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Ligand regulation of retinoic acid receptor-related orphan receptors: implications for development of novel therapeutics

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Abstract

Purpose of review—In the late 1980s, the cloning of several nuclear receptors led to the intense search and isolation of new members of this superfamily. Despite their identification, many of these receptors were dubbed ‘orphan’ receptors, as their physiological ligands remained unknown. Recent reports have presented evidence for one family of orphan receptors, the retinoic acid receptor-related orphan receptors (RORs), in several pathologies, including osteoporosis, several autoimmune diseases, asthma, cancer, diabetes and obesity. The present review summarizes the studies identifying ligands for the RORs and evaluates their role as targets for potential therapeutics.

Recent findings—Significant progress was made in the initial identification of ligands for the RORs when X-ray crystallographic studies identified several molecules within the ligand-binding pockets of ROR α and ROR β . Recently, we identified endogenous and synthetic ligands for ROR α and ROR γ , thereby solidifying their function as ligand-dependent transcription factors.

Summary—Recent studies have established roles for the RORs in physiological development and the advent of disease. Identification of ligands for the RORs, both endogenous and synthetic, has established these receptors as attractive new therapeutic targets for the treatment of ROR-related diseases.

Keywords

lipid; nuclear receptor; orphan receptor; oxysterol; steroid receptor; sterol

Introduction

Nuclear receptors comprise a superfamily of ligand-dependent transcription factors, including receptors for thyroid hormones, steroid hormones, retinoids, fatty acids, sterols, as well as receptors with no currently known ligand. In the late 1980s, the identification of the canonical domain structure and conserved sequence of members of this superfamily led to an intense effort to identify additional members of this superfamily. The retinoic acid receptor-related orphan receptors alpha, beta, and gamma (ROR α , ROR β , and ROR γ , also referred to as NR1F1-3) comprise a distinct subfamily of nuclear receptors and are

considered ‘orphan’ receptors, as they have no known, or generally agreed upon, endogenous ligands.

The first member of the ROR subfamily, *RORα*, was identified in the early 1990s based on sequence similarities to the retinoic acid receptor (RAR) and the retinoid X receptor (RXR), thereby giving this subfamily the name ‘retinoic acid receptor-related orphan receptor’ [1,2]. Highly similar receptors, *RORβ* and *RORγ*, were identified soon after [3,4]. Several orthologs to the RORs have been identified in lower species, including *Drosophila* hormone receptor 3 (DHR3) in *Drosophila melanogaster*, CHR3 in *Caenorhabditis elegans*, and MHR3 in *Manduca sexta* [5–7].

Retinoic acid receptor-related orphan receptor structure and activity

The *RORα* gene maps to human chromosome 15q22.2, encompassing a relatively large genomic region of 730 kb containing 15 exons. The *RORβ* and *RORγ* genes map to 9q21.13 and 1q21.3 and cover 188 and 24 kb, respectively. The RORs display significant sequence similarities and each ROR gene generates several isoforms, differing only in their amino termini, as a consequence of alternative promoter usage and exon splicing [2,4,8–10]. Four isoforms of *RORα* have been identified in humans, *RORα*1–4, whereas only two isoforms, *RORα*1 and *RORα*4, have been identified in mice. Two isoforms of *RORβ* have been reported in mice, *RORβ*1 and *β*2, whereas humans appear to express only one form, *RORβ*1 [8]. *RORγ* is expressed in two isoforms in humans and mice, *RORγ*1 and *RORγ*2, with the later also referred to as *RORγ*t. Most isoforms exhibit distinct patterns of expression and regulate different physiological processes and distinct target genes. However, due to the significant sequence similarities, cells co-expressing RORs may exhibit functional overlap.

RORα is widely expressed in many tissues, including cerebellar Purkinje cells, liver, thymus, skeletal muscle, skin, lung, adipose tissue, and kidney [9,11]. Studies of the *staggerer* mice (*RORα*^{sg/sg}) have revealed *RORα*’s role in the regulation of metabolism and cerebral development. This mutant mouse strain carries an intragenic deletion within the *RORα* gene, resulting in a frameshift and premature stop codon rendering *RORα* inactive [9]. *Staggerer* mice display ataxia and severe cerebral atrophy and more detailed examination of these mice revealed significant alterations in lipid metabolism characterized by low levels of total plasma cholesterol, triglycerides, and HDL as well as decreased expression of *apoCIII* and *apoA1*. *Staggerer* mice also display an increased susceptibility to atherosclerosis when fed a high-fat diet, have an altered immune response, and display disturbances in their circadian rhythm [12^{••},13,14]. Finally, mice lacking *RORα* have also revealed a role for this receptor in the formation and maintenance of bone tissue [15].

The expression of *RORβ* is much more restricted than that of *RORα* and its precise role has yet to be determined. *RORβ* is expressed in regions of the central nervous system involved in the processing of sensory information, the retina, and the pineal gland [8]. Mice deficient in *RORβ* display abnormalities in their circadian rhythm, suggesting a role for *RORβ* in the regulation of this physiological process [8,16].

ROR γ is most highly expressed in the thymus, but is also expressed in significant amounts in a pattern similar to that of ROR α . Mice deficient in ROR γ lack lymph nodes and Peyer's patches, suggesting that ROR γ is essential for lymph node development [17,18]. Additionally, mice deficient in ROR γ are significantly less susceptible to experimental autoimmune encephalomyelitis (EAE), the murine equivalent of multiple sclerosis [19]. ROR $\gamma^{-/-}$ mice exhibit normal levels of plasma cholesterol and triglycerides [20]. Crossing *staggerer* mice with ROR $\gamma^{-/-}$ mice, essentially creating ROR α/γ -deficient mice, illustrated a role for these receptors in the maintenance of blood glucose homeostasis and suggested a functional redundancy between the two receptors [20].

The ROR genes encode proteins of 459–556 amino acids in length, depending on the isoform of the gene expressed. Like all nuclear receptors, RORs display a typical and conserved domain structure consisting of four major functional domains. The variable amino-terminal (A/B) domain is followed by the central, highly conserved DNA-binding domain (DBD), which contains two zinc fingers (C domain). Adjacent to the C domain is the hinge region (domain D), followed by a carboxy-terminal ligand-binding domain (LBD; E domain) [21] (Fig. 1). Note that several nuclear receptors such as the estrogen receptor contain an additional C-terminal F domain whose role is poorly defined. The LBDs of the nuclear receptors are multifunctional and have secondary domain structure that is characteristic of nuclear receptors [22–24]. The ROR LBDs have the typical 12 canonical α -helices (H1–H12) with two additional helices, H2' and H11'. The LBD facilitates coactivator and/or corepressor binding to the receptor. In many nuclear receptors, corepressor and coactivator interaction is ligand-dependent. However, in the case of ROR α and ROR γ , which have constitutive or intrinsic activity in the absence of ligand, the role of ligand binding is less clear. The activation function-2 domain (AF2) in H12 consists of the sequence PLYKELF, is 100% conserved among RORs, and, as determined from the crystal structure of ROR α , Y507 and H484 are likely to form a strong hydrogen bond capable of keeping H12 in the active conformation. Deletion or point mutations in H12, specifically Y507A, causes a reduction in transcriptional output and results in a dominant negative ROR [25,26]. These data support the notion that RORs are constitutively active and that coactivators can bind to the AF2 surface in the absence of ligand.

All RORs regulate gene expression by recognizing and binding to specific DNA sequences, termed ROR response elements (ROREs), consisting of the core consensus 'half-site' AGGTCA preceded by a 5' 6-base pair A/T-rich sequence in the regulatory region of the target gene [2–4,8]. RORs bind to DNA as monomers and are not capable of forming heterodimers with the retinoid-X-receptor (RXR) [2,3,8]. Structure analysis has revealed the presence of a 'kink' in H10 of the LBD of ROR α and ROR β . This kink affects the dimerization capability of RORs and is consistent with the conclusions that RORs do not form homodimers with other RORs or heterodimerize with RXRs [22–24]. When monomers of RORs are bound to their elements within the promoter of their target genes and are associated with transcriptional coactivators, constitutive activation of their target genes is detected. The positive and negative transcriptional regulation of the RORs occurs through the recruitment of nuclear receptor coactivators and corepressors, proteins with intrinsic chromatin remodeling or integration activity. ROR α has been shown to interact with the

nuclear corepressors NCoR, RIP140, and SMRT, and the coactivators GRIP, PBP, SRC-1, SRC-2, CBP, and PGC1 α [27*,28–32].

Regulation of retinoic acid receptor-related orphan receptors

Regulation of ROR-mediated gene expression is a complex and dynamic process. Interestingly, another orphan nuclear receptor subfamily, the REV-ERBs (REV-ERB α and REV-ERB β , or NR1D1 and NR1D2, respectively), also recognize and bind a subset of RORE sites and are often coexpressed with RORs. A ‘cross-talk’ between these two nuclear receptor subfamilies involves the competition between the RORs and REV-ERBs for binding of this common element [33,34]. Until recently, the REVERBs were thought to be constitutively active repressors of transcription. This is in contrast to the observation that the RORs are constitutive activators of transcription. Recent work has demonstrated that the porphyrin heme functions as a ligand for both REV-ERB α and REVERB β and is required for REV-ERB repressor activity [35,36]. Because REV-ERBs act as ligand-dependent transcriptional repressors, they are able to inhibit ROR-mediated transcription by competing with RORs for RORE binding.

The dynamic interplay between the RORs and REVERBs, resulting in the positive and negative regulation of gene transcription, has been demonstrated by their roles in the control and regulation of brain and muscle ARNT-like 1 (BMAL1 or ARNTL) expression. The *Bmal1* promoter contains two ROREs and *Bmal1* transcription is activated by RORs and repressed by REV-ERBs, thus regulating the mammalian clock. Consistent with these data, mice deficient in ROR α , ROR β , or REV-ERB α display abnormal circadian behavior patterns [37–39]. It is conceivable and highly likely that other feedback loops may be regulated by the interplay between these two receptor subfamilies.

Although the ROR/REV-ERB regulatory mechanism involves the competition for ROREs, regulation of ROR-mediated gene transcription can also occur through direct interaction and competition for transcriptional cofactors. The nuclear receptors LXR α and LXR β have been defined as sterol sensors that can be activated by endogenous cholesterol derivatives, the hydroxycholesterols (OHCs). LXRs regulate a large array of metabolic genes. Gene expression profiling of livers from ROR α ^{sg/sg} mice and LXR-activated mice demonstrated a substantial overlap of affected genes, suggesting crosstalk between ROR α and LXRs. In LXR-activated mice, the expression of *Cyp7b1*, a ROR α -regulated gene, was suppressed, whereas the expression of *CD36*, a fatty acid uptake transporter and LXR-regulated gene, was induced [40*,41]. A similar pattern of gene expression was observed in the ROR α ^{sg/sg} mice, suggesting that ROR α and LXR may be mutually suppressive *in vivo*. The authors proceeded to examine the mutual suppression between ROR α and LXR on *Cyp7b1* gene expression. They discovered that LXR activity on the *Cyp7b1* promoter was reciprocally suppressed by ROR α [40*]. Further studies suggested that the mutual suppression between ROR α and LXR was due to a competition for nuclear receptor cofactors, adding another degree of complexity to ROR-mediated metabolic regulation. Our recent discovery that ROR α and ROR γ function as oxysterol receptors (described below) suggests that the RORs and the LXRs are more closely related than previously believed. They both appear to function as oxysterol sensors and receptors.

Retinoic acid receptor-related orphan receptors as ligand-dependent transcription factors

Identification of ligands that potentially regulate the activity of RORs has met with controversy. As early as 1995, two putative ligands for ROR α were suggested, melatonin and a thiazolidinedione [42], but these data have not been confirmed. Elegant X-ray crystallographic studies provided significant insight into the nature of a putative physiological ligand. The resolution of the structure of ROR α 's LBD revealed the presence of cholesterol in the binding pocket (Fig. 2). A second crystallographic study established that in addition to cholesterol, cholesterol sulfate could be bound within the LBD of ROR α . These studies described that both cholesterol and cholesterol sulfate bound ROR α in a reversible manner and could modulate the transcriptional activity of ROR α when changes in the intracellular cholesterol level occurred. Additionally, when residues involved in cholesterol binding were mutated, ROR α transcriptional activity was affected. This study suggested that cholesterol and cholesterol sulfate were putative agonists for ROR α and that ROR α could play a key role in the regulation of cholesterol homeostasis and function as a lipid sensor [22,43]. This concept is particularly intriguing, given that ROR α regulates the expression of several genes involved in lipid metabolism [12[•],14,20,22]. These results suggested that ROR α was not constitutively active, rather cholesterol (an agonist) was present during most conditions leading to the appearance of constitutive activity in cells. Using electrospray ionization–mass spectrometry (ESI–MS), a third group identified the presence of another ligand, 7-dehydrocholesterol, in the purified LBD of ROR α from Sf9-insect cells. Using ligand-exchange experiments, this same group confirmed the binding of cholesterol and cholesterol sulfate to the LBD of ROR α and also found that 25-OHC bound ROR α 's LBD as well [44]. These data suggest that ROR α may bind to a number of sterols, leaving the question of which sterol(s) are physiologically relevant ligands.

The X-ray crystallographic studies of the ROR β LBD identified stearic acid within the LBD. However, it is believed that this ligand was a fortuitous one, as it appeared to act as a stabilizer filling the ligand-binding pocket rather than acting as a functional ligand [24]. Further work on ROR β identified several retinoids, including all-*trans* retinoic acid (ATRA) and the synthetic retinoid ALRT 1550 (ALRT), as ligands for ROR β functioning as inverse agonists [23]. ATRA and ALRT were able to bind ROR β reversibly, with high affinity, and reduced GAL4-ROR β -mediated transcriptional activity in cotransfection assays. It is unclear whether the retinoids regulate the transcriptional activity of the full-length ROR β or whether these ligands regulate ROR β target genes in an ROR β -dependent manner.

Although the idea that ROR α functioned as a receptor for cholesterol and cholesterol sulfate was attractive, it was still unclear whether cholesterol or a derivative of this sterol was truly a physiological ligand for ROR α . We recently demonstrated that 7-oxygenated sterols function as high-affinity ligands for both ROR α and ROR γ with K_i values in the range of 10–20 nmol/l as determined by radioligand binding assays. Our data indicate that 7-oxygenated sterols (7 α -OHC, 7 β -OHC, and 7-ketocholesterol) bind ROR α and ROR γ with higher affinity than cholesterol sulfate, whereas cholesterol binding is barely detectable. We found that ROR α and ROR γ , produced in *Escherichia coli*, were devoid of any endogenous

sterols and still displayed constitutive activity in terms of their ability to bind to coactivator nuclear receptor box peptides, suggesting that it is an inherent property of these receptors to activate transcription in the absence of a ligand (i.e., cholesterol). In cell-based assays, we found that the 7-oxygenated sterols functioned as inverse agonists of both receptors (both GAL4-LBD and full-length receptors), whereas cholesterol and cholesterol sulfate failed to modulate the activity [45**]. We also found that 7 α -OHC modulated the expression of ROR α / γ target genes in a receptor-dependent manner (Fig. 2). These data would strongly suggest that although cholesterol and cholesterol sulfate can bind to RORs, they do not induce sufficient perturbation in cofactor interaction surfaces on the LBD to alter the receptors intrinsic activity.

Recently, we identified a synthetic ROR α / γ inverse agonist in a nuclear receptor specificity screen of known nuclear receptor ligands. T0901317, a known and well characterized LXR α and LXR β agonist, has also been found to be a potent agonist to farnesoid X receptor (FXR) and the xenobiotic receptor, pregnane X receptor (PXR). The promiscuity of T0901317 indicates that there are structures that can bind to a range of nuclear receptors [46,47]. We demonstrated that T0901317 binds directly to and modulates the transcriptional activity of ROR α and ROR γ . T0901317 repressed ROR α / γ -dependent transcription and modulated the receptors ability to interact with transcriptional cofactor proteins. Since this report, we have demonstrated direct binding of radio-labeled T0901317 to RORs and analogs of this compound have been made that are devoid of LXR activity. Thus, T0901317 represents a novel chemical probe to examine ROR α / γ function and lays the groundwork for medicinal chemistry to develop ROR-selective modulators [48**].

Silent ligands?

The most rigorous definition of a true endogenous ligand is that of a molecule that binds the ligand-binding pocket (LBP) *in vivo* under physiological (or potentially pathological) conditions and this binding alters the activity of the receptor. For nuclear receptors, this would entail that a ligand binds with sufficient affinity to the LBP and that the binding mode of the ligand is such that critical contacts are made with the receptor to alter its conformational dynamics so that cofactor interaction is modulated. Modulation of nuclear receptor cofactor protein interaction will directly impact transcriptional output of the receptor. Additionally, other mechanisms are possible, including ligand-induced perturbation in conformational dynamics that modulate post-translational modification (i.e., sumoylation, phosphorylation) of the receptor that may impact its localization or stability leading to an alteration of transcriptional activity. Although many ligands have been proposed to be 'endogenous' for the RORs, our data suggest that apo-ROR α is constitutively active and retains the ability to interact with a range of coactivators in the absence of binding any ligand. A range of ligands have been demonstrated to bind within the LBP of ROR, but whether these ligands induce perturbation of receptor conformation resulting in alteration of receptor function is questionable. For receptors that have constitutive activity, it is conceivable that a class of silent ligands (functionally dead) may exist that have affinity for the receptor and bind efficiently but do not induce a conformational change that results in an alteration in receptor activity. We have clearly observed this with 25-OHC for ROR α , in which we used 25-OHC as a high-affinity radioligand in the binding assay ($K_d = 3.3$ nmol/l)

but it does not modulate ROR α transcriptional activity [45^{**},48^{**}]. We believe that cholesterol and cholesterol sulfate behave similarly. This notion of a spectrum of ligand activity in which ligand binding occurs but does not alter functional activity (Fig. 3) is supported by recent work with another nuclear receptor, hepatocyte nuclear factor 4 alpha (HNF4 α). Using affinity isolation/mass spectrometry, Yuan *et al.* [49^{*}] demonstrated that the LBD of HNF4 α is occupied by linoleic acid, but that ligand occupancy was not sufficient to have a significant effect on the transcriptional activity of HNF4 α . This group also established that linoleic acid binding was reversible and exchangeable for another ligand.

Further support of this theory comes from recent work performed in *D. melanogaster*. DHR96 is a *Drosophila* nuclear receptor that is considered to be the ortholog of PXR, constitutive androstane receptor (CAR), and vitamin D receptor (VDR). Previous studies defined roles for these three receptors in sensing xenobiotic compounds and in the direct regulation of genes involved in detoxification [50,51]. However, further characterization of DHR96 revealed an unexpected role for this receptor in the regulation of genes involved in lipid and carbohydrate metabolism, functions similar to mammalian LXR and ROR [52]. Three recent reports have identified DHR96 as an ancestral regulator of fat and cholesterol metabolism [53–55]. More importantly, these studies found that although cholesterol co-purified with DHR96, it had little effect on DHR96 activity in response to exogenous cholesterol, suggesting that cholesterol was not a functional ligand for this receptor [53] and thus exhibited characteristics of a ‘silent’ ligand. Although the studies emphasized that the sequence of DHR96 is most closely related to mammalian PXR and CAR, it shares significant functional characteristics with LXRs and RORs. Consequently, additional studies are required in order to determine whether another related sterol may function as a regulatory ligand to modulate the activity of DHR96 in a manner similar to what we have noted for ROR α/γ .

So what is the role of these silent ligands? It is possible that these silent ligands (ligands that are conformational change inept) might be functioning as neutral antagonists. Their role would be controlled by affinity toward the receptor where for instance a tight binding silent ligand would compete out any endogenous agonists or inverse agonist such as 7 α -OHC. The role of the silent ligand might be important to maintain the constitutive transcriptional output level of the receptor and prevent hyperactivation via agonist or inadvertent repression by endogenous inverse agonists. As required, endogenous repressor with higher binding affinity would compete out the silent ligand and repress the receptors’ transcriptional output. This concept would add another level of complexity to the control of nuclear receptors.

Therapeutic potential of retinoic acid receptor-related orphan receptor modulators

Animal models in which RORs have been deleted or mutated have revealed various roles for RORs in disease. As previously mentioned, *staggerer* mice develop severe atherosclerosis when placed on a high-fat diet, suggesting that ROR α plays an atheroprotective role [14]. ROR α is expressed in human aortic smooth muscle cells and endothelial cells and there is a significant decrease in expression of ROR α in atherosclerotic arteries [56]. The 7-

oxygenated sterols, which we identified as ROR α / γ inverse agonists, play a very important role in the pathology of atherosclerosis [45^{**}]. 7 β -OHC and 7-KC are the two most enriched oxysterols found in atherosclerotic plaques following 27-OHC. 7-KC is the most enriched oxysterol in oxidized LDL and in the macrophage foam cells [57,58] and has been shown to exert an atherogenic activity by inhibiting sterol efflux from these cells [59]. One mechanism by which 7-oxygenated sterols exert their atherogenic activity is by decreasing the atheroprotective effects of ROR α . Plasma levels of 7 β -OHC and 7-KC are elevated by as much as 370% in patients with familial combined hyperlipidemia and treatment with statins or a fibrate significantly reduces these levels in addition to LDL, VLDL, and triglycerides [60]. Chopra *et al.* [27^{*}] recently identified a role for ROR α in the regulation of glucose metabolism. This group demonstrated that the loss of the murine nuclear receptor coactivator SRC-2 led to a phenotype similar to von Gierke's disease. This disease is associated with severe hypoglycemia and abnormal accumulation of liver glycogen. The loss of the enzyme *glucose-6-phosphatase* (*G6Pase*) is responsible for 80% of von Gierke's disease cases. This group demonstrated that SRC-2 was required for ROR α to regulate this gene in a normal manner. With these recent data in hand, it is evident that ROR α and ROR γ play distinct roles in the regulation of lipid and glucose homeostasis and it becomes clear that there is potential for use of synthetic ligands that modulate ROR α / γ activity for treatment of metabolic diseases.

Littman and colleagues [61,62] were the first to report that ROR γ t is required for the differentiation of naive CD4⁺ T cells into a specific lineage of helper T cells called Th17 cells [19,21,61,62]. ROR γ t is induced during the differentiation process of Th17 cells in response to interleukin (IL)-6 or IL-21 and transforming growth factor β . Cells deficient in IL-6, STAT3, or ROR γ either do not express ROR γ t, IL-17F, and IL23R, are severely impaired in ROR γ t activation and Th17 differentiation, or exhibit a marked reduction in their ability to undergo differentiation into Th17 cells, respectively [21]. Subsequent studies showed that like ROR γ t, ROR α is highly induced during Th17 cell differentiation in a STAT3-dependent manner [63]. Deficiency in ROR α led to decreased IL-17 and IL23R expression [64]. Conversely, exogenous expression of ROR γ t or ROR α in T helper cells or Jurkat cells increased the expression of IL-17 cytokines and IL23R [63]. These studies indicate that not only does ROR α function as a positive regulator of Th17 differentiation; they suggest a degree of functional redundancy between ROR γ t and ROR α . This was consistent with findings in ROR α /ROR γ t double knockout mice in which Th17 differentiation was completely impaired [63,65]. Recent studies have shown that Th17 cells are crucial effector cells in diseases that were considered to be caused by a different lineage of CD4⁺ helper T cells, called Th1 cells. Th17 cells are now considered the effector T-cell subset that leads to several autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus (SLE) [66]. A synthetic ROR α / γ inverse agonist may have utility in treatment of autoimmune disorders by suppressing Th17 cell development. As indicated above, we found that the LXR agonist T0901317 displays significant ROR α / γ inverse agonist activity, and interestingly, it has been recently shown that this compound has efficacy in treatment of EAE in mice [67]. It is unclear what role the ROR α / γ activity of this ligand plays in this efficacy.

Several studies have provided evidence suggesting a role for RORs in cancer. Mice deficient in ROR γ exhibit a high incidence of thymic lymphomas that can metastasize to the liver and spleen [68,69]. Gastric tumors and ovarian cancers show an increased population of circulating Th17 cells and elevated expression of Th17-associated genes [70,71]. Although no direct links between ROR α and cancer have been established, a number of studies have indicated a possible role for ROR α in cancer development. Common fragile sites, those that are highly unstable genomic regions that are hotspots for genetic alterations, have been implicated in several diseases and a number of different types of cancer. The ROR α gene is located in the middle of the common fragile site FRA15A. Genomic instability of FRA15A may lead to changes in ROR α expression and may play a role in the development of certain cancers [72,73]. Indeed, ROR α mRNA expression is often down-regulated in tumor cell lines and primary cancer samples [73]. Whether RORs truly play a direct role in cancer development and progression is still debatable and will require more extensive research.

Conclusion

RORs play crucial roles in lipid metabolism, circadian rhythm, glucose homeostasis, and immune function and recent studies have revealed that the activity of these nuclear receptors can be regulated by both endogenous and synthetic ligands. It is currently unclear what role the endogenous ligands play in the physiological function of these receptors. Recent breakthroughs in identification of a synthetic ligand that regulates both ROR α and ROR γ promises to provide chemical tools that can be used to probe the function of the RORs, and our recent work indicates that it is possible to develop high-affinity, selective modulators of ROR activity.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 267–268).

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Figure 1. Comparison of the human receptor-related orphan receptor nuclear hormone receptors
Numbers indicate the amino acid identity relative to receptor-related orphan receptor alpha (RORα). Splice variants are also indicated.

Figure 2.
Examples of several endogenous receptor-related orphan receptor ligands and a synthetic receptor-related orphan receptor ligand

Figure 3. Spectrum of receptor-related orphan receptor ligands indicating degrees of inverse agonism that have been observed

Agonists of receptor-related orphan receptor activity are predicted.