowel disease (IBD) (Nijmeijer et al., 2011) and several cancers (**see section 3.1**).

2.2.1 Structure of FXR

FXR is composed of an N-terminal AF1 domain, a DNA binding domain, a variable hinge region, followed by a C-terminal ligand binding domain (LBD) containing activation function 2 domain (AF2) (Downes et al., 2003). There are two FXR genes, alpha and beta, but in humans only the alpha paralogue is protein coding, beta is a pseudogene (Otte et al., 2003). Of the 49 mouse NRs, FXRβ is the only gene not expressed as one of the 48 human NRs. Alternative promoter usage and splice site slipping gives rise to four different transcript variants harbouring two different N-terminal AF1 domains, each of which can have inclusion or exclusion of a four amino acid addition to the hinge region. The organization of protein domains that are encoded by the FXRα gene and for the four splice variants experimentally validated in humans are shown in **Fig1C**.

2.2.2 FXR ligands

BAs are cholesterol derived molecules synthesised in the liver, and activate multiple NRs including, but not limited to, farnesoid X receptor (FXR), pregnane X receptor (PXR), and vitamin D receptor (VDR). Here we focus on their role in FXR regulation, but further information regarding their role as PXR and VDR receptors can be found here (Koutsounas et al., 2013; Makishima et al., 2002). Around 90% of the primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesised via the classic pathway using microsomal cholesterol 7alpha-hydroxylase (CYP7A1). The remainder are produced via the alternative LXR and LRH1 mediated sterol-27-hydroxylase (CYP27A1) pathway. Liver BAs are conjugated with glycine or taurine and secreted into bile where they function as lipid emulsifiers that carry sterols and phospholipids. BAs are potent signalling molecules driving FXR dependent regulation of signalling pathways that converge to influence lipid, glucose and energy metabolism, drug detoxification, and liver regeneration. Once in the gallbladder, cholecystokinin (CCK) stimulates bile release in the duodenum to favour digestion of cholesterol, triglycerides and liposoluble vitamins. BAs can be reabsorbed in the ileum, and return into the liver via the portal vein (enterohepatic circulation), or proceed into the colon and be further metabolised by gut microbiota, which is responsible for their transformation into secondary BAs (for further information the reader is directed to (Di Ciaula et al., 2017). BAs are natural ligands for FXR and CDCA is the most potent ligand, followed by deoxycholic acid (DCA), lithocholic acid (LCA), and finally CA (Sepe et al., 2016). Differences in BAs serum levels were observed among healthy subjects and on-alcoholic steatohepatitis (NASH)

patients. Subjects with NASH showed total serum BAs levels 3 times higher than healthy controls. In particular, NASH patients have 4 times higher levels of serum DCA and lower levels of serum CDCA. These BAs differences are paired with upregulation of liver FXR gene expression (Jiao et al., 2018).

2.2.3 Fine-tuning of FXR signaling

FXR is regulated by miRNAs and post-translational modifications. Reducing FXR activity is a common feature of liver disease and concequeently, several of these pathwyas have been explored in the context of steatosis, non-alcholic fatty liver disease (NAFLD), or hepatocellular carcinoma. MiR-192 (Krattinger et al., 2016), miR-194 (Nie et al., 2017), and niR-382-5p (Nie et al., 2021) all target and downregulate FXR expression – typically exacerbating liver pathologies such as NAFLD. Acetylation of FXR at K157 and K217 by p300 reduces dimerisation potential with RXRα and consequently its transcriptional activity (Kemper et al., 2009). SUMOylation of FXR occurs at several different amino acids and is also associated with suppression of FXR signaling, downregulation of FXR target genes (Balasubramaniyan et al., 2013) and progression of liver disease (Zhou et al., 2020).

2.3 Peroxisome proliferator-activated receptors (NR1C1, NR1C2, NR1C3)

PPAR target genes typically regulate carbohydrate and lipid metabolism and homeostasis, and give PPARs control and influence over cell fate decisions and tissue remodeling processes. The three genes show overlapping expression patterns in normal physiology, and all three have been implicated in a range of diseases. PPARα is expressed by metabolically active tissues that need high fatty acid oxidation to produce energy, like liver, brown adipose, and skeletal tissue. To facilitate the functions of these tissues, PPARα gene targets are involved in FA mobilization and oxidation, ketogenesis and plasma lipoprotein metabolism (comprehensively reviewed by (Grabacka et al., 2016; Pawlak et al., 2015; Tahri-Joutey et al., 2021). PPARα suppresses inflammation by downregulating expression of pro-inflammatory genes and upregulating anti-inflammatory gene expression (Ramanan et al., 2008; Shin et al., 2016; Zúñiga et al., 2011).

PPARδ is expressed more ubiquitously than the other PPARs with highest expression in gastrointestinal system, skeletal muscles, and kidneys. A major role of PPARδ is in coordinating reverse cholesterol transport and removal of triglycerides (Ooi et al., 2011; Vrins et al., 2009). PPARδ also promotes FA oxidation and energy uncoupling, reducing the risk to develop obesity (Wang et al., 2003). PPARγ is mainly expressed by adipose tissue, and moderately expressed in intestine and spleen. PPARγ, especially the PPARγ2 variant (**see**

section 2.3.1), controls the balance of adiponectin and leptin secretion (adipokines that are typically out of balance in the adipose tissue of obese individuals), and helps to maintain insulin sensitivity. PPARγ also regulates the expression of genes involved in FA efflux, transport, and storage (e.g., LPL and FAT/CD36) and as such prevents lipotoxicity and lipid overload in liver, skeletal and other tissues (further details available (Grygiel-Górniak, 2014).

2.3.1 Structure of PPARs

PPARs are ligand-activated NRs composed of an N-terminal DNA binding domain and C terminal ligand binding domain (LBD) containing activation function 2 domain (AF2) (Fyffe et al., 2006). There are three paralogous PPAR genes in humans, which are further extended by expression of transcripts encoded from alternative promoters and splicing. PPARs are probably subject to significantly more alternative splicing than has been experimentally evaluated to date (Annalora et al., 2020). Annalora and colleagues annotated 28, 33, and 23 alteratively spliced transcripts for PPARα, PPARδ, and PPARγ respectively, which had been listed in Ensembl, AceView, or PubMed databases. Interestingly, and somewhat unusually, all three PPARs lack modular cassette exons. In the absence of modular cassette exons, alternative splicing is likely to result in a shift of the open reading frame creating truncated or non-functional variants. This perhaps explains why so few of these PPAR transcript variants have been experimentally observed at protein level to date. PPARγ has alternative promoters, resulting in the expression of four PPARγ transcripts that produce two distinct proteins (PPARγ1, 3, and 4 differ only in their 5'UTRs) (further details available (Knouff and Auwerx, 2004). PPARγ2 has an additional 30 amino acids at the N-terminal domain leading to an extended AF1 domain similar to the α4 variant of the LXR splice family. The organization of protein domains that are encoded by the PPAR genes and the major protein coding variants are shown in **Fig1D**.

2.3.2 PPAR ligands

Essential fatty acids (EFAs) and eicosanoids are specific endogenous agonists of the Peroxisome Proliferator-Activated Receptor (PPAR) family. Fatty acids (FAs) are components of many lipids involved in energy storage and metabolism, cell structure and signaling. FAs are structurally composed by a terminal carboxyl group and a hydrocarbon chain of various lengths, made up by an even number of carbon atoms, with (unsaturated) or without (saturated) double bonds. FAs can be endogenously produced by fatty acid synthases (FAS). However, some FAs cannot be synthesized by animals and they must be introduced with diet. These EFAs are the two polyunsaturated fatty acids (PUFAs), alpha-linolenic acid (ALA) and linoleic acid (LA), belonging to the omega-3 (ω3) and omega-6 (ω6) families, respectively.

Once ingested, ALA can be converted into eicosapentaenoic acid (EPA), and subsequently form docosahexaenoic acid (DHA). LA is converted to γ-linolenic acid (GLA) that can be elongated to dihomo-GLA (DGLA), which is the precursor of arachidonic acid (AA). AA, EPA and DGLA, can be further metabolized into eicosanoids, which have a physiological role in inflammation and immunity, circulatory and female reproductive system, kidneys and gastrointestinal functions (reviewed by (Calder, 2020). PUFAs and especially, EPA and DHA, as well as eicosanoids (15-HETE, PGJ2, and 15-deoxy-Δ12,14-PGJ2) and oxidized metabolites of LA (9-HODE and 13-HODE) are ligands for PPAR. PPARα is activated by unsaturated FAs, especially omega-3, and the eicosanoids leukotriene B4 and (8S)-hydroxyeicosatetraenoic acid (8(S)-HETE) (further details available (Grygiel-Górniak, 2014), and its activation plays a role in energy combustion and metabolism regulation. PPARδ is activated by unsaturated fatty acids, especially EPA, and eicosanoids.

2.3.3 Fine-tuning of PPAR signaling

The PPAR genes are predicted to produce relatively few alternative transcripts (Annalora et al., 2020) and few have been experimentally validated to date. However, the PPARs are subject to extensive post-transcriptionally regulation by miRNAs (Seiri et al., 2019), post-translational regulation by a variety of enzymes that modify the protein function through covalent modifications (Brunmeir and Xu, 2018), and through co-factor expression levels. Expression of miR-9 in monocytes and macrophages downregulates PPARδ contributing to polarization of pro-inflammatory M1 macrophages (Thulin et al., 2013). M1 macrophages are further activated by increased miR-27b expression that targets PPARγ (Jennewein et al., 2010). In adipocyte stem cells miR-138 (Wang et al., 2019), miR-130 (Lee et al., 2011a), miR-548d-5p (Sun et al., 2014), and miR-27b (Karbiener et al., 2009) have all been shown to prevent differentiation via PPARγ. miR-27 is of particular interest in disease of lipid metabolism and has been implicated in the pathogenesis of NAFLD (Zhang et al., 2021). PPARγ can be phosphorylated by multiple kinases leading to pathway specific effects. For example, when phosphorylation occurs via the MAPK-JNK1/2-p38 pathway, transcriptional activity is reduced (Adams et al., 1997; Camp et al., 1999; Hu et al., 1996; Tang et al., 2006), but when phosphorylation occurs via CDK7 or CDK9, transcriptional activity is enhanced (Compe et al., 2005; Iankova et al., 2006). SUMOylation within the hinge region of PPARα enhances recruitment of NCOR leading to inhibition transcriptional activity (Pourcet et al., 2010).

2.4 Other lipid-sensing NR

Depending on how the term ‘lipid’ is defined, it is arguable that all NRs are lipid sensing. However, there are several examples of NRs that belong to the high affinity steroid hormone

classes, such as glucocorticoid and estrogen receptor alpha, that can bind and be activated by the same lipids that also activate the lipid-sensing NRs described above.

The side-chain hydroxycholesterol 27-OHC is produced from cholesterol by a single hydroxylation on the final C atom by the CYP27A1 enzyme and is estrogenic. 27-OHC was originally identified as an antagonist for the ER when added in the presence of estradiol (Umetani et al., 2007), but since emerged as a selective estrogen receptor modulator (DuSell et al., 2008). When bound to ERα, 27-OHC drives proliferation of ERα expressing cells in culture, and growth of ERα positive cancers *in vivo*. 27-OHC has an affinity for ERα orders of magnitude less that estradiol (27-OHC Ki = 1.32 μm, E2 Kd = 0.1 nm) in vascular endothelial cells (Umetani et al., 2007), yet is present in circulation at concentrations orders of magnitude greater (ca. 30-990 ng/mL (Stiles et al., 2014)) than estrodiol (30-400pg/mL in premenopausal women). This balance between affinity and concentration likely means that endogenous 27-OHC can antagonise endogenous E2 activity.

The plasma membrane is a complex structure that contains a plethora of lipids such as sphingosines, phosphatidylinositols and phosphatidylcholines that act as ligands for the NR5A subgroup. The biophysical properties of phospholipids influence ordering of nanoregions in the membrane but are also released from the membrane, accumulating and acting within the nucleus, and even as part of chromatin and transcriptional complexes with LRH1 (NR5A2) (Krylova et al., 2005; Lee et al., 2011b; Sablin et al., 2015). This membrane-lipid NR links the plasma membrane composition to lipid homeostasis, lipid diversity (Miranda et al., 2018), stem cell maintenance via pluripotency factor Oct4 (Gu et al., 2005), cellular stress responses (Sun et al., 2021), and estrogen synthesis (Clyne et al., 2002). Dysregulation of phospholipid and LRH1 function is therefore associated with several pathologies, including NAFLD (Sun et al., 2021), cancers of the liver (Sun et al., 2021) and breast (Clyne et al., 2002; Soteriou et al., 2021). Steroidogenic Factor 1 (NR5A1/SF1) is also able to directly bind and respond to phospholipids (Blind et al., 2014). When bound to SF1, sphingosine acts as an antagonist and suppresses expression of aromatase, the rate limiting enzyme in the synthesis of estrogen. Deeper discussions of how phospholipid influence nuclear receptor signalling and the plasma membrane structure and function are available in the following review articles (Crowder et al., 2017; Musille et al., 2013; Soteriou et al., 2021).

3. Emerging trends for lipid-sensing nuclear receptors

3.1 Cancer theranostics

Cancers initiate from imbalances in proliferative and differentiation factors, driven by oncogenic transformation and loss of tumour suppressor function. However, metabolic imbalances are as readily measured as gene status by genomic profiling, and therefore the complexity of NR activity regulation means that simple measures of either NR expression or even genomic binding, may mean the centrality of lipid metabolites to many cancer processes is overlooked.

Expression of NR co-factors that strongly influence response to ligand are disrupted in cancers of the prostate (Battaglia et al., 2010; Doig et al., 2012; Long et al., 2014), bladder (Abedin et al., 2009), breast (Hutchinson et al., 2019b) and others. Accumulation or depletion of NR ligands can occur via changes in expression of the CYP family resulting in variation in NR ligand bioavailability. Such differences that impinge on NR activity can alter energy and cellular metabolism within the tumour mass. The actions of lipid-sensing, and indeed other NRs, in tumour cell energy regulation has been reviewed previously (Thorne and Campbell, 2015). Assessing activity of the lipid-sensing NRs is perhaps more challenging than assessing activity of steroid hormone receptors levels (e.g. ERs and AR). Although all are subject modulation of activity by selective modulation, co-factors, and miRNAs, the mere presence or absence of hormone receptors such as ERα and AR is sufficient for clinical classifications. The greater complexity of measuring and understanding activity the NRs that sense a diverse range of ligands will most likely be more clearly resolved as technologies improve and allow them to be therapeutically and diagnostically exploited in the coming years.

3.1.1 LXR in breast and prostate cancer

Breast tissue is rich with a heterogenous mixture of cell types that store and metabolise lipids. Epithelial and ductal cells, adipocytes, fibroblasts, tissue resident macrophages and others combine to regulate the synthesis, storage, metabolism, and movement of many lipid species. During tumouriogensis these non-cancer cells form the tumour microenvironment and may be co-opted to provide a range of selective advantages that enhance tumour growth, including utilization of lipids.

In fibroblasts CYP27A1 converts cholesterol into 27-OHC (Axelson and Larsson, 1995; Lange et al., 2009), which owing to its estrogenic potential drives breast cancer (BCa) cell proliferation via activation of the estrogen receptor (Wu et al., 2013a) and allows the tumour to evade anti-estrogen therapy. Contrary to this, when 27-OHC (or indeed several other oxysterols) activates LXR in BCa pro- and anti-tumourogenic effects occur. The LXR-oxysterol pathway slows proliferation (Vedin et al., 2009) and activates apoptosis, yet exacerbates metastasis (Baek et

al., 2017b; Nelson et al., 2013) and drives chemotherapy resistance (Hutchinson et al., 2021). There appears to be a clear selective advantage for the tumour to maintaining these apparent idiosyncrasies, the OHC-LXR axis drives expression of both pro-tumour and anti-tumour pathways. Evaluating the cholesterol metabolic role of fibroblasts across the BCa subtypes has not yet been systematically evaluated, although their presence does drive activation of interferon-β1 signaling (Broad et al., 2021) which has recently been shown to be downstream of LXR in macrophages (la et al., 2021).

In macrophages, 25-hydroxylase (CH25H) and converts cholesterol into 25-OHC (Blanc et al., 2013) which can be secreted (Bauman et al., 2009). This oxysterol is a potent activator of LXR in BCa cells (Hutchinson et al., 2019b), leading to acute chemotherapy resistance in triple negative BCa (Hutchinson et al., 2021). In the OXYTAM study 25-OHC was found to be a potential diagnostic indicator of disease relapse as it was significantly elevated in the serum of BCa patients who had disease recurrence compared to those with primary disease (Dalenc et al., 2017). Interestingly, CH25H and LXR are involved in a feed-forward loop. LXR can bind the CH25H promoter and induce its transcription (Liu et al., 2018), leading to a rise in synthesis of 25-OHC from cholesterol. The CH25H enzyme may therefore be a useful clinical theranostic that indicates activity of the LXR pathway in a tumour, and could be targeted by existing therapeutics such as statins that lower circulating levels of several endogenous LXR agonists (Thelen et al., 2006). Other clinical studies, particularly centered around exploring the diagnostic, prognostic, and therapeutic potential of the oxysterol:LXR axis are required in the coming decade. Systematic reviews and meta-analyses support the proposal that the LXR pathway is important in breast cancer; pharmacological (Liu et al., 2017) interventions and dietary patterns (Jiang et al., 2019) that lower circulating cholesterol, and by extension oxysterol levels (Dias et al., 2018), consistently indicate there is a reduced risk of developing and dying from breast and other cancers.

Contrary to a tumour promoting role for the side chain oxysterols in some BCa subtypes, the situation is reversed in prostate cancer. LXR is anti-proliferative in prostate cancer (PCa) cell lines (Chuu et al., 2006; Chuu et al., 2007; Chuu and Lin, 2010) but in mouse models, LXR activity prevents features of benign prostate hyperplasia appearing (Viennois et al., 2012) and can restrain hyper-proliferation induced by a high cholesterol diet (Pommier et al., 2013). In the absence of LXR, cholesterol esterification is drastically enhanced and expression of an array of genes involved in metabolism of cholesterol, fatty acids, and triglycerides are lost (Pommier et al., 2013) and AR target genes involved in secretory cell-cell communication are induced (Viennois et al., 2012). Cross-talk between the androgen receptor (AR) and LXR is an established phenomenon. Treatment of PCa cells with AR ligands reduces expression of the

canonical LXR target ABCA1 (Fukuchi et al., 2004) and reciprocally, LXRα activation within the liver accentuates circulating testosterone levels, although perhaps not in the prostate directly (Viennois et al., 2012). These observations led to a series of human clinical studies that again indicate that PCa tumours are divergent to BCa tumours in the context of oxysterol and LXR signaling. In BCa, 27-OHC levels are significantly elevated in tumour tissue compared to adjacent normal breast tissue (Wu et al., 2013b), whereas in PCa, results from the CHOMECAP study revealed the opposite, 27-OHC level were markedly lower in tumour tissue compared to adjacent normal tissues (Celhay et al., 2019). Low levels of both the 27-OHC metabolite and the gene that codes for its synthesizing enzyme *CYP27A1*, were predictive of higher grade PCa and relapse. The expression level of neither LXRα nor LXRβ were different between normal and tumour prostate tissue in the CHOMECAP study.

3.1.2 FXR in cancer

In vitro models of BCa suggest that pharmacological activation of FXR reduces the tumour promoting effects of cancer associated fibroblasts (Barone et al., 2018; Giordano et al., 2016). CDCA treatment and FXR consequent activation, increase BCa cells cytotoxicity (Alasmael et al., 2016), and in patients affected by invasive breast carcinoma, expression of FXR represents an independent prognostic factor of overall and disease patient's survival (Giaginis et al., 2017). Conversely, other studies showed that CDCA increase BCa cells proliferation and metastasis (Absil et al., 2020; Journe et al., 2009). FXR activation has also pro tumorigenic potential, as well as pro proliferative and anti-apoptotic effects in gastric, esophageal, kidney and lung cancer (Fujino et al., 2017; Guan et al., 2013; You et al., 2017; Zhou et al., 2018). FXR expression is correlated with early colorectal cancer onset (Yu et al., 2020), proliferation and progression (Fu et al., 2019), and clinical outcome (Lax et al., 2012). In FXR-null mouse models of hepatocellular carcinoma (HCC) however, 90% of mice developed liver tumours that was linked to significant increases in *Myc* oncogene expression (Takahashi et al., 2018). FXR activation by CDCA increases chemosensitivity of biliary tract cancers (Wang et al., 2016), and in cholangiocarcinoma, CDCA correlates with tumour differentiation (Erice et al., 2018). Processing of BA associated with FXR activation is therefore generally associated with reduced risk of oncogenic transformation.

3.1.3 PPAR in cancer

An anti-tumorigenic effect of PPARγ activation has been proposed following the discoveries of multiple mechanisms where it can induce terminal differentiation, apoptotic signaling, cell cycle arrest promotion, and inhibition of pro-inflammatory signaling (reviewed in (Peters et al., 2012)). Despite these observations some synthetic PPARγ agonists may promote onset of

colon and bladder cancer (Peters et al., 2012). In murine models, long term administration of PPARα synthetic agonist induce liver cancer (Hays et al., 2005), however, this mechanism was not observed in humans (Peters et al., 2005; Peters et al., 2012). Currently, PPARα is under investigation in multiple cancer prevention studies owing to the ability to inhibit tumorigenesis (Luo et al., 2019; Morinishi et al., 2019), cancer cell proliferation (Chen et al., 2020; Liang et al., 2014; Morinishi et al., 2019), angiogenesis (Garrido-Urbani et al., 2011) and interfere with the Warburg effect (Chang and Huang, 2019; Huang and Chang, 2016). PPARδ is consider pro-tumorigenic for several cancers, promoting cancer hallmarks, including angiogenesis, tumorigenesis, cell death resistance and metastasis (reviewed in (Wagner and Wagner, 2020). The transcriptional coactivator peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) impairs PCa metastatic process in oestrogen-related receptor alpha (ERRα)-dependent mechanisms, (Torrano et al., 2016; Valcarcel-Jimenez et al., 2019). On the contrary, in BCa PPARGC1A promotes lung metastasis and helps adaptation to metabolic drugs (Andrzejewski et al., 2017).

3.2 Lipid-sensing nuclear receptors and immuno-oncology

Immune evasion is considered as an emerging hallmark of cancer. Programmed death protein 1 (PD-1) and its ligands (PD-L1/2), as well as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are the immune checkpoints that become activated and impair the immune system's response against cancer cells (Yearley et al., 2017; Zerdes et al., 2018). During tumorigenesis, CD8+ cytotoxic T-cells, which play a significant role in cancer immune detection and elimination, lose the ability to produce effector molecules in a process termed exhaustion. These 'silenced' T-cells are marked by overexpression of PD-1, CTLA-4 and other inhibitory markers. There is now a clear association between cancer-initiated T-cell exhaustion with metabolism of cholesterol and fatty acid via the LXRs and PPARs (Bu et al., 2011; Cioccoloni et al., 2020; Cochain et al., 2014; Gotsman et al., 2007; Ma et al., 2019).

Oxysterols can promote tumour growth by creating a pro-tumorigenic microenvironment. In fact, tumour cells derived oxysterols can recruit neutrophils in the tumour microenvironment (TME) promoting angiogenesis and immune suppression (Raccosta et al., 2013; Soncini et al., 2016). Vice versa, oxysterols enzymatic inactivation by sulfotransferase 2B1b (SULT2B1b) reduce neutrophils presence in breast tumours and increase the ratio between CD8+IFNγ+ cytotoxic T-cells and CD4+IL-4+ T-cells, which are known to be pro tumorigenic, in favour of the effector cells (Moresco et al., 2018). Activated T-cells present elevated levels of SULT2B1 leading to suppression of LXR (Bensinger et al., 2008) and a consequential increase in proliferation, differentiation, and expansion of T-cells, and reduction of PD-1 expression

(Bensinger et al., 2008; Carpenter et al., 2019). LXRα activation increases Treg populations and differentiation that maintain immune tolerance in cancer (Carpenter et al., 2019; Pardoll, 2012; Wood and Sawitzki, 2006) and reduce dendritic cell migration to lymphoid organs and expression CC chemokine receptor-7 (CCR7) (Villablanca et al., 2010). The oxysterol-LXR axis also mediates resistance to immune destruction via the myeloid population. LXR activation by 27-OHC in myeloid cells accelerates T-cell apoptosis thus enhancing immune suppression while blocking 27-OHC synthesis via CYP27A1 inhibition improves the efficacy of anti-PDL1 checkpoint inhibitors (Ma et al., 2020). The expression of CYP27A1 in myeloid cells is needed to guarantee a pro-tumorigenic microenvironment in ovarian cancer, and mice treated with 27-OHC increase myeloid derived suppressor cells (MDSCs) production, which suppress cancer immune response, and alters T-cells population composition (He et al., 2019). The pro-metastatic effect of 27-OHC also involves myeloid immune cell function. In fact, 27-OHC increases the activation/recruitment of polymorphonuclear-neutrophils and γδT-cells, with a reduction of CD8+ cytotoxic T-cells in distal metastatic sites (Baek et al., 2017a). Many studies therefore support the hypothesis that the oxysterol-LXR axis leads to immune resistance.

Evidence of a role for PPARs in cancer immune response is less clear than for LXR. PPARα activation supports anti-PD1 immunotherapy, enhances CD8+ T-cell activity, and increases survival time. This occurs via a change in their primary metabolism pattern from glycolysis to mitochondrial fatty acid oxidation and oxidative phosphorylation (Chowdhury et al., 2018). Activation of PPARγ impairs the inflammatory responses of MDSCs, mainly by production of reactive oxygen species and the RAGE pathway, leading to a reduction of tumour growth in melanoma cells (Riehl et al., 2009; Zhao et al., 2016). Moreover, PPARγ activation orients tumour associated macrophages (TAMs) toward an anti-inflammatory phenotype in BCa (Gionfriddo et al., 2020), reduces Treg response, and enhances GM-CSF–secreting tumour-cell vaccine (GVAX) power. Collectively this leads to an increase cancer rejection and improvement in tumour immunity when in combination with anti-CTLA-4 (Goyal et al., 2018). PPARγ activation also has synergistic activity with anti-PD1 in mouse colon cancer model (Chamoto et al., 2017). Conversely, in lung cancer activation of PPARγ stimulates tumour progression and metastasis via promotion of Arginase 1 expression in TAMs, which is considered an effector and a marker of pro-inflammatory (M2) phenotype macrophages (Li et al., 2011). Also, the activation of PPARγ mediated by paracrine Wnt5a/β-catenin signalling in DCs increases IDO activity and Tregs production, leading to immunotolerance (Zhao et al., 2018). In bladder cancer, high PPARγ expression impairs CD8+ T-cell infiltration, and consequently sensitivity to immunotherapies (Korpal et al., 2017). In line with these findings,

also PPARδ seems to promote cancer immune resistance. In fact, PPARδ macrophages recruitment and proliferation in colon cancer TME (Jeong et al., 2014), and it is involved into TAMs pro-tumorigenic polarization in ovarian carcinoma (Schumann et al., 2015).

3.3 Therapeutic ligands targeting lipid-sensing nuclear receptors

Besides the classic natural and synthetic LXR ligands, several ligands have recently been developed or discovered during investigations to evaluate if ligands of lipid-sensing are effective therapeutic targets in metabolic and cardiovascular diseases. For example, N-(4-trifluoromethylphenyl) 3,4-dimethoxycinnamamide (TFCA) derived from cinnamide, and the plant triterpenoid ursolic acid, act as LXRα antagonists reducing lipogenic genes activation and drug induced cellular lipid content in hepatic cells, potentially decreasing the risk to develop fatty liver and drug-induced hepatic steatosis (Lin et al., 2018; Sim et al., 2015). Ouabagenin, an aglycone of the steroid hormone Ouabain isolated from Strophanthus gratus, was reported to be a selective agonist for LXRβ and downregulates the expression of the LXR target gene epithelial sodium channel (ENaC), making it a potential diuretic treatment for hypertension (Tamura et al., 2018). Activation of LXRs transcription by the dietary plant oxysterol 28-homobrassinolide (28-HB) has positive metabolic effects reducing glycaemia and cholesterol levels in diabetic rats (Premalatha et al., 2014).

Other plant derived lipids, phytosterols, directly activate the LXRs (Plat et al., 2005) and interfere with oxysterol mediated activation of LXR (Hutchinson et al., 2019a). The array of LXR co-factors that can be recruited and/or exchanged in response to phytosterols remains largely unvalidated; only NCOA1/SRC1 has been experimentally confirmed as a phytosterol recruited co-activator and this was in a cell free assay (Plat et al., 2005). In cardiovascular disease models several novel LXR agonists with potential anti-atherosclerotic effects have been developed. The LXRβ agonist E17110 and the LXRα agonist IMB-170 increase ABCA1 and ABCG1 gene expression, reduce lipid accumulation and enhance cholesterol efflux in macrophages (Li et al., 2016; Li et al., 2014) and the dual LXRα/β agonist IMB-808 reduces macrophage lipid accumulation (Li et al., 2017).

In cancer, LXR ligands may confer different effects depending on the tissue. In liver cancer, LXRs activation by plant-derived product bergapten inhibits hepatocarcinogenesis by regulating PI3K/Akt and IDOL/LDLR pathways (Pattanayak et al., 2018), while the synthetic LXRs inverse agonist and degrader GAC0001E5 inhibits pancreatic cancer cells proliferation through the inhibition of oxidative stress and glutamine anaplerotic reactions (Karaboga et al., 2020; Srivastava et al., 2020). Similarly, two novel PPARγ ligands, lobeglitazone (LGZ) and CB11, were studied in papillary thyroid cancer (PTC) and non-small cell lung cancer (NSCLC).

Through the inhibition of TGF-β1 and p38 MAPK phosphorylation, LGZ impairs epithelial to mesenchymal transition and reduced migration and invasion of PTC cells (Jin et al., 2021). CB11 increased cell death, ROS production, cytotoxicity and cell cycle arrest in NSCLC cells via PPARγ activation (Kim et al., 2020).

In terms of FXR control of cholesterol and glucose metabolism, the anti-parasitic drug ivermectin, reduces glycaemia and cholesterol levels through the induction of FXR transcriptional activity (Jin et al., 2013). At the hepatic level, activation of FXR by hedragonic acid protects form drug induced liver injury and reduce liver inflammation in mice (Lu et al., 2018), while the FXR steroidal agonist BAR704 protects liver from inflammation and fibrogenesis through the downregulation of genes involved in these pathways (Carino et al., 2018). The plant-derived product isotschimgine activating FXR transcription has anti-steatotic and insulin-sensitizing properties in obese mice, suggesting it may be a potential NAFLD therapy (Li et al., 2020). Hepatic steatosis and inflammation in NAFLD can also be reduced by FXR ligand and immunomodulatory drug vidofludimus (Zhu et al., 2020). Obeticholic acid (OCA) was recently investigated as FXR agonist in liver diseases clinical trials, like NASH and biliary cirrhosis but its administration seems to increase cholesterol levels side-effects (further details available (Gege et al., 2014). However, OCA in combination with nitazoxanide has a synergistic tumour suppressive effect in colon cancer (Yu et al., 2021).

PPAR agonists have been investigated and developed into therapies for dyslipidaemia and diabetes (e.g. fibrates). The wide range of PPAR functions in metabolically active tissues means their value as therapeutic targets should extend to multiple diseases. In disease of glucose and lipid imbalance, the dual PPARα/γ partial agonist LT175 is an attractive candidate as it impairs adipogenic activity and improves glucose and lipid metabolism (Gilardi et al., 2014). Lipid levels are normalized with several dual PPARα/γ phytocannabinoid agonists derived from *C. Sativa*, which target PPAR gene transcription in adipocytes and hepatocytes (D'Aniello et al., 2019). Insulin-sensitizing and glucose-lowering properties have been attributed the PPARγ agonists UNIST HYUNDAI compound 1 (UHC1) and F12016 in obese and diabetic animal models respectively (Choi et al., 2014; Liu et al., 2015).

4. Perspectives

There are both experimental challenges and gaps in understanding that need to be addressed in the coming decade. For example, experimentally it remains unclear of the best way to measure activity of lipid-sensing NRs; should this be done by measuring either recruitment of co-activators (in cell based or cell free assays), the concentration of ligand, expression of the NR itself with/without co-factors, expression of NR target genes, or a combination?

Furthermore, distinct disciplines need to come together to address hypotheses that are intractable within a single discipline. For example, measuring lipid levels and type in cells, tissues or model lipid membranes, especially at the level of -omics or short angle x-ray scattering, is not in the domain of the typical clinical research setting or molecular biology wet lab. Computational modeling can give insight into lipid behaviour and protein function, but must be iteratively developed through pairing with 'wet-lab' experimentation to both validate findings and improve and revise hypotheses. Computational atomistic and coarse-grained modeling of intra and inter lipid-NR interactions, combined with methodologies such as x-ray scattering, atomic force microscopy, and cryo-EM will improve understanding at the molecular level of how lipid-NR moieties are formed and dissolved, and how they are regulated by other cellular processes and deregulated. Delicate bioinformatic approaches are required to combine different high dimensional data sets, so collaborative efforts between scientists in the fields of molecular biology, biophysics, and computational modeling and bioinformatics should be encouraged to address these challenges. More broadly, such combinatorial approaches most likely will have distinct statistical challenges including how data density is considered across sets, inevitable nomenclature issues (species-level, RNA, protein, metabolite relationships), and ultimately data visualization and availability.

There are also key gaps in understanding that remain. Newly identified nuclear receptor splice variants can be catalogued (in databases such as GTEX and TSVbd) but their experimental validation at peotein level is limited by detection of unique peptide sequences in proteomics, or antibodies that recognize alternative variants. Predictions based on genomic databases suggest extensive splicing in the NR family, with LXRα perhaps one of the most widely spliced genes, let alone members of the NR family. Only upon careful examination of primary tissues has the existence of some of these predicted splice variants been validated at the protein level. This suggests that either the spliceosome of cell lines are overly simplified, or that distinct cell populations within tissues contribute different splice variants. A major goal within the field therefore, is to define the exact range of protein variants that are expressed across different tissues and during development of different diseases. The methods to achieve such a goal for this are increasingly accessible. Specialized and targeted methods such as rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) (Mohammed et al., 2016), or integration of genomic and proteomic data (proteogenomics) (Komor et al., 2017) will yield more information than has been possible in previous efforts that had to rely on antibodies raised against peptides with a priori sequences. Indeed, unexpected bands in westerns may be a subtle clue that the NR of interest may be subject to unknown alternative splicing patterns and should not be immediately disregarded.

Another major gap in understanding is the role of pools of ligand. This is a particularly pertinent concept for the lipid-sensing NRs owing to their broad substrate repertoires, complexity in the type and quantity of different lipid species present in most tissues, and differing affinities or EC50 values for target NRs. Many studies to date have focused on the levels of individual NR ligands, but with improvements in lipid-omics and targeted mass-spectrometry methods, understanding how the pool of lipids is interacting the pool of lipid-sensing NRs is possible. The lipid-sensing NRs are broad affinity, and unlike the hormone or seco-steroid receptors, a cell may contain many variants of ligand-NR combinations leading to localized differences in transcription factor complexes. This again takes us back to the experimental challenge of how do we best measure genome wide activity of the lipid-sensing NRs.

The lipid-sensing NRs remain underexploited in the clinical setting. This is due to the complexity of their regulation rather than their functional involvement in disease processes. Indeed, given these complexities it is likely we may see the emergence of a wider range of lipid-based therapeutics and diagnostics as precision medicine becomes increasingly possible. Integrating disciplines and gaining a full understanding of ligand pools and the array of functional protein variants will be crucial in realizing the potential of these broad sensing mediators of cell and tissue physiology.

5. Figure legends

Figure 1: Structure of lipid-sensing nuclear receptors. The main transcripts and splice variants for LXRα (A), LXRβ (B), FXRα (C), and PPARs (D). AF = activation function domain; DBD = DNA binding domain; H = hinge region; LBD = ligand binding domain. Numbers above protein indicate feature position by amino acid number. Numbers with plus/minus sign indicate number of additional or lost amino acids relative to first variant. Data for figure acquired by Mr Alex Websdale and Ms Priscilia Lianto.

Figure 2: Complexity of the endogenous liver x receptor ligand pool. Sulfonated and esterified oxysterols are represented with [S] or [E] following their name. A sequence of three arrows in a row indicates a series of enzymatic functions leading to the synthesis of the next product. LXR ligands are shown in blue boxes, depth of colour indicates LXR transactivation potential according to (Hutchinson et al., 2019a; Hutchinson et al., 2019b; Janowski et al., 1999; Janowski et al., 1996; Plat et al., 2005). Compounds with unreported ability to induce a response from LXR shown in thatched boxes. Figure generated by Mr Alex Websdale.

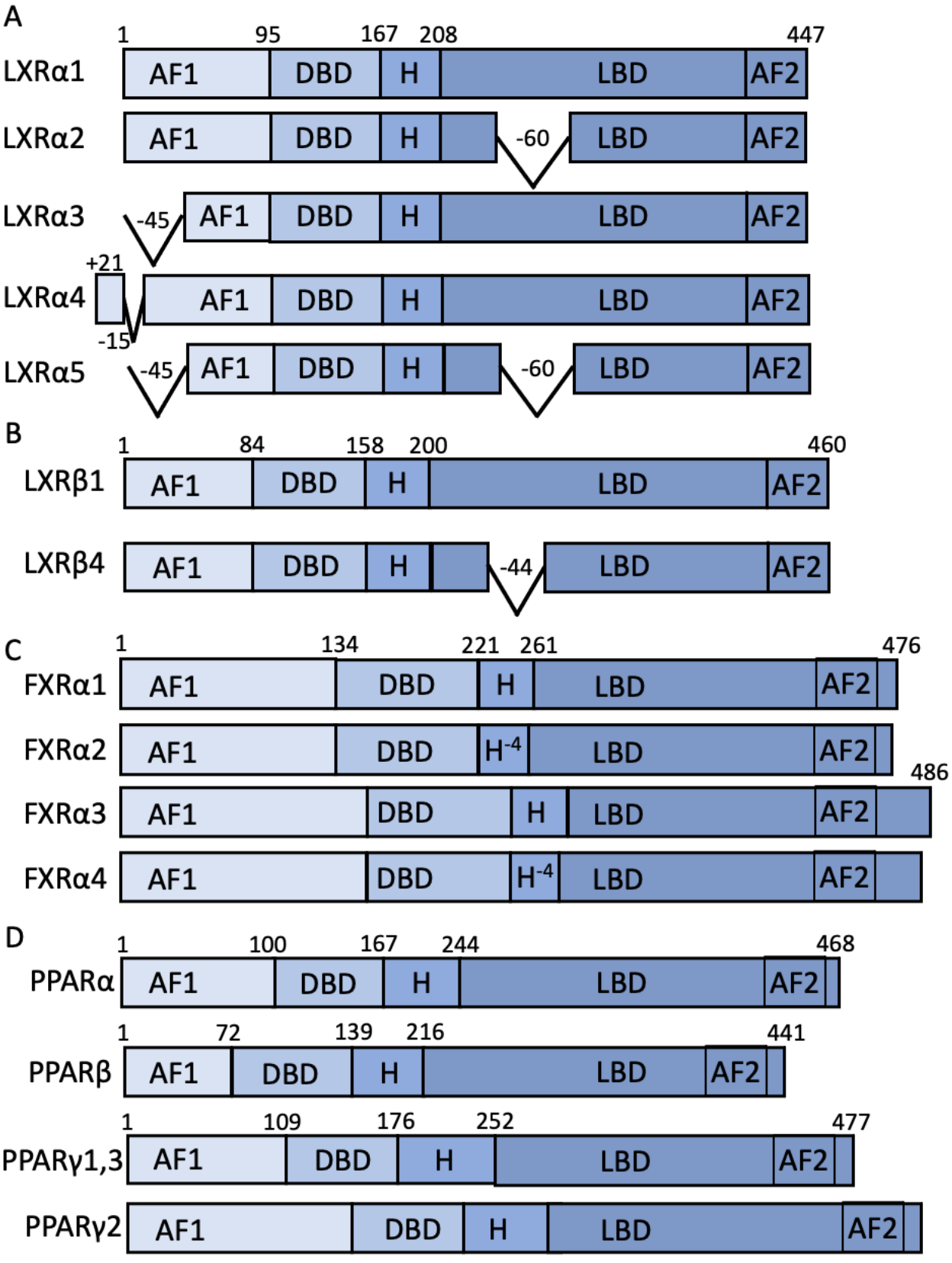
A
1
95
167
208
447
LXRα1
AF1
DBD
H
LBD
AF2
LXRα2
-60
LXRα3
-45
+21
LXRα4
-15
LXRα5
B
1
84
158
200
460
LXRβ1
LXRβ4
-44
C
1
134
221
261
476
FXRα1
FXRα2
H-4
486
FXRα3
FXRα4
D
1
100
167
244
468
PPARα
1
72
139
216
441
PPARβ
1
109
176
252
477
PPARγ1,3
PPARγ2

Figure 1

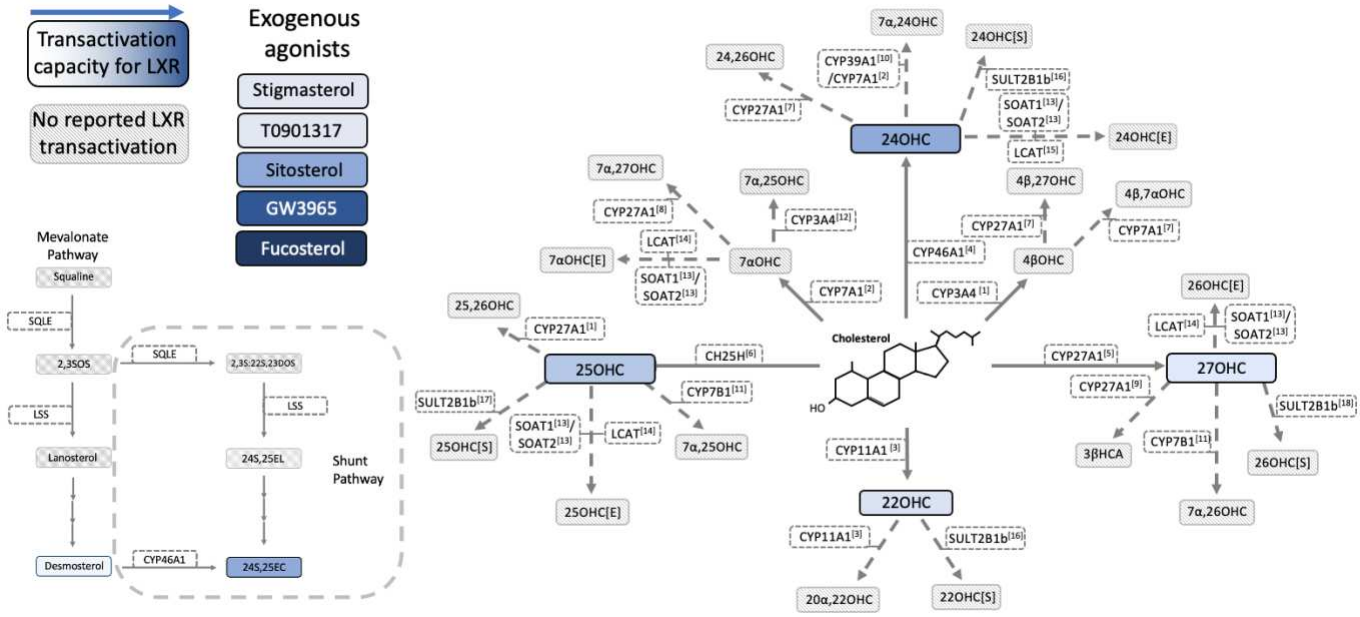
Transactivation capacity for LXR
No reported LXR transactivation
Exogenous agonists
Stigmasterol
T0901317
Sitosterol
GW3965
Fucosterol
Mevalonate Pathway
Squaline
SQLE
2,3SOS
2,3S:22S,23DOS
LSS
Lanosterol
24S,25EL
Desmosterol
CYP46A1
24S,25EC
Shunt Pathway
Cholesterol
HO
24OHC
24,26OHC
7α,24OHC
24OHC[S]
24OHC[E]
25OHC
25,26OHC
7α,25OHC
25OHC[S]
25OHC[E]
27OHC
26OHC[E]
26OHC[S]
7α,26OHC
3βHCA
22OHC
20α,22OHC
22OHC[S]
7αOHC
7α,27OHC
7αOHC[E]
4βOHC
4β,27OHC
4β,7αOHC
CYP39A1[10] /CYP7A1[2]
CYP27A1[7]
SULT2B1b[16]
SOAT1[13]/ SOAT2[13]
LCAT[15]
CYP3A4[12]
CYP27A1[8]
LCAT[14]
CYP46A1[4]
CYP7A1[7]
CYP7A1[2]
CYP3A4[1]
CYP27A1[1]
CH25H[6]
CYP7B1[11]
SULT2B1b[17]
CYP27A1[5]
CYP27A1[9]
SULT2B1b[18]
CYP11A1[3]
CYP11A1[3]
SULT2B1b[16]

Figure 2

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