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Orphan Nuclear Receptors as Targets for Drug Development

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Abstract

Orphan nuclear receptors regulate diverse biological processes. These important molecules are ligand-activated transcription factors that act as natural sensors for a wide range of steroid hormones and xenobiotic ligands. Because of their importance in regulating various novel signaling pathways, recent research has focused on identifying xenobiotics targeting these receptors for the treatment of multiple human diseases. In this review, we will highlight these receptors in several physiologic and pathophysiologic actions and demonstrate how their functions can be exploited for the successful development of newer drugs.

Keywords

agonist; antagonist; ligand binding domain; orphan nuclear receptor

INTRODUCTION

Nuclear receptors (NRs) define the largest superfamily of ligand-dependent transcription factors. They are involved in a wide variety of biological functions, including cell proliferation, differentiation, development and homeostasis (1, 2). Since the discovery of the first NR in the 1970s (3), several more structurally similar receptors were discovered, defining a new class of NRs called the “orphan nuclear receptors” (ONRs) (Fig. 1) (1). ONRs are defined by a lack of identifiable ligands controlling their physiological functions *in vivo*. In recent years, low affinity ligands have been discovered for some of the orphans and were subsequently classified as “adopted” ONRs (1, 2). The ligand-binding pocket of these adopted receptors (e.g. pregnane X receptor (PXR; NR1I2), liver X receptor (LXR; NR1H2 and NR1H3), farnesoid X receptor (FXR; NR1H4), constitutive androstane receptor (CAR; NR1I3 and NR1I4), peroxisome proliferator activated receptor (PPAR; NR1C2, NR1C3, and NR1C4), etc.) are larger than classical NRs and bind to a large diversity of molecules with lower affinity (1).

Functionally, ONRs are very similar to classical NRs. The classical function of NRs is to transcriptionally regulate expression of target genes by the recruitment of co-activators or co-repressors. Ligand binding to these receptors recruits the co-activators (activation) or co-repressors (repression), thereby regulating the coordinate expression of their target genes (Fig. 2) (4, 5). Generally, NRs bind to co-repressors in their un-liganded apo-form with histone deacetylase (HDAC) activity and act as a transcriptional suppressor (6, 7). With agonist ligand binding, conformational change in the helix 12 (H12) of the ligand-binding

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domain creates an open conformation of the NR holo-form favoring co-activator binding with histone acetyltransferase (HAT) activity for its activation properties (7). Discovery of antagonist molecules has been targeted to this H12 structure with the hypothesis that molecules that inhibit folding of H12 can act as antagonist for the corresponding NR (7). Though the classical mechanism of NR action is called transactivation, alternative mechanisms of NR action have also been reported (e.g., transrepression, where NRs, instead of binding directly to DNA, interact with other promoter-specific transcription factors to deactivate the target gene (8, 9), and non-genomic mechanisms, such as the very fast actions of NRs via membrane-associated signal transduction machineries (10, 11)).

Structurally, classical NRs contain four distinct functional domains: (1) AF1 or ligand-independent activation domain or A/B domain at the amino-terminal end, (2) DBD or DNA-binding domain containing two conserved C4-type zinc-finger motifs, (3) a highly variable flexible hinge region connecting the DBD with the (4) LBD or ligand-binding domain that is associated with second activation domain (AF2) at the extreme carboxy-terminal end (Fig. 3) (12). NRs bind to the target gene DNA response element using their conserved DBD. These response elements contain conserved hexameric sequences that can be arranged in several configurations, such as inverted or direct repeats (13, 14). Though the majority of ONRs possess all the functional domains common to the classical NRs, diversity is present in some ONR structures (1, 15). The structures of ONRs within the LBD in general and highly diverse (e.g. the ligand-binding pocket of PXR is very large and flexible due to the presence of two additional strands of β sheet, which explains the promiscuous nature of PXR binding to diverse range of compounds) (16–18). Some orphan receptors contain only one of the two characteristic domains (DBD or LBD) of the NR superfamily. In vertebrates, DAX-1 (NR0B1) and small heterodimer partner (SHP/SHP-1; NR0B2) contain only a LBD and lack a classic DBD (19, 20). In other species, such as *Drosophila* Knirps, KNRL and EGON (NR0A1, 2, 3) lack either of these domains (21, 22). The size of the domains in ONRs also varies, e.g., the A/B domain of some receptors is short (e.g., RORβ (NR2F2) and TLX (NR2E1)), whereas the same domain is quite large for NGFI-B/NR4A group members (15). The diversity of ONRs is also present in the modes of their DNA binding. While most of them bind to DNA as homodimers on direct repeat elements (e.g. HNF4 (NR2A1, A2, A3), COUP-TFs (NR2F1, F2, F3), TLX, and TR2/4 (NR2C1, C2)), some bind to DNA by interacting with retinoid X receptor (RXR) as a heterodimer partner (e.g. PXR, CAR, FXR, LXR and PPAR) (23), some oligomerize (e.g. GCNF (NR6A1)) on binding to a direct repeat (24), whereas several other orphan receptors (e.g. Rev-erbs (NR1D1, D2), RORs (NR2F1, F2, F3), SF-1 (NR5A1), NURR1 (NR4A2), NOR1 (NR4A3) and ERRs (NR3B1, B2, B3)) have been shown to bind DNA as a monomer to a half-site sequence (25). Additionally, estrogen receptor α, a ligand-activated NR, contains an additional carboxy-terminal F domain with unknown function (26). Furthermore, the NR diversity is also evident in their expressions in different species both vertebrates and invertebrates, such that there are 21 and more than 270 NR-like genes identified in *Drosophila melanogaster* and in *Caenorhabditis elegans*, respectively (27, 28). A list of vertebrate ONRs is presented in Table I, information that will be very useful to study individual receptor in these research animals for drug design and *in vivo* testing (1).

Because of this structural and functional diversity, promiscuous nature of DNA and ligand-binding properties (e.g. CAR and PXR) and their ability to bind to a broad range of molecules, thereby regulating a vast array of target genes, ONRs have become an attractive target for drug development. In this review, we will discuss how individual ONRs can be exploited (using agonist and antagonist molecules of these receptors) for a successful drug discovery in various human diseases (Table II).

NUCLEAR RECEPTOR DRUG DISCOVERY TOOLS

Identification of potential drug candidates for NRs traditionally has been done by using several drug development strategies (Fig. 4). The various strategies are described below.

In cell-based reporter assays where NR LBD is fused with DBD of yeast transcription factor Gal4, cells are transfected with this construct along with a reporter construct containing Gal4 upstream activation sequences (UAS) upstream of a reporter gene (e.g., luciferase, β-galactosidase). The activation and/or inactivation of the NRs by binding of a ligand is monitored by the expression of the reporter gene. Multiple modifications of this assay have been made since its discovery to allow for the measurement of receptor activation or inhibition by compounds and also to determine compound selectivity and potency (29).

In vitro ligand binding assays (e.g., scintillation proximity and fluorescent polarization assays) screen potential ligands by their competition with radiolabeled known ligands for the LBD of respective NR (30, 31).

Yeast and mammalian are two hybrid assays in which ligand-dependent NR-coactivator interactions are determined to identify potential compounds that augment/inhibit NR-coactivator interactions (32, 33).

Similarly, NR-coactivator interaction properties are exploited for more specific, sensitive and high-throughput assays, such as the fluorescence resonance energy transfer (FRET, or more specifically time-resolved FRET or TR-FRET) (34), ligand-sensing assay (LiSA) (35) and AlphaScreen assay (Amplified Luminescent Proximity Homogenous assay) (36). In FRET assays, NR and coactivator are labeled with proper fluorescent donor-acceptor probes (e.g., europian cryptate [Eu (K)]-cross linked conjugate of allophycocyanin (XL665) or cyan fluorescent protein (CFP)-yellow fluorescent protein (YFP)), and ligand-induced NR-coactivator interactions are measured by the energy transfer between the fluorophores. In TR-FRET assay, this energy transfer is time-gated in order to reduce short-lived fluorescent background, thereby increasing the sensitivity of the detection (37, 38). Additionally, the rapid fashion in which TR-FRET assay is accomplished gives the advantage of screening large drug samples in high-throughput format. In LiSA, similar to FRET assay, biotin-streptavidin interaction properties are used for fluorophore labeling of NR and coactivator. AlphaScreen is a bead-based assay where NR is coupled to donor beads and coactivator to acceptor beads. Ligand-induced conformation change in NR and hence its binding to co-activator triggers the donor-acceptor beads to come in proximity with subsequent generation of a signal.

Another assay, known as amide hydrogen/deuterium exchange, coupled with proteolysis and liquid chromatography-mass spectrometry (H/D-Ex), is gaining popularity as a drug development tool for the analysis of NR-ligand interactions (39). In this assay, the rate of amide hydrogen exchange depends on local fluctuations in the protein structure, and, for this reason, the rate of H/D-Ex exchange is a good indicator of protein conformational change. Hence, H/D-Ex is used to detect differences of protein dynamics in apo and holo forms of NR LBD.

Structural detailing of NR LBD has triggered the development of *in silico* approaches, like virtual ligand screening (VLS) for NR drug development. VLS is a knowledge-driven approach based on the structural information of either ligand (ligand-based approach) or NR target (receptor-or target-based approach). This method expands the concept of central similarity-property principle, which depicts that similar molecules exhibit similar properties. Based on this, similarity calculations can be performed, and molecules from large chemical

libraries can be screened and subsequently scored and ranked using computational methods (40).

Computational methods have become an extremely powerful tool not only to provide insights into novel agonist-antagonist interactions with NRs but also have facilitated the identifications of off-target interactions of NRs responsible for undesirable side effects (41). Results from computational methods have shown that NRs undergo significant conformational changes upon ligand binding or release, and the key factor of such NR-ligand interaction depends on the quaternary state of the receptor (41, 42). Computational studies have also been helpful to understand and characterize the structural domains of NR required for the recognition and specificity of interacting partners for its transcriptional activities (43, 44). Similarly, computational analysis of the three-dimensional coordinates of NR protein structure has helped to identify newer functional residues using the evolutionary trace computational methods, which are important for NR-coregulator interactions (45, 46).

Combined *in silico-in vitro* approaches have also shown their importance in studying evolutionary change in the LBD of different NRs responsible for their varying ligand specification across species (47). Finally, docking studies with structural similarity comparison methods have been helpful to understand the molecular mechanisms of adverse drug effects. This *in silico* approach, therefore, can be used to identify early off-target side effects of newly discovered drugs. This approach can also be expanded to drug repurposing or repositioning, where newer related therapeutic applications of older marketed drugs are exploited while eliminating the activity at the original target, thereby reducing the cost and time associated with the drug development processes (48, 49).

LIMITATIONS OF ONR-BASED PHARMACOLOGIC THERAPY

Drug adverse effects are a common cause of morbidity and mortality world-wide, and it is estimated that 20% of all adverse drug effects are due to drug-drug interactions (50, 51). Drug-drug interactions are a process by which administration of one drug alters the systemic drug levels (i.e. increasing or decreasing effective concentration) of another co-administered drug. Among the mechanisms of drug-drug interactions, drug metabolism and transport are considered to be most important, since they directly affect the therapeutic plasma concentrations of drugs administered, thereby determining drug toxicity or loss of drug response (52, 53).

Tissues that are mostly responsible for drug metabolism and transport are liver and the intestine. In these tissues, oxidative metabolism of drugs occurs through CYP450 group of enzymes, and drug efflux transport is mediated via MDR1 (also known as p-glycoprotein, ABCB1, ATP binding cassette subfamily B member 1) (52, 54). ONRs are critical in regulating these important mediators. Arguably, the most important ONR involved in drug-drug interaction is PXR, since it can be activated by a wide array of chemicals because of its promiscuous nature of ligand binding. Moreover, PXR regulates expression of the two most important drug metabolizing proteins—CYP3A4 (cytochrome P450 family 3 subfamily A member 4, the most abundant of the CYP450 isozymes in the liver and intestine) and MDR1—and is thereby responsible for the metabolism of ~50% of marketed drugs (55, 56). CAR is activated by fewer compounds than PXR but is responsible for similar effects on drug interactions via CYP3A4 and MDR1 (57). Additionally, FXR has also become an important ONR involved in drug interactions, since functional FXR response elements have been identified in CYP3A4 and another drug transporter MRP2 (58, 59). Since any given drug can activate more than one receptor and NR themselves regulate each other, this complex drug-drug interaction network regulated by ONRs should be very carefully exploited for safer pharmaceutical applications (60, 61).

While the numbers of compounds that can activate orphans have rapidly increased over the last decade, a few prescription drugs also cause clinically relevant drug-interactions. Drugs in these categories are anticonvulsants (e.g. phenobarbital, carbamazepine, phenytoin, valproic acid), antibiotics (e.g. rifampicin, nafcillin, rifabutin), human immunodeficiency virus protease inhibitors (ritonavir, nelfinavir, tipranavir) and non-nucleoside reverse transcriptase inhibitors (e.g. nevirapine, efavirenz) (62–66). Additionally, several herbal medicines (e.g. St. John's Wort, Ginkgo biloba) similarly affect drug-drug interactions through ONR signaling pathways (67, 68). In several disease conditions (e.g. tuberculosis, HIV infection, epilepsy) where chronic treatment is required, drugs used for these purposes can also activate ONRs as an off-target effect causing multiple side effects. For example, anticonvulsants and rifampicin (anti-tuberculosis drug) can cause hypothyroidism by increasing thyroid hormone turnover in liver via induction of glucuronidation, sulfation and biliary excretion (69, 70). Rifampicin treatment results in bone-loss and osteomalacia by interfering with vitamin D signaling via PXR (71). Additionally, HIV protease inhibitors by activating PXR and CAR are associated with the development of fatty liver (72).

Classical estrogen receptor exerts its effect on various tissues, and synthetic estrogens have shown its potential to act in tissue-specific manner (e.g. selective estrogen receptor modulators) (73, 74). While this effect is beneficial for receptor drug targeting to prevent off-target adverse effects, unfortunately, no tissue-specific pharmacologic agents targeting ONR have been described to date.

Since ONRs are very crucial as a drug development target, predictions of *in vivo* drug interactions have become very important for successful drug discovery. Several experimental systems have been employed that include cultured primary human hepatocytes, humanized mouse models, transformed cell lines (e.g. DPX-2, a derivative of HepG2 cells, that harbors human PXR and luciferase-linked CYP3A4 promoter and Fa2N-4 immortalized human hepatocyte clone), reporter gene assays, coactivator recruitment assays and receptor binding assays (55, 75–78).

Therefore, it is evident that both ONR agonist/antagonist therapies and prescription drugs causing off-target ONR effects could affect the therapeutic outcomes of treatment. Hence, serious considerations should be given while designing ONR-based pharmaceutics to avoid drug adverse effects.

PREGNANE X RECEPTOR (PXR): AGONIST AND ANTAGONIST—IMPLICATION FOR MULTITUDE OF DISEASES

Pregnane X Receptor (PXR) is an ONR encoded by the NR1I2 gene. It is involved in drug metabolism, bile acid transport, cancer, cholesterol metabolism and inflammation (79–81). While it is highly expressed in the liver and proximal small intestine, reduced levels of expression are seen in the large intestine (80, 82). PXR ligands, such as rifampicin, pregnenolone and phenobarbitone, are typically activators, although a small number of antagonists have also been identified, such as the ketoconazole (and related azoles), suphoraphane, ecteinascidin (ET-743) and coumesterol (83, 84).

Crystal structure of PXR LBD has shown that the ligand binding pocket of PXR is highly unrestricted because of its larger volume (more than 1,600 Å). Hence, it is able to function as a broad-specificity sensor of lipophilic xenobiotics and therefore regulate a vast array of target genes (16).

PXR has a substantial cross-species difference in terms of ligand binding. PXR LBD amino acid sequence identity has shown only 75% identity among human and rodents and 50%

among human and chicken or zebrafish sequences (85). Since PXR and CAR (discussed later in the article) play a major role in drug metabolism and they show interspecies sequence variation in terms of ligand-mediated gene transcription, drug bioavailability testing, efficacy and toxicity evaluations become difficult in animal models of human disease. To address these issues better, humanized mouse models of PXR, CAR and double humanized (both PXR and CAR) models have been created, where mouse PXR and CAR genes have been exchanged with their human counterparts (75, 86, 87).

PXR Functions and Therapeutic Implications

The metabolism of xenobiotics via CYP3A4 and MDR1 pathways are regulated by PXR (88, 89). By binding to the promoter region of CYP3A4 gene, PXR brings about its activation, leading to enhanced xenobiotic metabolism. Possible roles of PXR antagonist (e.g., ketoconazole) are that they may bind to PXR LBD and inhibit its interaction with its coactivator (SRC-1, steroid receptor coactivator 1), thereby inhibiting target gene activation (90). Inhibition of PXR causes decreased metabolism and, hence, increased bioavailability of bioactive compounds. This provides a novel mechanism (PXR antagonist) to increase bioavailability of the chemotherapeutic drugs, while induction of PXR by agonists can lead to drug resistance (91, 92).

Recent investigations have shown that PXR has an anti-apoptotic role in colon cancer cells, implicating its role in tumorigenesis. Anti-apoptotic role of PXR is independent of the xenobiotic metabolizing role. Instead, it is associated with up-regulation of multiple anti-apoptotic genes, including BAG3 (BAG family molecular chaperone regulator 3), BIRC2 (Baculoviral IAP repeat-containing protein 2) and MCL-1 (Induced myeloid leukemia cell differentiation protein), and down-regulation of pro-apoptotic genes, such as BAK1 (Bcl-2 homologous antagonist/killer) and TP53/p53 (Tumor protein 53/protein 53) (93). This antiapoptotic role is also seen in normal colonic epithelial cells. For the treatment of colon cancer, this information will be very useful in developing PXR antagonist. PXR transcriptionally activates organic anion transporter OATP1A2 (mediates cellular uptake of estrogen metabolites), and this effect leads to increased proliferative potential of estrogen in breast tissues. Specific PXR antagonists have been shown to inhibit proliferative effects of estrogen (94). Additionally, PXR activation has been shown to increase proliferative potentials of ovarian cancer cell-lines, further strengthening the basis for finding novel non-toxic inhibitors of PXR activation to control cell growth (91).

PXR transcriptionally activates dehydroepiandrosterone (DHEA) sulfotransferase (SULT2A1; phase II drug conjugating enzyme) and regulates bile acid metabolism by facilitating elimination of lithocholic acid from the body (95). Additionally, a complex network of ONRs (PXR, FXR, CAR, LRH-1(liver receptor homolog/NR5A2), HNFs, SHP) regulates genes involved in organic anion uptake (NTCP, OATPs), bile canalicular export (BSEP, MRP2) and alternative basolateral export (MRP3, MRP4) in liver (96–98). Furthermore, PXR has also been shown to inhibit cholesterol 7α hydroxylase (CYP7A1, rate-limiting enzyme in bile acid biosynthetic pathway) transcription via a complex regulatory mechanism involving HNF4α (hepatocyte nuclear factor α) and PGC-1α (peroxisome proliferators-activated receptor γ coactivator) (99). This describes a novel protective mechanism of PXR activation against bile acid-induced cholestasis. SULT2A1 also has a role in energy and lipid homeostasis, and this involvement may highlight treatment potentials for many metabolic disorders targeting PXR.

PXR also activates fatty acid uptake transporter CD36 (in a complex interregulatory network involving PXR/LXR/PPARγ) and several accessory lipogenic enzymes, such as stearoyl CoA desaturase-1 (SCD-1) and long-chain free fatty acid elongase (FAE) (100). This is

related to fat accumulation in the liver cells. It indicates that antagonism of PXR-related pathways may be utilized to treat alcoholic and non-alcoholic hepatic steatosis (101).

Other examples where PXR anatagonism would be beneficial are in disease conditions such as osteomalacia and acetaminophen-induced hepatotoxicity. PXR activators lead to osteomalacia by increased clearance of 1, 25 dihydroxyvitamin D_3 (102). Similarly, activation of hepatic PXR increases conversion of acetaminophen to hepatotoxic metabolites (103, 104).

Intestine-specific PXR/CYP27A1/LXRα pathway regulates intestinal cholesterol efflux and high-density lipoprotein (HDL) assembly, targeting mitochondrial sterol 27-hydroxylase (CYP27A1), which catalyzes oxidative cleavage of the sterol side chain in the bile acid biosynthetic Orphan Nuclear Receptor and Drug Development 1447 pathway in the liver and 27-hydroxylation of cholesterol in most tissues. PXR transcriptionally activates CYP27A1 to produce 27-hydroxycholesterol in intestine, which in turn activates liver X receptor α (LXRα) to induce cholesterol efflux transporters ABCA1 and ABCG1 in macrophages (105). Therefore, this provides the evidence of PXR as a target for regulation of cholesterol efflux and HDL assembly, indicating its role in hyperlipidemia.

Vitamin K_2 plays an important role in bone formation. It has been found that vitamin K_2 binds to and transcriptionally activates PXR. PXR mRNA is expressed in osteosarcoma cell lines, and vitamin K_2 with known PXR agonists induces the expression of the prototypical PXR target gene CYP3A4 in these cells (106). Thus, PXR is likely to be involved in the maintenance of bone homeostasis. This reveals a novel biological function of PXR and indicates that PXR agonists can function as effective therapeutic agents in the treatment of osteoporosis.

An inverse relationship was found between the PXR level and estrogen receptor (ER) status in breast cancer cells. It has been shown that PXR level is lower in ER+ breast cancer cells than in ER− cells. However, the level is the same in ER− cells as in normal cells. But there is no relation between the progesterone receptors. This may point to the fact that PXR has a role in breast cancer and may be utilized for the treatment for ER− tumor cells (107).

Promoter region of inducible nitric oxide synthase (iNOS) gene contains responsive elements for PXR. Since an iNOS-induced production of nitric oxide (NO) is known to influence inflammation and apoptosis, a PXR-regulated iNOS activity may explain a modulatory effect of steroids and xenobiotics on these cellular processes (108).

Besides that, PXR has also been shown to down-regulate NF-κB activation to inhibit inflammatory processes (109). Thus, potent PXR agonists can be used for the treatment of inflammatory diseases.

CONSTITUTIVE ANDROSTANE RECEPTOR (CAR): DRUG TREATMENT FOR LIVER CANCER AND METABOLISM

Constitutive androstane/active receptor is another NR from the same subfamily of NR1I as PXR. Apart from its lower levels of expression in heart, muscle, kidney and lung, it is predominantly expressed in the liver and small intestine. CAR can be activated by a wide variety of xenobiotics and is involved in phase I and II detoxification of drugs, steroid hormones, thyroid hormones and bilirubin. What sets it apart from classical NRs is that it is constitutively active even without any ligand, and ligand binding modulates its activity (12, 110). Other ONRs that show similar constitutive activities are RORα, LXRα, LRH-1, HNF4, NR4A subfamily of receptors and ERR (111–116). CAR remains sequestered in the

cytoplasm by binding to chaperone proteins, and after binding to its agonist, it translocates to the nucleus to bind (as a dimer with RXR) to its target gene regulatory element (62).

Discovery of the crystal structure of CAR LBD provides information about its constitutive activity and the molecular basis for inverse agonism. Additionally, it was also found that CAR ligand binding pocket is smaller (~675 Å) and less flexible than in PXR, making it less promiscuous (117). The structure of CAR bound to androstenol (inverse agonist) showed that this androstenol binding sterically blocks the constitutive active position of helix 12 (110, 118). While phenobarbitone (PB) and TCPOBOP (1, 4-Bis [2-(3, 5-dichloropyridyloxy)] benzene) are well known activators of CAR, there is a good number of CAR antagonists also available, including clotrimazole and androstenol (57, 110, 119). PB is not an agonist but a well-known activator of CAR. It facilitates nuclear translocation of CAR with subsequent activation but without directly binding to CAR (120). PB exerts its tumorigenic effects by causing sustained activation of CAR (121, 122). Besides PB and TCPOBOP, steroid hormones also modulate the CAR-mediated regulation of target gene transcription. Estrogens activate mouse CAR and induce the CYP2B10 gene in mouse liver, whereas androgens and progesterone repress estrogen-activated mouse CAR (123).

Similar to PXR, CAR also shows species specificity in regards to some of its agonists. For example, human CAR, unlike mouse CAR, does not respond to steroid hormones, and a single residue difference in the C-terminal region of the mouse *versus* human CAR (T350M) has been shown to be responsible for this species specificity (110, 117).

CAR receptor associates itself with many other NRs to exert its effects via many cross-regulatory pathways, some of which are discussed as follows with possible implications for drug discovery: PB causes liver cell growth and tumor promotion and regulates glucose metabolism, steroid and thyroid hormone metabolism, drug metabolism and bile acid synthesis. Upon activation by PB and numerous PB-type inducers, the orphan receptor CAR mediates those pleiotropic actions by regulating various target genes, utilizing multiple regulatory mechanisms. This provides an idea about the pleiotropic effects of CAR (124). Tumorigenesis by PB is induced by the DNA methylation, which is mediated by CAR as well. This methylation may have a role in the carcinogenesis. Hence, similar to PXR, CAR is also a potential candidate for tumor drug development, especially for hepatocarcinogenesis (125).

CAR induces metabolism of the thyroid hormones T4 and T3. With CAR agonist therapy, it was found that both T4 and T3 levels dropped. CAR participates in the molecular mechanisms contributing to homeostatic resistance to weight loss by regulating lipid and glucose metabolism (126–128).

Activation of CAR suppresses lipid metabolism by reducing lipogenic transcription factor SREBP-1 (sterol regulatory element binding protein 1) protein levels (129). CAR induces Insig-1, a protein with anti-lipogenic properties, which lead to reduced levels of active SREBP-1 with subsequent reduction in target gene expression involved in triglyceride synthesis (130). Hence, this information implies that CAR represents a novel therapeutic target to uncouple metabolic rate from food intake and has implications in obesity and its associated disorders. Through a multiplex promoter spanning 218 kb, the phase II UDP-glucurono-syltransferase 1A (UGT1A1) gene encodes at least eight differently regulated mRNAs whose protein products function as the principal means to eliminate a vast array of steroids, heme metabolites, environmental toxins, and drugs. It was found that CAR, in association with PXR, activates this pathway of metabolism as well, in addition to the P450 system (131). UGT1A1 is also associated with many genetic diseases associated with bilirubin clearance, like Crigler Najjar syndrome and Gilbert's disease. These findings may

provide new dimensions to the understanding of these disorders with newer treatment options.

LIVER X RECEPTOR (LXR): ROLE IN CHOLESTEROL HOMEOSTASIS AND INFLAMMATION

LXR is a member of the NR1H NR subfamily involved in the regulation of cholesterol, fatty acid and glucose homeostasis (132). Two isoforms of LXR have been described as LXRα and β. LXRα is expressed in the liver, kidney, intestine, fat tissue, macrophage, lung and spleen, while LXRβ is expressed in almost all tissues (hence, earlier it was called as ubiquitous receptor) (132, 133). Both LXRα and LXRβ can be activated by endogenous oxidized derivatives of cholesterol, oxysterols (132).

X-ray crystal structures of both isoforms of LXR are reported in complex with synthetic LXR agonist T0901317 (can also activate FXR, PXR and CAR). LXRβ LBD shows that T0901317 can adopt two distinct conformations in the ligand binding pocket because of the pockets larger size (~830Å). This explains the molecular basis of LXR activation by a wide range of endogenous ligands. The conservation of amino acid sequence among human LXR isoforms is also very high, which makes it difficult to design isoform-selective agonists (134, 135).

LXR Functions and Pharmacological Implications

LXR activates fatty acid synthase (FAS) gene expression through binding to a direct-repeat 4 (DR-4) element in the promoter. Another orphan receptor, LRH-1 binds to a distinct element of FAS gene 21 bases downstream to DR-4 element, which is critical for the maximal response of LXR towards FAS expression, and this binding of LRH-1 is blocked by SHP (136). Hence, LXR plays a role in fatty acid synthesis along with two other orphan receptors, LRH-1 and SHP. Fatty acids serve many specialized functions, including cholesterol esterification, lung surfactant production, mammary gland secretions, signaling molecules and many others, including energy storage. Fatty acid biosynthesis is regulated mainly through two enzymes: acetyl-CoA carboxylase (ACC, the rate-limiting enzyme in fatty acid biosynthesis) and FAS. Besides being regulated by LXR, FAS can also be independently regulated by a large number of signals, including insulin, fatty acids, thyroid hormone, sterols, oxysterols, glucocorticoids, growth factors and cyclic AMP. These signals exert effects via FAS promoter with binding sites for E-box binding proteins USF1 & 2, sterol regulatory element binding proteins (SREBPs), thyroid hormone receptor (TR), LXR and carbohydrate response element binding protein. Additionally, LXR activation by oxysterols has been shown to up-regulate transcription of SREBPs (particularly SREBP-1c), which explains a mechanism of coordinate regulation of homeostatic balance between fatty acids and sterols (137).

It has been found that LXR promotes reverse cholesterol transport and inhibits atherosclerosis. In the presence of LXR agonists T0901317 and 22(R) hydroxycholesterol, fluid phase pinocytosis of low-density lipoprotein (LDL) by the macrophages are suppressed (138). Recently, it has also been observed that NAD-dependent deacetylase SIRT1 (sirtuin 1, silent mating type information regulation 2 homolog) deacetylates and activates LXR, thereby potentially regulating reverse cholesterol transport (139). This shows that there are mechanisms to inhibit macrophage cholesterol accumulation and atherosclerosis, by inhibiting macrophage uptake of LDL by activated LXR. Hence, as a transcriptional regulator of genes, such as the ABC transport proteins and genes involved in fatty acid and cholesterol metabolism, LXR has become an attractive target for the development of drugs for atherosclerotic disorders.

However, though the desirable therapeutic effects of increasing reverse cholesterol transport and cholesterol catabolism on activating LXR-mediated gene transcription can be achieved, the undesirable side effect of increasing hepatic lipogenesis can occur in the presence of LXR agonists. Therefore, searching for a LXR antagonist has identified fenofibrate esters (not fenofibric acid), which represses LXR activation-mediated lipogenesis but without negating the beneficial roles of LXR in increasing reverse cholesterol transport and cholesterol secretion (140).

Androgen ablation therapy is the mainstay therapy for certain prostate cancers. But many patients who receive the ablation therapy develop androgen-independent tumors. It has been found that androgen paradoxically inhibits the Orphan Nuclear Receptor and Drug Development 1449 proliferation of these cells partially by down-regulating c-myc and inducing the CDK-inhibitor p27kip1, which causes cell-cycle growth arrest (141). It was also found that LXR agonists decrease proliferation of both androgen-dependent and -independent prostate cancer cells. This effect of LXR is mediated by the ATP binding cassette transporter A1 (ABCA1) as well as by LXR signaling (LXR/ABCA1/27-hydroxylase) in the retardation of progression of prostate cancer growth (142). This indicates that LXR may be involved in the prostate cancer progression *in vivo* and suggests LXR signaling may be a useful target for prostate cancer treatment.

Similarly, LXR may also play a role in breast cancer. LXR controls estrogen homeostasis by regulating the basal and inducible hepatic expression of estrogen sulfotransferase (EST or SULT1E1), which is critical for metabolic estrogen deactivation. Estrogen has an important role in normal physiology, as well as in breast cancer and other hormonal disturbances. Therefore, this represents LXR to be a novel target for drug development in the treatment of breast cancer (143). Recently, it has been shown that LXR activation suppresses colon cancer proliferation in a β-catenin-dependent pathway, further implicating its role in the regulation of cancer development (144).

Macrophages have a central role in innate immunity, by functioning as a scavenger for pathogens and apoptotic cells as well as by coordinating inflammatory response through production of cytokines and inflammatory mediators. It has been found that LXR inhibits genes involved in innate immune response and stimulate those involved in lipid metabolism, serving as a link between these two pathways. Additionally, LXR in macrophages can be transcriptionally activated by oxidized lipoproteins. LXR then acts to promote cholesterol efflux (via ATP-binding cassette transporters ABCA1 and ABCG1) from the cell to prevent lipid overload and limit the production of inflammatory mediators (145, 146). Thus, LXR may function to integrate metabolic and immune signaling. Many of the genes inhibited by LXR are established targets of NF-κB signaling (e.g., iNOS, COX-2 (Cycloxygenase-2), MMP-9 (Matrix metallopeptidase 9) etc.) (145). This role may be important in atherogenesis. Oxidized lipids may act as inducers or inhibitors of inflammatory gene expression, depending upon the context—the inhibitory effects being mediated via LXR. Since the anti-inflammatory effect of LXR is not limited to promoting cholesterol efflux (which has a direct implication in atherosclerosis) but to decrease the inflammatory mediators as well, LXR agonists may have the utility in the treatment of other chronic inflammatory disease where macrophages play an important role (e.g., osteoarthritis, contact dermatitis, etc.).

Cholesterol is an important constituent of mammalian cell membranes and is a major constituent of myelin. It plays an important role in central nervous system (CNS) synapto-genesis and is essential for optimal neurotransmitter release. Most of the cholesterol is synthesized *in situ*, and to maintain homeostasis, cholesterol leaves CNS in the form of 24(S)-hydroxycholesterol, which is secreted across blood-brain barrier in high

concentrations. It was found that patients with Alzheimer's disease have high circulating levels of 24(S)-hydroxycholesterol, which might be a reflection of ongoing neurodegeneration. LXR, which has a close relationship with cholesterol metabolism, is expressed in the brain and is related to cholesterol efflux from CNS. Therefore, it has been suggested that LXR may have an implication in neurodegenerative diseases as well by modulating CNS cholesterol content, cholesterol efflux and inflammation. In fact, it was found that astrocytes treated with synthetic LXR ligands exhibit enhanced cholesterol efflux and increased expression of LXR target genes (ABCA1, ABCG1 and apoE) (147), whereas loss of the two isoforms of LXR in mice led to degenerative processes in brain characterized by enhanced lipid accumulation, astrocyte proliferation and disorganized myelination (148).

FARNESOID X RECEPTOR (FXR): IMPLICATING TREATMENT OPTIONS FOR HYPERTRIGLYCERIDEMIA

Farnesoid X Receptor is a nuclear hormone receptor encoded by the NR1H4 gene. It is similar in form to PPAR, LXR and RXR. When activated by its ligands, such as chenodeoxycholic acid, it translocates to the cell nucleus. One important function of FXR is to suppress the expression of cholesterol alpha hydroxylase (CYP7A1), which is a rate-limiting enzyme in bile acid synthesis (37).

Crystal structure of FXR LBD (~720 Å) has been reported in complex with coactivator peptide (GRIP-1) and two different bile acids. With the availability of FXR crystal structures, newer potent therapeutic bile acids and non-steroidal FXR modulators can be designed for the treatment of hyperlipidemia and cholestasis, where FXR plays a major regulatory role (149). FXR is highly expressed in the liver, gut, kidney, and adrenal cortex and at low levels in heart, lung, stomach, and adipose tissue.

FXR Functions and Therapeutic Implications

It has been found that bile acids repress the transcription of CYP7A1, which catalyzes the rate-limiting step in bile acid biosynthesis. It was shown that bile acids activate FXR, which leads to the expression of orphan receptor SHP-1. SHP-1 in turn inhibits LRH-1, which is another orphan receptor known to regulate CYP7A1 expression positively. Therefore, FXR plays an important role in the coordinated regulation of cholesterol and bile acid synthesis in FXR/SHP-1/LRH-1 cascade (150).

FXR is a good therapeutic target for cholesterol gall stone disease, and this effect is mediated by the FXRmediated expression of bile acid transporter ABCB11 and ABCB4 (151). A new class of FXR agonist, 1, 1-bis-phosphonate esters has been discovered that up-regulates the bile acid transporter intestinal bile acid binding protein (I-BABP), which, like other agonists, increases the degradation of HMG-CoA reductase leading to hypocholesterolemia in normal animals (37).

In another study, FXR has been found to negatively regulate serum HDL and apolipoprotein A-I (apoA-I) levels. Ligand-activated FXR directly binds to apoA-I promoter and decreases its expression (152). As serum levels of HDL and risk of coronary heart disease (CHD) are inversely correlated, potent FXR antagonists could be promising in the treatment of CHD by raising serum HDL levels.

Syndecan-1 (SDC1) is a member of the family of transmembrane heparan sulfate proteoglycans, which are widely expressed in many cell types and tissues and has a significant contribution to the lipoprotein metabolism. Their principal function is to modulate the ligand-dependent activation of primary signaling receptors at the cell surface, leading to an increase in binding and/or internalization of extracellular ligands. It is highly

expressed in the liver. In liver heparan sulfate proteoglycans (HSPGs) bind to lipoproteins via several accessory proteins, such as the lipoprotein-lipase, apoE, and hepatic lipase, which are crucial for binding and sequestering of lipoprotein remnants before transfer to specific receptors (such as the LDL receptor) with subsequent endocytosis. It has been found that hepatic SDC1 is induced in a FXR-dependent manner. Therefore, increased expression of SDC1 may be one mechanism by which administration of chenodeoxycholic acid (naturally occurring FXR ligand) and synthetic FXR ligand GW4064 leads to hypotriglyceridemic effect (153).

The plant sterol guggulsterone, which has been used to treat hyperlipidemia, has been found to be an antagonist of FXR and to decrease expression of FXR target genes (154). Although acting as an antagonist, it enhances FXR agonist-induced transcription of bile salt export pump (BSEP), which is a major hepatic bile acid transporter. It has been proposed that it mediates its effects via SHP, a known FXR target. Therefore, guggulsterone defines a novel class of FXR ligands characterized by antagonistic activities by coactivator association assays but at the same time can enhance the action of agonists on BSEP expression *in vivo*.

Human kininogen belongs to plasma kallikrein-kinin system. High molecular weight kininogen is the precursor of two-chain kinin-free kininogen and bradykinin. The former has properties of anti-adhesion, anti-platelet aggregation, anti-thrombosis, while the latter is a potent vasodilator and mediator of inflammation. It has been found that human kininogen gene is strongly up-regulated by agonists of the FXR. FXR response element (inverse repeat, IR-1) was also found in the promoter of kininogen at −66/−54, where FXR-RXR heterodimer binds. Hence, it means that FXR and its agonists (e.g., bile acids) may play a role in vasodilatation and anti-coagulation process (155).

FXR directly regulates the expression of FGF-19 (FGF-15, mouse ortholog), which is a member of the fibroblast growth factor family of signaling molecules (156). FGFs bind to the extracellular domain of their cognate cell surface receptor (FGFRs) and induce receptor dimerization and tyrosine kinase phosphorylation, which, in turn, leads to the activation of a number of intracellular pathways. This FXR/FGF-19 pathway is also involved in the bile acid homeostasis. In mice lacking apical ileal bile acid transporter (*Asbt* −/− mice, animal model of bile acid malabsorption), it was found that the FXR/FGF-15 pathway is disrupted. With the treatment of *Asbt* −/− mice with either FXR agonist or FGF-15 peptide, CYP7A1 expression becomes less. This suggests that FXR agonist (and/or FGF-15 peptide) could be used in the treatment of patients of bile acid malabsorption to decrease excessive bile acid synthesis (157). It is understood that the FGFs regulate cell growth, differentiation, and morphogenesis; however, it is now apparent that some of these proteins are also important components of specific homeostatic pathways (e.g., bile acid homeostasis). It means that FXR has more implications than previously thought which expands its importance in drug discovery targeting FXR/FGF-19 pathway. FXR has also been shown to play a role in tumor formation. It was also observed that FXR deficiency results in increased colon cell proliferation and tumorigenesis, which is accompanied by the up-regulation of the genes involved in cell-cycle progression and inflammation (e.g. cyclin D1 and interleukin-6) (158). Additionally, in breast cancer cells, FXR plays a pro-apoptotic role by regulating the expression of genes involved in the transport of bile acids, amino acids and xenobiotics (159). Hence, activation of FXR could be a novel intervention strategy for the protection of intestinal and breast carcinogenesis.

FXR also regulates the expression of various transport proteins and biosynthetic enzymes crucial to the physiological maintenance of lipids, cholesterol and bile acid homeostasis (38).

PEROXISOMAL PROLIFERATOR-ACTIVATED RECEPTOR (PPAR): DRUG TREATMENT FOR DIABETES, LIPID DISORDERS, ACNE AND INFLAMMATION

PPAR is another class of ONR which has been very well studied. It was initially described in *Xenopus* frogs, where it leads to the proliferation of peroxisomes (160). PPAR was shown to increase peroxisomes in the rodent liver apart Orphan Nuclear Receptor and Drug Development 1451 from improving insulin sensitivity; hence, they were called peroxisome proliferator activator receptors. There are three types of PPAR described so far: PPARα, γ and δ/β, the latter two being closely related (161).

X-ray crystal structures of the PPARα, γ and δ ligand binding domains (LBDs) have revealed that the receptors contain a much larger ligand binding pocket (~1,300 Å) compared to other NRs (162–165).

The size of this pocket may explain the ability of the PPARs to bind a variety of naturally occurring and synthetic lipophilic acids. Known endogenous ligands for PPARs are free fatty acids and eicosanoids (166). Leukotrienes B4 is specific for PPARα, while prostaglandin J2 (PG-J2) is specific for PPARγ (167). Each class of PPAR has its own modulators targeting their functions. PPARα is targeted by the fibrates, such as clofibrate, fenofibrates, gemfibrozil, etc., which are used in the treatment of hyperlipidemias. PPARγ modulators include thiazolidinediones (TZD), which are used as anti-diabetic drugs, although some interactions with NSAIDs have been demonstrated as well (168–170). PPARδ modulators, still in the experimental stage, include GW501516 (171). A new experimental class of PPAR modulators that affect more than one class of PPARs includes muraglitazar and tesaglitazar, which are being developed for metabolic syndromes (172). PPARα is distributed mainly in the liver, kidney, heart, muscle, and adipose tissue. PPARδ/β are mainly expressed in the brain, adipose tissue and skin. PPARγ has a very wide distribution in the body and is found in three forms formed by alternate splicing in the same gene. PPARγ1 is expressed in heart, muscle, kidney, colon, pancreas and spleen, while PPARγ2 is expressed mainly in adipose tissue, and PPARγ3 is expressed in macrophages and adipose tissue (161).

PPAR Functions and Therapeutic Implications

Cannabinoids (CB) are a group of terpenophenolic compound present naturally in nervous and immune systems of mammals (173). CB activate G-protein coupled receptor CB1 (cannabinoid receptor) to inhibit calcium-induced neurotoxicity (174). Studies in CB receptor knock-out mice have revealed non-CB receptor-mediated responses both in CNS and periphery (175). These non-CB responses are shown to be mediated via PPARs (176). The monounsaturated analog of the endocannabinoid anandamide oleoylethanolamide regulates feeding and body weight, stimulates fat utilization and has neuroprotective effects mediated through activation of PPARα. Other CBs, like palmitoylethanolamide, anandamide, virodhamine and noladin, also act via PPARα to regulate lipid metabolism. Few (anandamide and 2-arachidonoylglycerol) act on PPARγ to exert anti-inflammatory activities. This opens a new domain of application of PPARs as a target of naturally occurring CB. This also supports the importance of PPARs as a target for lipid disorders and also for neuroprotective and cardioprotective tretaments (177).

PPARγ agonist TZDs are used clinically to treat insulin resistance and diabetes, disease conditions strongly associated with obesity (178). It is believed that elevated fatty acids produced by adipose tissues promote insulin resistance, resulting in increased hepatic gluconeogenesis and decreased glucose utilization in the periphery (179). TZDs induce the

expression of genes involved in adipocyte differentiation and lipogenesis through PPARγ activation, and these mechanisms are responsible for the insulin-sensitizing actions of these drugs in the treatment of type II diabetes (180, 181). Additional mechanisms of TZD action in diabetes have also been discovered, where TZDs target adipocyte gluconeogenesis (182). Gluconeogenesis is a process, where glycerol-3-phosphate is produced from precursors other than glycerol or glucose in adipose tissue when glucose utilization is reduced during fasting. Hence, gluconeogenesis in adipocytes acts as a fatty acid homeostatic pathway that allows the re-esterification of fatty acids (FAs) from glycerol-3-phosphate for triacylglycerol synthesis at the time when their breakdown is occurring through lipolysis. This action therefore prevents the release of FAs into the blood. Phosphoenol pyruvate carboxy kinase enzyme (PEPCK) is the central regulator of gluconeogenesis and is being closely coupled with cytosolic aspartate transaminase (cAspAT) in the liver. It was found that TZD responsiveness of cAspAT is dependent on PPARγ and protein synthesis. This effect of TZD on PEPCK and cAspAT leads to hypolipidemia by inducing gluconeogenesis, which is a novel mechanism of anti-diabetic drug therapy by TZDs (179, 182).

Recently, many NRs expressed in skeletal muscle have been shown to improve glucose tolerance, insulin resistance, and dyslipidemia. Skeletal muscle and NRs are rapidly emerging as critical targets in the battle against cardiovascular disease risk factors. It has also been found that estrogen receptor-related receptor (ERR) stimulates PPARα-mediated energy metabolism in cardiac and skeletal muscle cells via activation of medium chain acyl-CoA dehydrogenase (183). Understanding the function of NRs in skeletal muscle has enormous pharmacological utility for the treatment of cardiovascular disease and for obesity (184).

Activation of orphan receptors, in particular activation of PPARα and PPARγ, can regulate lipogenesis in human sebaceous glands. PPARγ activation induces COX-2 expression in sebocytes, which leads to sebocyte proliferation and/or lipogenesis (185). As suppression of sebum secretion is associated with reduced acne activity, the NRs involved may open new avenues in the development of novel acne treatments.

Both PPARα and PPARγ receptor subtypes have been reported to regulate inflammatory responses, both *in vivo* and *in vitro*. Leukotriene B4 (LTB4) is an endogenous ligand for PPARα, which leads to transcription of genes of the ω- and β-oxidation pathways that can catabolize LTB4 itself. Activation of PPARα by NSAIDs contributes to the anti-inflammatory, antipyretic, and analgesic properties of these drugs through stimulation of oxidative pathways involved in the catabolism of eicosanoids. PPARγ regulates the activity of iNOS, and activation of this PPAR subtype controls inflammation by diminishing nitric oxide production. PPARγ has shown involvement in other inflammatory pathways as well. This provides a new avenue to develop treatment for autoimmune diseases, which has not been well explored so far (186).

All-trans retinoic acid (ATRA) leads to activation of apoptotic pathways via its interaction with RAR/RXR dimers. But it was also found to bind to PPARβ/δ with nanomolar affinity, modulating the conformation of the receptor, promoting interaction with the coactivator SRC-1, and efficiently activating PPARβ/δ-mediated transcription, leading to activation of growth promoting and antiapoptotic pathways. While the current use of retinoids as chemotherapeutic agents is due to its growth-inhibitory effects mediated via RAR, it can also promote growth via activation of PPARβ/δ (187).

PPARδ has a wide expression pattern in adult animal and is expressed very early during embryogenesis (188). PPARδ gene disruption is lethal due to placental defect. The surviving

knock-out mice are smaller than their control, and they have reduced body fat mass, skin defects and alterations in myelinisation (189).

PPARδ also plays important role in lipid absorption in the intestine by directly regulating genes involved in lipid uptake, such as fatty acid binding protein and fatty acid translocase (190). Furthermore, activation of PPARδ has been shown to regulate lipid metabolism by activating fatty acid oxidation (191). Consistent with this role, it was observed that PPARδ expression increases in muscle during physical exercise with subsequent increase in fatty acid burning (192). Hence, PPARδ agonist treatment could have therapeutic usefulness in metabolic syndromes to increase insulin sensitivity and obesity (193).

Human immunodeficiency virus long terminal repeat (HIV-1 LTR)-driven transcription is regulated by NR-responsive element (NRRE) located in its promoter. This NRRE contains tightly clustered binding sites for RXRα, RARα, apolipoprotein AI regulatory protein, HNF-4, NGFI-B and PPAR. These findings suggest that a complex network of NR signaling pathways that include 9-cis- and all-trans-retinoic acid, fatty acids, peroxisome proliferators, growth factors, membrane depolarization, and possibly other signals, converge onto the HIV-1 NRRE and may participate in modulation of viral gene expression (194). PPARγ activation has also been shown to suppress HIV-1 replication in an animal model of encephalitis, further implicating the role of PPARs in anti-viral drug treatment (195).

NURR1: ROLE IN DOPAMINERGIC DYSFUNCTION AND SYNOVIAL INFLAMMATION

NURR1 belongs to the NR4A subfamily of ONRs and is expressed predominantly in the CNS, especially in the substantia nigra, the ventral tegmental area, the midbrain and limbic areas (196, 197). Recent reports have indicated its essential role in the development and survival of dopaminergic neurons (198). The purine anti-metabolite 6-mercaptopurine is reported to act as an agonist of NURR1, and it activates NURR1 transcription (199). Crystal structure of the NURR1 LBD has been reported and shows its two distinctive features. First, the NURR1 lacks a classic ligand binding pocket because of the tight packing of side chains. Second, NURR1 lacks a classical binding site for coactivators. Despite these features, the structure shows that NURR1 can be transcriptionally activated in a ligand-independent fashion (200).

Biological Importance of NURR1 as a Drug Target

Studies with NURR1 knock-out mice have shown that NURR1 deficiency results in impaired dopaminergic activity and apoptosis in midbrain dopaminergic neurons, which degenerate in Parkinson's disease (PD). Mutations in the gene encoding NURR1 and decreased NURR1 expression have been associated with disorders related to dopaminergic dysfunction, including PD, schizophrenia and manic depression (201, 202). Therefore, selective NURR1 agonist with high potency could be exploited for the prevention and treatment of PD.

Additionally, NURR1 is found to be expressed in inflamed synovial tissue. It was shown that enhanced binding of NF-κB and cAMP response element binding protein (CREB) to NURR1 promoter by inflammatory mediators (e. g., IL-1β, TNF-α, PG-E2) increases local production of NURR1 in the inflamed joints. NURR1 in turn acts as the mediator of an autocrine regulatory inflammatory cascade to amplify the inflammatory response by increasing synovial corticotrophin-releasing hormone (CRH) expression (203, 204). Hence, NURR1 transcriptional regulation can be selectively modulated using antagonist compounds for the prevention of inflammatory joint diseases.

NUR77: CANCER TREATMENT OPTIONS

Another member of the NR4A superfamily of ONRs, nerve growth factor IB (NGFIB or Nur77), is involved in many Orphan Nuclear Receptor and Drug Development 1453 biological processes, such as cell-cycle regulation, apoptosis and inflammation (205). However, a physiological ligand for Nur77 has not been identified. The octaketide cytosporone B (Csn-B) has been found to be a naturally occurring agonist for Nur77. Csn-B specifically binds to the LBD of Nur77 (modeling is based on the crystal structure of Nur77, PDB code 2QW4) and stimulates Nur77-dependent transactivation (206).

Csn-B has been found to elevate blood glucose levels in fasting mice by inducing multiple genes involved in gluconeogenesis pathway. These biological effects were not observed in Nur77-null mice, indicating that Csn-B regulates gluconeogenesis through Nur77 (206).

Nur77 has been shown to induce cytochrome c release and apoptosis through interaction with anti-apoptotic protein Bcl-2. Nur77 binding to Bcl-2 induces a conformational change in Bcl-2, resulting in conversion of Bcl-2 from a protector (anti-apoptotic) to killer (apoptotic) protein (207, 208). This suggests that Nur77 could be a new target for novel therapeutic applications for cancer.

Very recently it was also shown that a short Nurr77-derived peptide NuBCP-9 has a protective role in drug-resistant breast tumors (209). Novel compounds with Nur77 agonistic activity, such as 1, 1-bis (3′-indolyl)-1-(phenyl) methane (DIM-C-Ph) and 1, 1-bis (3′-indolyl)-1-(*p*-anisyl) methane (DIM-C-pPhOCH$_3$) have been discovered recently and have been shown to act as anti-colon-cancer drugs (210).

TLX (HUMAN HOMOLOG OF *DROSOPHILA* TAILLESS GENE): INVOLVEMENT IN NEURODEGENERATION AND RETINAL DEGENERATION

Orphan receptor TLX, encoded by the NR2E1 gene, is specifically expressed in the brain and has an important role in vertebrate brain and eye functions (211–213). It is the human homolog of *Drosophila* tailless gene. It plays an essential role in the maintenance of neural stem-cell proliferation and self-renewal in the adult mouse brain (214, 215). Additionally, TLX is a key component of retinal development and is essential for vision (216). Though the crystal structure of TLX is not available to date, identification of endogenous and synthetic TLX ligands are under investigation using novel affinity/GC-MS technology, chemical screening and cell-based assays.

Targeting TLX for Neuro- and Retino-degenerative Therapeutics

TLX is important for the formation of superficial cortical layers in embryonic brains, regulation of neurogenesis and patterning of lateral telencephalic progenitor domains during development. TLX-expressing neural cells can proliferate, self-renew and differentiate into all neural cell types, whereas TLX-null neural cells show a significant reduction of cell proliferation (217). This shows the importance of TLX in neural development (214).

TLX is also found to be expressed in retinal progenitor cells in the neuroblastic layer during the period of retinal layer formation and is crucial for controlling the generation of appropriate numbers of retinal progenies. The TLX knock-out neural retinas were significantly thinner than controls (218). It is well known that malformations in the eye can be caused by either an excess or deficiency of retinoids, by regulating the expression of its early target gene, retinoic acid receptor β (RARβ). A TLX response element has been identified in RARβ promoter, which is important for retinoic acid-mediated induction of RARβ. These results show an important role for TLX in autologous regulation of the RARβ

gene in the eye, critical for its development (219). TLX-Pax2 (paired box homeodomain transcription factor) regulatory network has also been identified as involved in vertebrate eye development. It has been shown that TLX by binding to Pax2 gene promoter represses its expression, which is essential for retinal development and vision (216).

RETINOIC ACID RECEPTOR-RELATED ORPHAN RECEPTOR (ROR): MAINTENANCE OF CHOLESTEROL HOMEOSTASIS

ROR is a member of NR1F subfamily of ONRs. The RORs are somewhat unusual in that they appear to bind as monomers to hormone response elements as opposed to the majority of other NRs which bind as dimers (1, 220). There are three subtypes of ROR: RORα, β and γ. RORα is expressed in specific areas of the brain, including purkinje cells in the cerebellum and the suprachiasmatic nucleus of the hypothalamus. It is also expressed in the spleen, thymus and macrophages (220, 221).

Crystal structure of RORα has been solved in complex with cholesterol-3-O-sulphate, suggesting that cholesterol sulphate could regulate RORα functions *in vivo* (222). Despite the high homology between RORα and RORβ LBD, cholesterol is not a ligand of RORβ; instead, RORβ LBD has been shown to bind all-trans retinoic acid (ATRA) in crystal structure (223). ATRA has been shown to work as an antagonist for the constitutively active RORβ.

ROR Disease Associations

RORα knock-out mice, which show a stagger phenotype, exhibit ataxia resulting from neurodegeneration in the cerebellum involving the purkinje cells (224). Furthermore, the staggerer mice display lowered plasma apoAI/II levels, decreased plasma HDL cholesterol and triglycerides, and develop hypo-α-lipoproteinemia and atherosclerosis (225).

It has been shown that the muscle carnitine palmitoyltransferase-1 and caveolin-3 promoters are directly regulated by RORα, implying that RORα could play an important role in the control of lipid homeostasis in skeletal muscle (226). It has also been reported that cholesterol and its sulfonated derivatives might function as RORα ligands. Additionally, it was shown that oxysterol 7α-hydroxylase (CYP7B1), which plays a critical role in cholesterol homeostasis, is a RORα target gene. Studies in RORα- and LXR-deficient mice have revealed an interesting functional crosstalk between them in endobiotic metabolic gene regulation (227). These data suggest that RORα could have an important role in the maintenance of cholesterol homeostasis and thus could be used for the treatment of cholesterol-related diseases (222, 228).

RORβ is expressed in areas of CNS that are involved in the processing of sensory information and the circadian rhythm. Therefore, it could be possible that target genes of RORβ play an important role in sensory input integration and maintenance of biological clock. Besides, RORβ knock-out mice display a duck-like gait, disrupted reproduction in males, blindness and abnormal circadian rhythm (229, 230). These observations suggest that RORβ ligands can become very useful for RORβ-related CNS disorders.

In contrast to other ROR genes, RORγ is not expressed in the CNS. Instead, RORγ is found at high levels in skeletal muscle and thymocytes. RORγ knock-out mice lack peripheral and mesenteric lymph nodes and peyer's patches (231). Though the functional role of RORγ is not fully characterized, its role in lymphoid organogenesis could be exploited for thymopoiesis and the maintenance of T cell homeostasis.

DOSAGE-SENSITIVE SEX REVERSAL-ADRENAL HYPOPLASIA CONGENITA CRITICAL REGION ON THE X CHROMOSOME GENE 1 (DAX-1): BONE DEVELOPMENT AND GLUCOCORTICOID RECEPTOR MODULATOR

DAX-1 is a member of the NR0B subfamily of ONRs. It acts by inhibiting the activity of other NRs, such as steroidogenic factor 1 (SF-1), estrogen receptor and androgen receptor by heterodimerization, thereby acting as a negative regulator of steroidogenesis. It is involved in controlling the development of the hypothalamic-pituitary axis, as well as in gonadal development and sex determination (232). DAX-1 is expressed primarily in reproductive tissues (ovary, testis, and uterus), endocrine tissues (adrenal gland) and the CNS (pituitary and hypothalamus) (232). While the crystal structure of DAX-1 is unavailable, DAX-1 LBD homology model have been constructed to get an idea about the position of mutations and deletions in DAX-1 gene that are responsible for gonadal dysfunction (233). It was found that missense mutations and codon deletion in DAX-1 all mapped to the predicted hydrophobic core of its LBD (233, 234).

DAX-1 is involved in various disease states, such as X-linked adrenal hypoplasia congenita (AHC), which is caused by mutations in the NR0B1 gene. More than 90 NR0B1 mutations that cause AHC have been identified, and several of these mutations delete all or part of the NR0B1 gene, preventing the production of DAX-1 protein. Loss of DAX-1 function leads to adrenal insufficiency and hypogonadotropic hypogonadism, which are the main characteristics of this disorder (235). DAX-1 knock-out mice are associated with delayed testis development and male sterility.

Additionally, DAX-1 expression has been detected in totipotent murine embryonic stem cells, which suggests an important function of DAX-1 in early embryonic development, which is independent of its role in steroidogenesis (236).

DAX-1 expression was found to increase with osteoblast differentiation and in a variety of tumor tissues (e.g. adrenal and pituitary adenomas, breast, ovarian and prostate cancer), implicating its potential role in bone cell development and malignancy (237).

Another important aspect of DAX-1 function is that it has been shown to physically interact with glucocorticoid receptor (GR) and thus acts as a novel selective GR modulator. It specifically inhibits ligand-dependent GR transactivation with little effect on GR-mediated transrepression. Clinically, glucocorticoids are extensively prescribed for their anti-inflammatory or immune-suppressive effects (i.e. transrepression). However, long-term use of steroids is often associated with a wide range of adverse effects. It is well known that GR-mediated transrepression of target genes, particularly pro-inflammatory cytokines and cytokine receptors are responsible for the beneficial effects of glucocorticoids in preventing inflammation, whereas the side effects are associated with GR-mediated transactivation. Therefore, selective GR modulators, like DAX-1 that enhances or has no effect on GR-mediated transrepression but reduce transactivation, are expected to have great a therapeutic value due to improved benefit-torisk ratio in various inflammatory conditions (238).

SMALL HETERODIMER PARTNER (SHP): DRUG TREATMENT FOR OBESITY

SHP belongs to the NR0B subfamily of ONRs. It is expressed primarily in endocrine organs (adrenal, pancreas), gastrointestinal organs (stomach, duodenum, ileum, colon and gall bladder), metabolic organs (liver, kidney), Orphan Nuclear Receptor and Drug Development

1455 reproductive organs (ovary and testis), cardiopulmonary organs (heart and lung), and CNS (cerebrum) (239). No crystal structure of SHP has been determined yet.

SHP dysfunction is associated with mild early-onset obesity, and early-onset type II diabetes (240, 241). Loss of SHP in mice causes abnormal accumulation and increased synthesis of bile acids due to de-repression of rate-limiting CYP7A1 and CYP8B1 hydroxylase enzymes in the bile acid biosynthetic pathway (242, 243).

SHP has also been shown to act as a transcriptional inhibitor of adipogenesis through inhibition of adipogenic transcription factors C/EBP alpha and PPARγ2 and other adipogenic stimulators Ebf3 and Stat5a (244). This property of SHP could be exploited (SHP agonists) for the treatment for obesity.

STEROIDOGENIC FACTOR 1 (SF-1): ROLE IN PROSTATE CANCER AND ADRENOCORTICAL TUMOR FORMATION

SF-1 is a member of the NR5A subfamily of ONR transcription factors that is essential for the development of adrenals and gonads and plays a role in sexual development. SF-1 is expressed primarily in the adrenal gland, hypothalamus, ovary and testes and acts by regulating the secretion of steroid hormones (245, 246). Crystal structure of SF-1 LBD has been solved in complex with non-bacterial phospholipids, and these phospholipids were shown to readily exchange bacterial phospholipids fortuitously bound to SF-1 ligand binding pocket (247–250).

SF-1 knock-out mice develop loss of pituitary gonadotrope function and fail to develop adrenal gland and gonads (245). Abnormalities in SF-1 function have been implicated in adrenocortical insufficiency, sex reversal, cryptorchidism, insulin resistance and type II diabetes (251, 252). Tandem Mass spectrometry and cell-based receptor selection and amplification technology (R-SAT) assays have identified sphingosine and 4-(heptyloxy) phenol (AC45594) as negative regulators of SF-1 activity, respectively (248, 253, 254). These properties of SF-1 modulators can be exploited to suppress both adrenal androgen and gonadal testosterone synthesis in the treatment of prostate cancer and in adrenocortical tumors (255, 256).

ESTROGEN RECEPTOR-RELATED RECEPTOR (ERR): TREATMENT OPTIONS FOR POSTMENOPAUSAL OSTEOPOROSIS, BREAST TUMOR AND TYPE II DIABETES

ERR is a member of the NR3B orphan subfamily of the NR superfamily of transcription factors. While ERR is structurally homologous to estrogen receptors and binds estrogen response elements, it is not activated by estrogens. It functions as a metabolic regulator by modulating the expression of enzymes involved in adipogenesis, energy metabolism, and lipid, eicosanoid, and steroid synthesis (257). It has three subtypes: ERRα, ERRβ and ERRγ. ERRα is expressed robustly in tissues in all major physiological systems (CNS, endocrine, metabolic, gastrointestinal, immune, reproductive, cardiovascular and respiratory) with particularly high levels in the olfactory bulb, jejunum, ileum, kidney, brown adipose tissue, heart and skeletal muscle. ERRβ is expressed specifically in the placenta, and ERRγ is expressed in brain, kidney, testis, lung, adrenal gland, pancreas, placenta and bone marrow (258–261).

Crystal structure of apo ERRα has revealed that it has a very small ligand binding pocket (~100Å) and shows constitutive activity (222, 262). Searches for ERRα ligand have mostly

identified inverse agonists, namely 4-hydroxytamoxifen and diethylstilbestrol (263). Crystal structures of the ERRγ LBD were determined in three distinct states: unliganded, inverse agonist bound (with 4-hydroxytamoxifen), and agonist bound (with GSK4716) (264). For ERRβ, also, crystal structure of ERRβ LBD with 4-hydroxytamoxifen has been reported (265).

Disease Conditions and Possible Clinical Interventions

Postmenopausal osteoporosis is a condition where serum estrogen levels decrease, resulting in decreased bone mineralization. Estrogen receptors (ERs) are expressed in osteoblasts. Strikingly, mice lacking ER show only minor skeletal deformities, suggesting other mechanisms or receptors (additional to ER) are involved in this process. ERRα is more widely distributed in osteoblast and osteoblast-like cells than ERs. It has also been shown to positively regulate osteopontin gene (extracellular matrix molecule secreted by osteblast) expression and thereby regulate bone remodeling (266).

ERR transcriptionally regulates estrogen-responsive breast cancer marker pS2, and it has been shown that ERR-responsive element in pS2 promoter is required for both estrogen and ERR response on pS2 expression. Transcriptional response of pS2 is completely abolished by diethylstilbestrol (DES) treatment, which is an inhibitor of ERR function, showing that DES treatment completely abolishes both ER+ and ER− breast tumor growth through ERR pathway (267). Several reports have shown that diabetics as well as individuals with a family history of diabetes have reduced mitochondrial oxidative phosphorylation (OXPHOS) capacity in muscle. OXPHOS genes are downstream targets of the transcriptional coactivator, PGC-1α. ERRα is an early target gene of PGC-1α. When activated by PGC-1α, ERRα expression is induced in an autocrine loop leading to increased OXPHOS target gene expression, as both ERRα and PGC-1α (they directly interact with one another) bind to promoter of OXPHOS target genes. Thus, as an ONR, targeting of ERRα with small molecules is an attractive strategy to increase mitochondrial OXPHOS function in type II diabetic patients. Moreover, because ERRα is involved in the regulation of fatty acid β-oxidation, activating ERRα can ameliorate the lipid accumulation in skeletal muscle, which is believed to contribute to insulin resistance (268).

ERRβ is specifically expressed in the chorion, suggesting that ERRβ may play a role in early placental development. The ERRβ knockout embryos have severely impaired placental formation and die owing to a lack of nutrients. The synthetic estrogen diethylstilbestrol (DES) has been shown to act as an inverse agonist of ERRβ, thereby affecting normal placental development (269).

ERRγ has been shown to play an anti-proliferative role in both androgen-sensitive and -insensitive prostate cancer by directly inducing cyclin-dependent kinase inhibitors p21 and p27, which results in cell-cycle arrest at G_1-S transition. Therefore, by selectively activating ERRγ by its synthetic agonist DY131, growth proliferation of prostate cancer cells can be prevented (270).

HEPATOCYTE NUCLEAR FACTOR 4 (HNF4): TARGET FOR ANTI-DIABETIC DRUG DISCOVERY

HNF4 is a member of the NR2A orphan subfamily of the NR transcription factors that is required for the development of liver (271, 272). Three subtypes of HNF4 are known: α, β and γ. Crystal structure of HNF4 LBD shows that it is structurally similar to other ONR LBD and can bind fatty acids (273). HNF4 is highly expressed in the gastrointestinal tract, liver, and kidney, with lower levels in adipose tissue and pancreas (274, 275).

While little is known about HNF4β and HNF4γ, HNF4α has been shown to constitutively bind fatty acids, and its dysfunction has been implicated in the development of maturity onset diabetes of the young (MODY) type I, adult onset type II diabetes mellitus, high serum lipid levels and chronic kidney failure (276). HNF4α knock-out mice show defects in embryonic, liver, biliary system and CNS development. It has been previously published that missense mutations of HNF4α LBD result in MODY-1, which can be rescued by fatty acid agonist activation of HNF4α (276). Hence, this property of HNF4α could be utilized for the treatment of MODY-1 patients by selective HNF4α agonist.

HNF4α also regulates coordinate nuclear-receptormediated response to xenobiotics and is involved in the PXR and CAR-mediated gene activation of CYP3A4, a drug-metabolizing enzyme with possible implications in drug metabolism (277).

GERM CELL NUCLEAR FACTOR (GCNF) AND LIVER RECEPTOR HOMOLOG (LRH-1)

GCNF (NR6A1) is a member of the NR6A subfamily of NR transcription factors and acts as a transcriptional repressor. It plays an important function in vertebrate embryogenesis and is highly expressed in oocytes and spermatogenic cells (278). In the absence of a GCNF crystal structure, very little is known about its ligand or any heterodimerization partner and cofactor.

It is predicted that GCNF regulates protamine gene expression in response to an unknown ligand, which is critical for testicular development. In the absence of a ligand, it is a repressor of transcription, and part of its repression is mediated by the corepressor N-CoR (279). Germ-cell-specific expression of GCNF, thereby regulating gametogenesis, could be exploited as a contraceptive target (280). The complex temporal and spatial expression pattern of GCNF suggests its involvement in different developmental processes in addition to its role in gametogenesis (281). Functional gene targeting studies have shown that GCNF knock-out mice are embryonic-lethal, and death is found to be due to severe cardiovascular and posterior developmental defects, suggesting its role in embryogenesis (282).

LRH-1 is a member of the NR5A subfamily of ONRs. It is expressed in liver, intestine and pancreas, where it is involved in bile acid metabolism, cholesterol homeostasis and liver development. LRH-1 transcriptionally up-regulates ATP binding cassette half-transporters ABCG-5 and ABCG-8 to facilitate biliary and intestinal removal of neutral sterols (283). It is also expressed in the preadipocytes, adrenal and sex glands including ovaries and placenta (284). Crystal structure of LRH-1 LBD has shown that it can exist in active conformation as a monomer without a ligand (285). But later findings from different groups have shown that ligand binding pocket of LRH-1 can be occupied by phospholipids (247, 248).

This receptor plays a role in cell proliferation by inducing the expression of cyclins D1 and E1 through both direct and indirect interactions with β-catenin (286). LRH-1 also regulates the transcription of genes responsible for hormone synthesis, including the expression of the estrogen-synthesis enzyme CYP19 in the pre-adipocytes of cancerous breast tissue (287).

LRH-1 has also been identified to play a role in colon cancer. It was shown that LRH-1 synergizes with β-catenin/T-cell factor 4 signaling to stimulate intestinal crypt cell renewal. LRH-1 haploinsifficiency thereby blunts intestinal tumorigenesis in $APC^{min/+}$ mouse and chemical carcinogen (azoxymethane) models of intestinal cancer (288). LRH-1 has also been shown to promote breast tumorigenesis by activating aromatase II promoter (CYP19 gene) activity. Hence, selective inhibition of LRH-1 Orphan Nuclear Receptor and Drug

Development 1457 may represent a novel strategy for the discovery of selective aromatase modulators (289).

Additionally, it has been observed that LRH-1 has a protective role in mouse models of inflammatory bowel disease by triggering local production of glucocorticoids (290). Structural and functional studies have shown that phospholipids bind to LRH-1 and regulate its downstream transcriptional events. GSK8470, a small-molecule high-affinity ligand for LRH-1, and SF-1 have been discovered by FRET-based biochemical assays to increase LRH-1 target gene SHP expression (113).

It has been found that both GCNF and LRH-1 compete for the same Oct-4 (member of POU homeodomain family of transcription factors) promoter elements during embryonic cell (ES) differentiation. LRH-1 induces expression of Oct-4 for the induction of ES cell differentiation, whereas GCNF represses Oct-4 to maintain the pluripotency of ES cells. Hence, agonist for LRH-1 and antagonist for GCNF could be used to maintain ES cell pluripotency and selfr-enewal for large scale culture of ES cells; similarly, LRH-1 antagonist and GCNF agonist could be used to silence pluripotency genes like Oct-4 for differentiation of ES cells to target cells for therapeutic purposes (291).

PHOTORECEPTOR-SPECIFIC NUCLEAR RECEPTOR (PNR): INVOLVEMENT IN AGE-RELATED MACULAR DEGENERATION (AMD)

PNR (NR2E3) is a member of the NR2E subfamily of ONRs which plays a role in retinal differentiation and degeneration. It is exclusively expressed in the rod photoreceptor cells of retina (not expressed in other tissues, including brain) (292). PNR gene was first identified through a search for genes related to TLX, which is involved in the development of midbrain and eye. *In vitro* assays have shown that PNR is capable of binding to a subset of TLX target sequences. TLX gene knock-out experiments in mouse revealed specific defects in forebrain derivatives; however, other regions, such as eye, develop normally, thus implicating compensation of loss of TLX function by other proteins in mice (211). Analysis of human PNR gene at the gene locus 15q24, a region susceptible for retinal degeneration, has supported a role for this receptor in retinal cell function (292, 293). Mutation of PNR gene gives rise to retinal degeneration in mice that resulted in abnormal development of rods and cones, enhanced S-cone and Goldmann-favre syndromes (inherited vitreoretinal dystrophy) (294, 295). The rd7 mice containing a sporadic deletion in PNR gene have abnormal development of rods and cones, leading to the development of age-related macular degeneration (AMD), the leading cause of blindness world-wide (296). Similarly, PNR gene mutations in humans have been correlated with various retinal diseases.

Based on the evidence regarding the involvement of PNR in retinal degeneration, agonist of PNR has become an attractive drug target for the treatment and prevention of AMD. It is noteworthy that 13-cis-retinoic acid, a receptor in retinal epithelium, has been found to be a weak PNR agonist in cell-based assays. While in the absence of a crystal structure of PNR LBD, recent high throughput screening has identified a new class of PNR agonist based on 2-phenylbenzimidazole core. This identification thus opens the door for future research into the development of PNR ligands (297).

CONCLUSIONS

ONRs are a group of important biological molecules, whose functions can be modified by the application of potent receptor-specific agonist and antagonist to regulate diverse biological processes. Discoveries of various ligands to these receptors will help us to modulate various important physiological processes for therapeutic purposes for various

human disease states. Thus, drug targeting of these ONRs is an important and viable means of treatment for various human disorders, and this will act as a challenge for better development of therapeutics to the pharmacological and pharmaceutical research.

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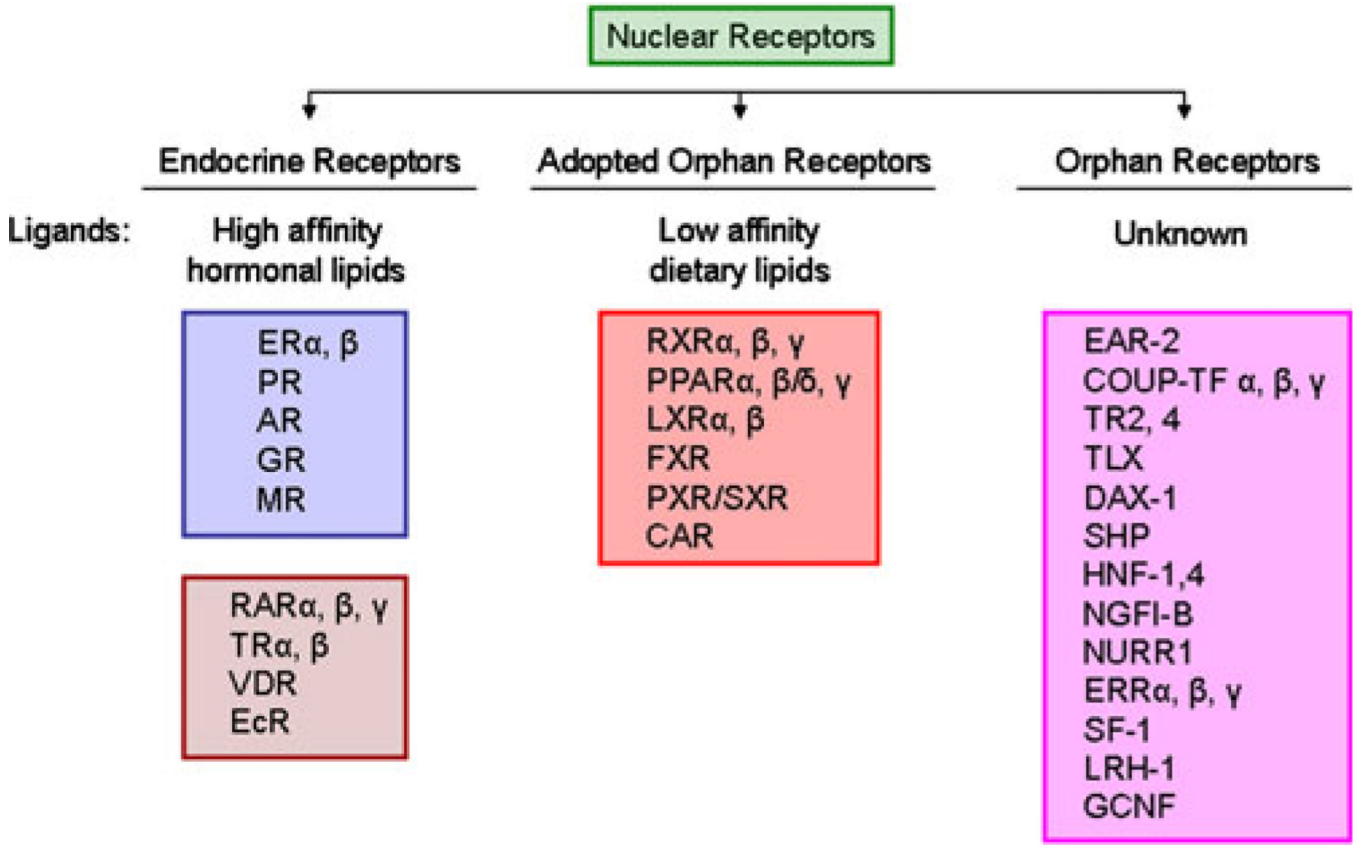

Fig. 1.
Classification of NR superfamily based on the identification of their respective ligands. This includes the classical NRs with known high affinity hormonal ligands, orphan receptors with no known identifiable ligands and adopted orphan receptors with low affinity dietary ligands.

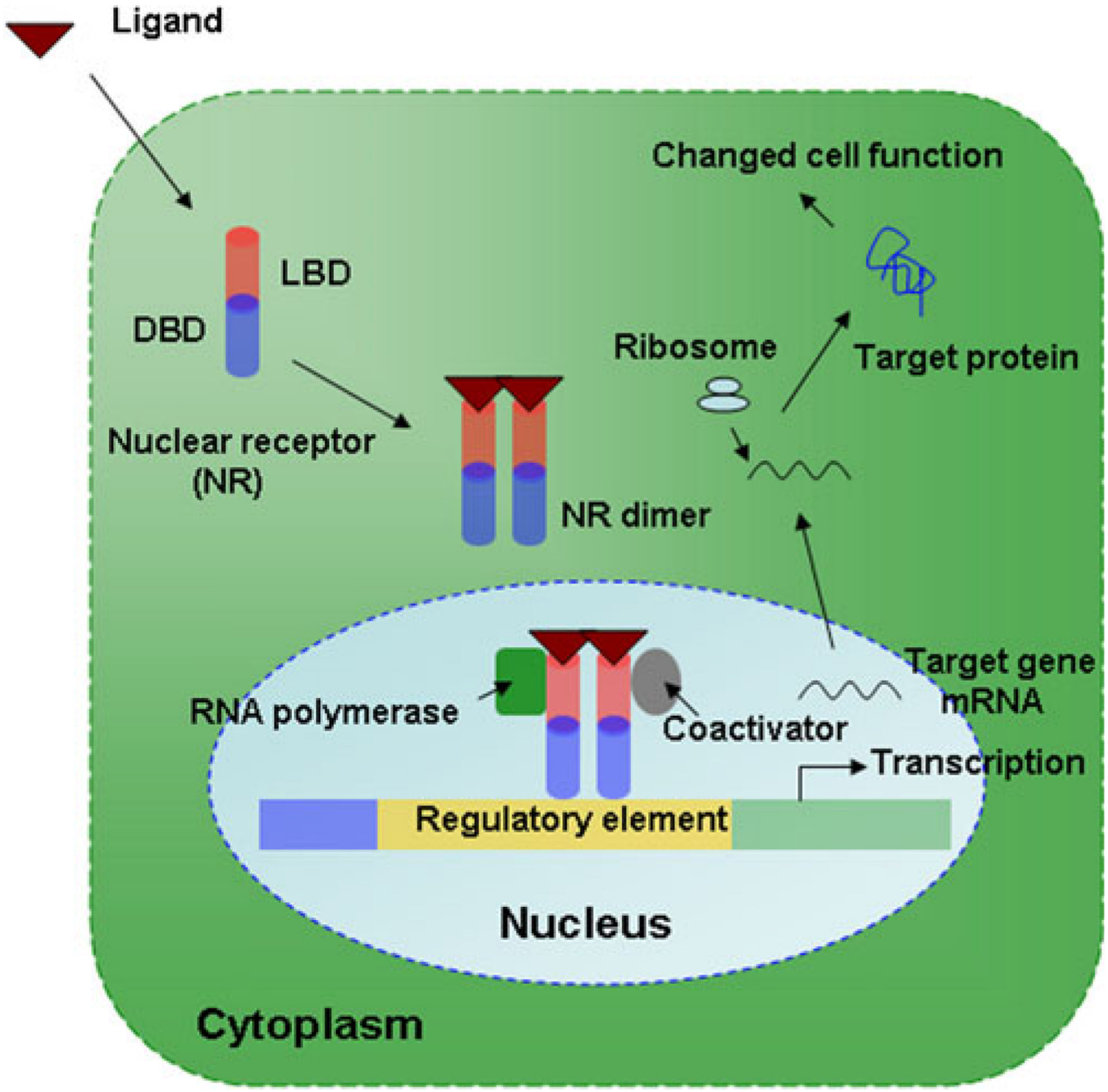

Fig. 2.

Schematic diagram describing classical NR function: NR binds to its ligand in the cytoplasm, which leads to its translocation into the nucleus. Ligand-bound NR dimerizes with its obligate partner to bind to the target gene regulatory element, with further recruitment of coactivators and RNA polymerase complex in the nucleus. This leads to target gene transcription with more protein production in the cytoplasm for gene specific activity.

Fig. 3.

Structural domain of the NR superfamily: extreme amino terminal domain is called the A/B domain that contains the AF1 region, followed by the conserved DNA binding domain (*DBD*), and linked by a hinge region with the ligand binding domain (*LBD*). The extreme carboxy terminal end of LBD is called the AF2 region.

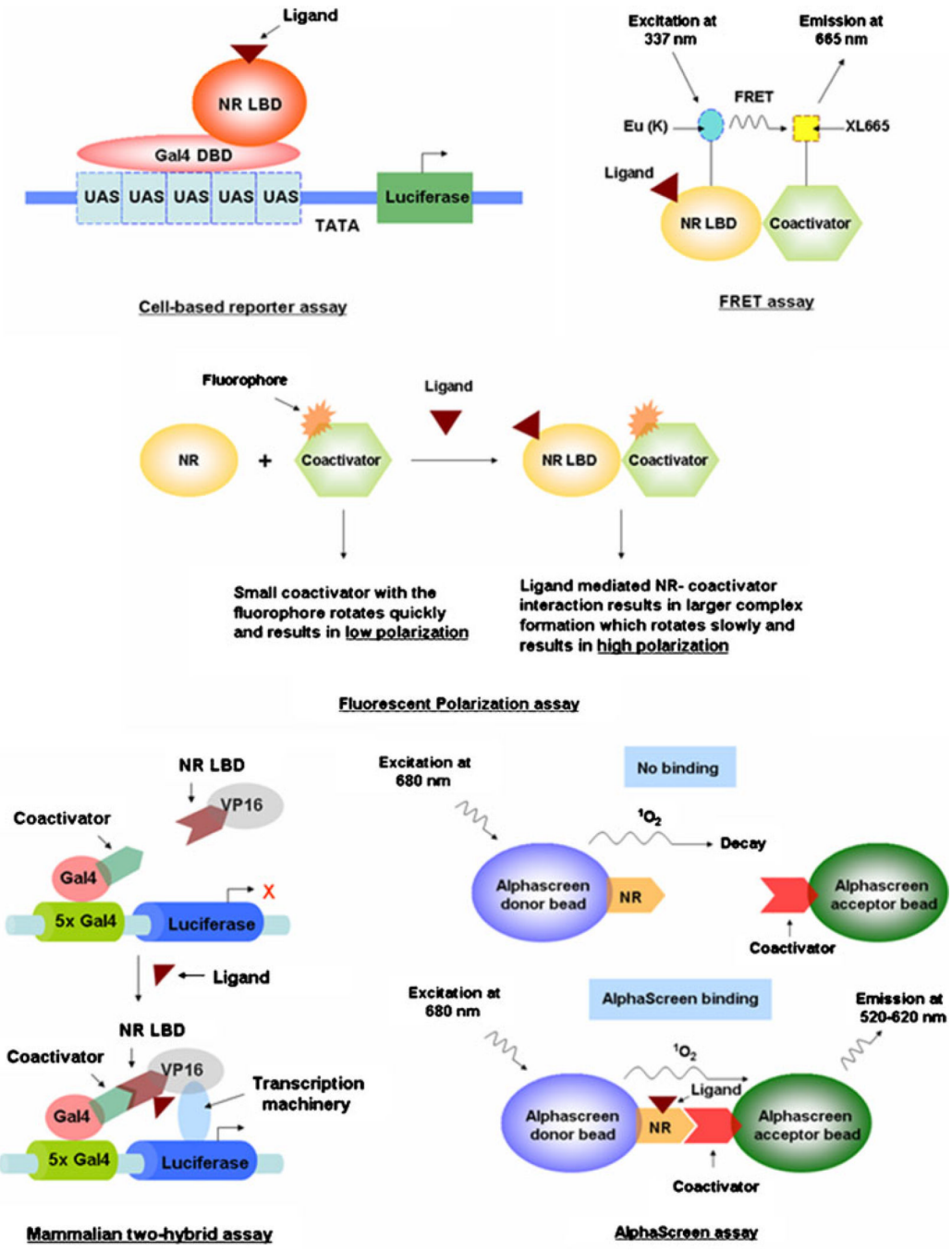

Fig. 4.

Schematic diagrams of various drug discovery tools. Detailed descriptions of each can be found in the text.

Table I

Known Vertebrate Orphan Nuclear Receptors

NR	Subtypes	Nomenclature	Species	References
PXR		NR1I2	h, m, x	(81,88,298)
CAR	α, β	NR1I3 (α), NR1I4 (β)	h, m	(299,300)
FXR		NR1H4	h, m, r	(301,302)
LXR	α, β	NR1H3 (α), NR1H2 (β)	h, m, r	(301,303–306)
PPAR	α, β/δ, γ	NR1C1 (α), NR1C2 (β/δ), NR1C3 (γ)	h, m, r, k, l, b, p, g, x	(166,307–314)
NURR1		NR4A2	h, m, r	(196,315,316)
Nur77		NR4A1	h, m, r, x	(317–319)
TLX		NR2E1	h, m, c, x, f	(212,320,321)
DAX-1		NR0B1	h, p, m, r	(19,322)
ROR	α, β, γ	NR2F1 (α), NR2F2 (β), NR2F3 (γ)	h, m, r	(323–326)
SHP		NR0B2	h, m, r	(20,327)
SF-1		NR5A1	h, m, c, b	(328–331)
ERR	α, β, γ	NR3B1 (α), NR3B2 (β), NR3B3 (γ)	h, m, r	(257,259,332)
HNF4	α, β, γ	NR2A1 (α), NR2A3 (β), NR2A2 (γ)	h, m, r, x	(272,333,334)
GCNF		NR6A1	h, m, x	(19,322)
LRH-1		NR5A2	h, m, r, c	(335–338)
PNR		NR2E3	h, m, r, c	(211,292,339,340)

Table II

Orphan Nuclear Receptor Phenotypes and Possible Therapeutic Interventions with Known Agonist and Antagonist Compounds for Various Human Diseases

Orphan Nuclear Receptor (ONR)	NRNC[a] symbol	Disease association	Possible interventions	
			Agonist	Antagonist
Pregnane X Receptor (PXR)	NR1I2	(1) Colon, breast and ovarian cancer (91, 93, 94) (2) Drug- drug interactions (91, 92) (3) Alcoholic and non-alcoholic hepatosis (101) (4) Acetaminophen-induced hepatotoxicity (103, 104) (5) Osteomalacia (102)		Ketoconazole, Suphoraphane, ET-743, Coumesterol
		(6) Intestinal inflammation (109) (7) Osteoporosis (106) (8) Bile-acid-induced cholestasis (99)	Rifampicin PCN, PB, SR12813	
Constitutive Androstane Receptor (CAR)	NR1I3 (α), NR1I4 (β)	(1) Liver cancer (125)		Clotrimazole, Androstenol
		(2) Hyperthyroidism (126) (3) Obesity (127) (4) Metabolic disorders (128-130)	PB, TCPOBOP	
Farnesoid X Receptor (FXR)	NR1H4	(1) Hypertriglyceridemia (153) (2) Cholesterol gall stone disease (151) (3) Colon and breast cancer (158, 159)	Chenodeoxycholic acid, 1,1-bisphosphonate esters, GW4064	
		(4) Hypo-HDL cholesterolemia (152)		Guggulsterone
Liver X Receptor (LXR)	NR1H3 (α), NR1H2 (β)	(1) Atherosclerosis (138-140) (2) Inflammation (145, 146) (3) Prostate, breast and colon cancer (142-144) (4) Cholesterol- related neurodegenerative disorders (148)	Oxysterol, 22(R)-hydroxycholesterol, GW3965	
		(5) Hepatic lipogenesis (136, 137)		Fenofibrate esters

Peroxisone Proliferator Activated Receptor (PPAR)	NR1C1 (α)	(1) Obesity (183, 184) (2) Inflammation (186)	Fatty acids, LT-B4, Fibrates		GW6471, MK886
	NR1C2 (β/ δ)	(3) Diabetes and obesity (193) (4) Metabolic disorders (192)	GW501516		
	NR1C3 (γ)	(5) Diabetes (179, 182) (6) Inflammation (186)	15d-PGJ2, TZD		GSK0660, GW9662 SR202
		(7) Acne (185)			SR202, GW9662
	NURR1	(1) Parkinson's disease (201, 202)	6-mercaptopurine		
		(2) Synovial inflammation (203, 204)			Unknown
	Nur77	(1) Breast and colon cancer (207, 209, 210)	DIM-C-Ph, DIM-C-pPhOCH3, Nurr77 peptide: NuBCP-9		
					Unknown
	TLX	(1) Neurodegeneration (214, 217, 218) (2) Retinal degeneration (216, 218)	Unknown		
					Unknown
	DAX-1	(1) X-linked adrenal hypoplasia congenita (AHC) (235) (2) Inflammatory conditions (238)	Unknown		
		(3) Bone tumor formations (237)			Unknown
	ROR	NR2F1 (α),	(1) Cholesterolemia (226)	Cholesterol-sulfate, 7-oxygenated sterols	Unknown
		NR2F2 (β),	(2) CNS disorders (229, 230)	Stearate	
					ATRA, ALRT 1550
		NR2F3 (γ)	(3) T-cell lymphoma (231)	7-oxygenated sterols	
					ATRA, ALRT 1550
	SHP	NR0B2	(1) Obesity (244) (2) Early-onset type II diabetes (240, 241)	Unknown	
					Unknown

SF-1	NR5A1	(1) Adrenocortical tumor formation (256) (2) Prostate cancer (255, 256)		Sphingosine, AC45594
			GSK8470 (Ref: (113))	
ERR	NR3B1 (α), NR3B2 (β), NR3B3 (γ)	(1) Post-menopausal osteoporosis (266) (2) Type II diabetes (268) (3) Prostate cancer (270)	DY131, phytoestrogen flavone, isoflavone	
		(4) Breast cancer (267)		DES, 4-hydroxytamoxifen, toxaphene, chlordane, kaempferol (inverse agonist)
HNF4	NR2A1 (α), NR2A3 (β), NR2A2 (γ)	(1) Diabetes (MODY I and type II) (276)	Myristic acid (C14:0), palmitic acid (C16:0), 9-cis-hexadecenoic acid (C16:1)	
				Polyunsaturated fatty acids (C 18:3,-3)-CoA, medica homologs
GCNF	NR6A1	GCNF knock-out mice is embryonic lethal (341)	Unknown	Unknown
LRH-1	NR5A2	(1) Breast and colon cancer (288, 289)		Unknown
		(2) Inflammatory bowel disease (290)	Phospholipids, GSK8470 (Ref:(113))	
PNR	NR2E3	(1) Age related macular degeneration (ARMD) (296) (2) Goldmann-favre syndrome (295) (3) Enhanced S-cone syndrome (294)	13-cis-retinoic acid, agonists based on 2-phenylbenzimidazole core structures	Unknown
				Unknown