Recent Advances in the Understanding of Nuclear Receptors- and Drug-Metabolizing Enzymes-Mediated Inter-Individual Differences

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The retinoic acid receptor-related orphan receptor α (RORα) is involved in the regulation of several physiological processes, including development, metabolism, and circadian rhythm. RORα-deficient mice display profound atherosclerosis, in which hypoalphalipoproteinemia is reportedly associated with decreased plasma levels of high-density lipoprotein, increased levels of inflammatory cytokines, and ischemia/reperfusion-induced damage. The recent characterization of endogenous ligands (including cholesterol, oxysterols, provitamin D3, and their derivatives), mediators, and initiation complexes associated with the transcriptional regulation of these orphan nuclear receptors has facilitated the development of synthetic ligands. These findings have also highlighted the potential of application of RORα as a therapeutic target for several diseases, including diabetes, dyslipidemia, and atherosclerosis. In this review, the current literature related to the structure and function of RORα, its genetic inter-individual differences, and its potential as a therapeutic target in atherosclerosis is discussed.

Key words retinoic acid receptor-related orphan receptor α; metabolism; cholesterol; statin; ischemia; atherosclerosis

1. INTRODUCTION

Lipid-soluble signaling molecules, including steroid hormones, lipids, and fatty acids, regulate the expression of target genes at the transcriptional level via cytoplasmic or nuclear receptors.1 As these receptors act as lipophilic ligand-induced transcriptional regulators, they play a central role in the mechanism of action of these lipophilic signaling molecules.2 Steroid hormones were first recognized as being mediated by nuclear receptors and the action of lipophilic ligand molecules.3 Following the cloning of nuclear receptors for estrogen and glucocorticoids in the 1980s, nuclear receptors for progesterone, mineralocorticoids, thyroid hormone, and vitamin D were cloned.3,4 Based on their amino acid sequence homology, these nuclear receptors were defined as a highly homologous, structurally similar, and molecularly evolved superfamily.5 Subsequently, homology screening was conducted using a synthetic DNA probe corresponding to five amino acid residues with extremely high homology in the DNA-binding region. This process identified retinoic acid receptor α (RARα) and retinoid X receptor (RXR) with all-trans retinoic acid (RA) and its isomer 9-cis RA as ligands, respectively.5,6 Other orphan receptors, such as retinoic acid receptor-related orphan receptor α (RORα), were also identified with unknown ligands.5,8

Nuclear receptors have a common structural organization; the N-terminal region (A/B domain) is highly variable and contains at least one constitutively activated activation function region 1 (AF-1), whereas the DNA-binding domain (DBD, C domain), a short motif responsible for DNA-binding specificity that contains two highly conserved zinc finger motifs, is conserved.9 Between the DBD and ligand-binding domain (LBD, E domain) is a less conserved region (D domain) that behaves as a flexible hinge between the C and E domains and contains the nuclear localization signal, which may overlap with the C domain. The characterization of the unliganded or liganded domains increases our understanding of the mechanisms involved in ligand binding. The C-terminal region of the LBD is the ligand-dependent AF-2.1,3,8 Thus, by elucidating the commonality and diversity of the structure and function of nuclear receptors, a better understanding of physiology is expected. This may be applicable to the medical field, as ligands can be used to target nuclear receptors involved in diseases (Fig. 1A).

2. STRUCTURE, DISTRIBUTION, AND FUNCTION OF RORS

RORα, also called NR1F1 for orphan monomeric receptors of unknown ligands, is a member of the NR1F superfamily of nuclear receptors, including receptors for thyroid and steroid hormones, retinoids, and vitamin D.10,11 The RORα gene is encoded on human chromosome 15q21–22 and mouse chromosome 9.12 It has three subtypes, each of which has a unique tissue-specific distribution; RORα is highly expressed in the brain, liver, thymus, heart, vessels, testes, and skin,10,13,14 whereas RORβ and RORγ are highly expressed in the brain and skeletal muscle, respectively.15,16 The tissue-specific distribution of ROR subtypes affects the development of these expressed tissues.14 Owing to alternative promoter usage and exon splicing,
Fig. 1. Structure of RORα Isoforms, Members of the Nuclear Hormone Receptor Superfamily

A. Domain structure of the nuclear receptor. B. Structure of the human RORα isoforms. In their isoforms (Protein database accession numbers; RORα1; NP_599023, RORα2; NP_599022, RORα3; NP_002934, RORα4; NP_599024), the NH2-terminus of each RORα generated by alternative promoter and/or splicing is the isoform-specific region, and the COOH-terminus, including the DBD and LBD, is the isoform common region. The numbers on the right refer to the total number of amino acids in the RORα. RORα, retinoic acid receptor-related orphan receptor α; AF-1, activation function region 1; AF-2, activation function region 2; DBD, DNA-binding domain; LBD, ligand-binding domain.

Each ROR gene generates several isoforms that contain a common DBD and LBD, which differ only in their amino termini. To date, four RORα isoforms, RORα1–4, have been identified in humans, whereas only two isoforms, α1 and α4, have been reported in mice. The human RORβ gene generates only the RORβ1 isoform, whereas that of mice appears to express two isoforms, β1 and β2. Both human and mouse RORγ genes generate the γ1 and γ2 (also γt) isoforms.

Most isoforms have a distinct tissue-specific expression pattern, regulate different physiological processes, and affect the expression of different target genes. For example, human RORα2 is located in the testis and skin, whereas human RORα3 is found exclusively in the testis. RORα1 and RORα4 are prominently expressed in the mouse cerebellum, whereas other mouse tissues predominantly express RORα4. Moreover, the expression of RORα4, but not RORα1, is highly induced by hypoxia-inducible factor (HIF)-1 under hypoxic conditions in various cells. Conditions that mimic the effects of hypoxia, including exposure to cobalt chloride and the iron chelator deferoxamine, also enhance RORα4 expression. In addition, RORα1 and RORα4 have unique intracellular localization; RORα1 is translocated to the nucleus and although RORα4 is primarily translocated to the nucleus, it is also partially localized in the cytoplasm.

Furthermore, the transcriptional regulation mechanism of Wnt/β-catenin signaling, depending on differences in the N-terminal domain of the RORα1 and RORα4 isoforms, has been clarified. The N-terminal domain of RORα1 specifically recognizes and binds to β-catenin, resulting in transcriptional activation via binding to the response elements of target genes by RORα1 through β-catenin as a coactivator. Meanwhile, transcriptional regulation by β-catenin with T cell factor (TCF)/lymphoid enhancer factor (LEF) complexes is suppressed via binding of RORα1 to β-catenin. In contrast, the N-terminal domain of RORα4 has no effect on β-catenin with TCF/LEF. Although the DBD and LBD are common, the transcriptional control function differs greatly depending on the difference in the N-terminal, and the role of each isoform differs. Although most RORα isoforms are under the control of different promoters, little is known about the transcriptional regulation of their tissue-specific expression (Fig. 1B).

Nuclear receptors activate or inactivate the transcription of primary target genes by binding to specific DNA sequences known as hormone-responsive elements, which are composed of 6-bp sequences from 5'-AGGTCA-3' or 5'-AGAACA-3'. These sequences are referred to as consensus half-site motifs and are arranged as direct, inverted, or everted repeats. As a monomer, nuclear receptor RORα binds to the ROR response elements (ROREs) containing a single core half-site motif RGGTCA (R; A or G) preceded by a 6-bp A/T-rich sequence. RORα is based on one half-site sequence, and the presence of dual elements that overlap with other hormone response sequences containing half-sites is expected. For instance, the RORE of the laminin B1 gene overlaps with that of the RA response elements (RAREs) and is transcriptionally activated by both RORα and RAR. In addition, the RORE of the CYP7B1 gene overlaps with liver X receptor (LXR) response elements (LXREs), resulting in the simultaneous transcriptional activation of LXR and repression of RORα; hence, the binding of RORα or LXR to the response elements functions as a competitor with each of the other receptors. The LXRE contains a direct repeat motif comprising two AGGTCA cores separated by four nucleotides. Furthermore, owing to the similarity in the binding sequences for peroxisome proliferator-activated receptor-γ (PPARγ) and RORα, RORα can modulate PPAR signaling by competing with PPARγ for PPAR response elements (PPREs). The PPRE contains a direct repeat motif comprising two AGGTCA cores separated by a single nucleotide. Recent global transcriptome studies have shown that RORα controls lipid homeostasis via the negative regulation of the transcriptional activity of PPARγ, which mediates hepatic lipid metabolism. Similarly, the reverse strand of ERβ1 (REV-ERβ), which has an amino acid sequence similar to that of RORα, binds to ROREs. Although REV-ERβ has been identified as an inactive monomeric receptor with DNA-binding ability, it lacks the capacity to activate transcription, instead functioning as a repressor of transcription (Fig. 2). Moreover, each nuclear receptor is dependent on specific DNA sequencing, whereas transcription is coordinated by DNA-binding affinity, expression level, and the presence or absence of a ligand.

3. LIGANDS AND MEDIATORS ACTING ON RORα

RORα was initially described as an orphan receptor and has long been considered a constitutive activator of transcription in the absence of exogenous ligands. A recent study has crystallized the RORα LBD and revealed the presence of cholesterol and cholesterol sulfate in the ligand-binding pocket (Protein Data Bank codes: 1N83 and 1S0X). Further experiments on purified RORα LBDs have shown the presence of ligands, such as cholesterol and 7-dehydrocholesterol (also provitamin D3), that represent the major ligands in the LBD. For instance, RORα LBD expressed in insect cells is in a liganded form with bound cholesterol, which stabilizes the receptor in an agonistic conformation. Furthermore, the oxidized form of cholesterol acts as an inverse agonist, one of the 7-oxygenated sterols, 7α-hydroxycholesterol (7α-OHC), functions as a high-affinity ligand for RORα isoforms by di-
Fig. 2. RORα Transcriptional Regulation System and Its Physiological Functions

RORα binds as a monomer to ROREs containing the RGGTCA (R; A or G) consensus motif preceded by an A/T-rich region. REV-ERB can compete with RORα for binding to ROREs. LXR and PPAR bind the LXRE and PPRE as overlaps to RORE, respectively. RORα interacts with coregulators as coactivators or corepressors to positively or negatively regulate gene transcription. Certain ligands (including cholesterol, oxysterols, provitamin D₃, and their derivatives) can modulate RORα transcriptional activity. RORα is critical in the regulation of several physiological functions and may have a role in various pathologies. RORα and its regulatory networks might serve as potential novel targets for therapeutic strategies to intervene in disease processes. RORα, retinoic acid receptor-related orphan receptor α; RORE, ROR response element; REV-ERB, reverse strand of ERβ4; LXR, liver X receptor; LXRE, LXR response elements; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response elements.

Cholesterol depletion in osteosarcoma cells by statins, which are inhibitors of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, in low-density lipoprotein (LDL)-free serum significantly decreases the transcriptional activity of RORα, implying that cholesterol is an endogenous ligand rather than merely a structural cofactor. In addition, the statin-induced reduction in cholesterol levels in hepatocytes with active cholesterol synthesis causes RORα target gene expression to decrease owing to the attenuation of the RORα transcriptional activation in the ligand form. Moreover, the ability of RORα to activate transcription is attenuated following the suppression of cholesterol synthesis by azoles acting as inhibitors of lanosterol 14α-demethylase (CYP51). Mice with hepatocyte-specific knockout of Cyp51 are characterized by the progressive onset of liver injury with fibrosis. Endogenous ligands of RORγ, a subtype of RORα, are situated downstream of lanosterol and upstream of zymosterol in the cholesterol synthesis pathway; the levels of these ligands are reduced by Cyp51 knockout. The reduction in RORα and RORγ transcriptional activity is greater in Cyp51 knockout mice and is correlated with their downregulated amino and fatty acid metabolism via the transcriptional attenuation of RORα target genes. Therefore, RORα may also impact transcriptional regulatory ability through the action of cholesterol derivatives as ligands. However, how nuclear receptors sense differences in the concentrations of multiple metabolites and interact with each other to activate or suppress the expression of target genes in vivo remains to be determined.

The conformational changes that occur following agonist binding cause receptors to enter an activated state, which induces chromatin remodeling via histone acetylase activity and, in turn, facilitates the recruitment of coactivator complexes that increase the transcription of the target gene. The recruitment of the corepressor complex induces chromatin
compression as well as the suppression of gene expression through histone deacetylase activity.\(^\text{55}\) RORs interact with coactivators and corepressors, suggesting that they function as activators and repressors of gene transcription.\(^\text{55}\) During ROR\(\alpha\)-mediated transcriptional regulation, RORs recruit different coactivator complexes when bound to ROREs in the promoter region of different genes,\(^\text{56}\) which suggests that their promoter functions play important roles in determining which coactivators are recruited by RORs.\(^\text{57}\) The coregulators found within ROR protein complexes include the coactivators steroid receptor coactivator-1 (SRC-1, NCOA1), SRC-2 (NCOA2, TIF2, GRIP1), SRC-3 (NCOA3), PARG coactivator-1 alpha (PGC-1\(\alpha\), PPARGC1A), cAMP response element-binding protein (CBP), p300, human immunodeficiency virus type 1 (HIV-1) Tat interactive protein (HTATIP, TIP60), and \(\beta\)-catenin with the following corepressors: nuclear receptor corepressor 1 (NCOR1, N-CoR), NCOR2 (SMRT), receptor interacting protein 140 (RIP140), Hairless (HR), cadherin 4 (CDH4), and neuronal interacting factor X (NIX1).\(^\text{45,57-60}\) (Fig. 2). Cell-specific interactions with specific coregulators may contribute to the molecular mechanism underlying the distinct physiological functions of RORA.\(^\text{57}\) Members of the p600 family containing SRCs were among the first coregulators to be cloned, based on their ligand-dependent recruitment to the nuclear receptor LBD through three-helical LXXLL (X, any amino acid) motifs located in their N-terminal region, and conserved leucine-rich motifs in their C-terminal activation domain that mediate interactions with additional coregulators.\(^\text{61}\) Moreover, ligands that affect the interaction between these nuclear receptors and coregulators are attracting attention as drug discovery targets for the treatment of various diseases.\(^\text{61}\) SRCs associate with nuclear receptors in a ligand-dependent manner to enhance transcriptional activity via the stabilization of the basic transcriptional complex by binding indirectly to DNA and play important roles in a variety of physiological processes.\(^\text{61}\) The role of RORs in the regulation of glucose metabolism is characterized by the loss of SRC-2 in mice, leading to a phenotype similar to von Gierke’s disease, which is associated with severe hypoglycemia and abnormal glycogen accumulation in the liver.\(^\text{62}\) Furthermore, the phenotypes observed in global or liver-specific SRC-2 knockout mice include hypoglycemia in the fasted state and reduced expression of hepatic glucose 6-phosphatase (G6PC) in both fed and fasted states.\(^\text{62}\) SRC-2 regulates G6PC expression by coactivating ROR\(\alpha\) at an evolutionarily conserved RORE sequence of the proximal G6PC promoter.\(^\text{62,65}\) Thus, the interaction of ROR\(\alpha\) with mediators and transcription factors is important in regulating the degree of gene expression.

4. PHENOTYPE OF ROR\(\alpha\)-DEFICIENT MICE

The staggerer (sg) mutation arose spontaneously in a stock of obese mice and is recognized by phenotypes characteristic of cerebellar lesions; the sg cerebellum is significantly smaller in obese mice than in control mice, containing fewer of each principal cell type.\(^\text{54}\) After 35 years of analysis, the sg mutation has been identified by positional cloning as a putative null allele of ROR\(\alpha\).\(^\text{17}\) Mutant mice contain a 6.4-kb intragenic deletion that removes the fifth exon, encoding the start of the LBD, loss of this exon predicts a frameshift in the mRNA, resulting in a premature stop codon. Reverse genetics analysis of ROR\(\alpha\)-null mice created by gene targeting produced phenotypes essentially identical to sg.\(^\text{65,66}\) ROR\(\alpha\) expression in the brains of adult mice is restricted to a few specific neuron classes, including the Purkinje cells of the cerebellum, and is temporally regulated during postnatal development.\(^\text{13,67}\) Moreover, the ROR\(\alpha\) gene is disrupted in sg mice, which show a cell autonomous defect in the development of Purkinje cells.\(^\text{17}\) Interestingly, further indications for a link between ROR\(\alpha\) and autism arose from a recent study demonstrating that microRNA-137, which has been implicated in autism and schizophrenia, targets the 3′-untranslated region (UTR) of ROR\(\alpha\).\(^\text{58}\)

In other pathophysiological conditions, sg mice have enhanced susceptibility to atherosclerosis and hypoalphapoproteinemia, displaying accelerated vascular lesion development in response to a proatherogenic diet.\(^\text{69}\) Sg mice also exhibit decreased fasting blood glucose levels, mildly improved glucose tolerance, increased insulin sensitivity, and ROR\(\alpha\) involvement in metabolic diseases.\(^\text{70}\) In addition to increased ischemia-induced angiogenesis in sg mice, ROR\(\alpha\) expression is observed in vascular cells, whereas ROR\(\alpha\) expression is decreased in atherosclerotic plaques.\(^\text{21,71,72}\) ROR\(\alpha\) also regulates gene expression in obesity-associated inflammation; sg mice fed a high-fat diet exhibit reduced adiposity and hepatic triglyceride levels compared with wild-type littermates and are resistant to the development of hepatic steatosis, adipose-associated inflammation, and insulin resistance.\(^\text{73}\) Further, RORs regulate key physiological pathways, is involved in pathogenic processes, regulates lipid and glucose metabolism, and is believed to play a protective role against the development of atherosclerosis.\(^\text{14}\) Indeed, cerebral ischemia/reperfusion (I/R) injury is associated with greater cerebral infarct size, brain edema, and cerebral apoptosis in sg mice compared with those in wild-type mice. In contrast, this effect is reduced in transgenic mice with brain-specific ROR\(\alpha\) overexpression, compared to nontransgenic controls.\(^\text{74}\) Furthermore, sg mice subjected to myocardial I/R injury show a significantly increased myocardial infarct size, myocardial apoptosis, and exacerbated contractile dysfunction compared with wild-type mice.\(^\text{75}\) Moreover, mice with cardiomyocyte-specific ROR\(\alpha\) overexpression are less vulnerable to myocardial I/R injury than the control mice.\(^\text{75}\) In a recent study on human-induced pluripotent stem cell-derived cardiomyocytes infected in vitro with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), ROR\(\alpha\) expression was reported to be upregulated, which was speculated to be a cardioprotective response to direct viral invasion.\(^\text{76}\)

Stenosis of blood vessels owing to atherosclerosis is a typical condition caused by modern unhealthy lifestyles (fat-rich, high-calorie diet, lack of exercise, and smoking). However, this condition also occurred in ancient human social groups.\(^\text{77}\) This phenomenon may be caused not only by environmental factors, such as unhealthy eating habits, but also by certain genetic factors.

5. ROR\(\alpha\) TARGET GENES

To identify atherosclerosis through metabolic disease-related genes, it is important to perform a forward genetics search based on the pathological conditions of atherosclerosis and reverse genetics evaluation based on the functional deletion of a specific gene. Based on transcriptional regulation analysis...
using an RORα functional deletion, target genes of RORα have been explored.

RORα regulates the circadian expression of several clock genes, including circadian locomotor output cycles kaput (CLOCK), aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL)/brain and muscle ARNT-like 1 (BMAL1), neuronal PAS domain protein 2 (NPAS2), and cryptochrome circadian regulator 1 (CRY1). Moreover, RORα competes with REV-ERBs for binding to their shared ROREs on certain clock genes, leading to a circadian pattern. Therefore, RORα influences the period length and stability of the circadian rhythm. Increasing evidence regarding how the cholesterol/oxysterol pathways are intertwined with the circadian clock has been reported. The identified contact points include different forms of the RORs, REV-ERBs, and LXRα. These receptors are regulated by sterols/oxysterols and the circadian clock, representing a complex interplay between sterol metabolism and the clock. Moreover, RORα binds to the ROREs of genes involved in lipid metabolism to regulate genes such as apolipoprotein A1 (APOA1), APOA5, and APOC3, the key regulators of sterol regulatory element-binding protein-1c (SREBP1c), and the reverse cholesterol transporters, ATP binding cassette subfamily A member 1 (ABCA1) and ABCG1. Synthesis enhancement of a subset of nuclear factor-kappa B (NF-κB)-regulated anti-inflammatory genes, including IL-1β, IL-6, and tumor necrosis factor (TNF), both at the transcriptional and transcriptional levels. RORα is a negative regulator of the inflammatory response, functioning via NF-κB inhibition through NF-κB inhibitor α (IκBα) activation by RORα via an RORE in the IκBα promoter. Furthermore, RORα is involved in TNF and IL-6 production upon macrophage activation and plays a key role in M1/M2 polarization of murine Kupffer cells, which are liver-resident macrophages. Kupffer cells with RORα deletion have the proinflammatory M1 phenotype, as the shift to anti-inflammatory M2 requires RORα activation. Additionally, RORα reduces lipid droplets via the upregulation of neutral cholesterol ester hydrolase 1 (NCEH1) in macrophages, suggesting that RORα functions to protect against atherosclerosis.

In brain endothelial cells, claudin domain containing 1 (CLDN1), which is involved in tight junction formation, is regulated at the transcriptional level by RORα binding to cholesterol derivatives as ligands and the myeloid zinc finger 1 transcription factor, as well as at the post-transcriptional level by microRNA-124. Moreover, decreased CLDN1 expression enhances vascular permeability, which consequently increases the risk of cerebellar hemorrhage. Within the liver, RORα specifically functions as a positive regulator of genes encoding phase I and phase II proteins, which are involved in the metabolism of lipids, steroids, and xenobiotics, such as sterol 12α-hydroxylase (CYP8B1), sterol 7α-hydroxylase (CYP7B1 and CYP39A1), aromatase (CYP19A1), and sulfotransferase family 2A member 1 (SULT2A1); these enzymes catalyze the metabolism of oxysterols, such as cytotoxic compounds, via SR1078 induces M2 polarization through Krüppel-like factor 4 (KLF4) gene regulation in macrophages, suggesting that RORα functions to protect against atherosclerosis.

6. DRUG TREATMENT OF DISEASES WITH RORα SYNTHETIC LIGANDS

Transcriptional conjugating factors in the cell nucleus bind to nuclear receptors in a ligand binding-dependent manner. The transcriptional conjugation inhibitor (corepressor) that controls transcription binds to the unbound state of the ligand and dissociates owing to ligand binding, after which the transcriptional conjugation activator (coactivator) is acquired. The role of LXR agonists and cholesterol derivatives in the regulation within the promoter region of human genes and gene expression analyses involving both loss-of-function and gain-of-function have been performed, identifying target genes associated with antiatherosclerosis. Uncoupling the ability of RORα chimerics to transactivate gene expression in a heterologous reporter assay. Moreover, the LXR agonist T0901317, a benzenesulfonamide derivative, suppresses the activity of RORα in macrophages, suggesting that RORα functions to protect against atherosclerosis.

In addition, the amide derivative SR1078, from the T0901317 scaffold, modulates RORα activity and increases coactivator recruitment in a dose-dependent manner. RORα activation via SR1078 induces M2 polarization through Krüppel-like factor 4 (KLF4) gene regulation in macrophages, effectively protecting against nonalcoholic steatohepatitis (NASH). This activation also promotes the removal of accumulated cholesterol, which is the cause of atherosclerosis, by regulating lipid metabolism-related genes. SR1078 ameliorates renal dysfunction and damage in a renal I/R injury mouse model; thus, RORα is a potential endogenous protector against renal I/R injury. Moreover, treatment of an autonomic mouse model with SR1078 reduces repetitive behavior. The thiourea derivative JC1-40, which is a RORα agonist, protects against oxidative stress through induction of the antioxidative enzymes, superoxide dismutase 2 (SOD2) and glutathione peroxidase 1 (GPX1) and attenuates the methio-
nine-choline deficient diet-induced NASH in mice.\textsuperscript{113} The selective and specific ROR\textalpha inverse agonist, SR3335, suppresses gluconeogenesis-associated ROR\textalpha target gene expression and decreases blood glucose levels in a diet-induced obesity mouse model.\textsuperscript{114} The inverse agonist SR1001 suppresses ROR\textalpha and ROR\textgamma, attenuates Th1 differentiation and insulin by inhibiting IL-17A production in a mouse model of type 1 diabetes. SR1001 also attenuates ROR target genes involved in hepatic gluconeogenesis and prevents hyperglycemia.\textsuperscript{107,115,116} Finally, RS-2982 is an ROR\textalpha agonist that increases the expression of microRNA-122 as an ROR\textalpha target in mouse livers. Mice treated with RS-2982 and fed a high-fat or atherogenic diet showed reduced hepatic lipotoxicity, liver fibrosis, and body weight compared to mice administered the vehicle.\textsuperscript{117} Hence, the regulation of ROR\textalpha activity may be a therapeutic strategy for treating several conditions, including atherosclerosis, NASH, type 2 diabetes, autoimmune disorders, and autism. In addition, gene regulation and polymorphisms of human ROR\textalpha are associated with the development of many conditions, including type 2 diabetes,\textsuperscript{118} multiple sclerosis,\textsuperscript{119} cerebellar ataxia,\textsuperscript{120} autism,\textsuperscript{121} bipolar disorder,\textsuperscript{122} Alzheimer’s disease,\textsuperscript{123} and the severity of coronavirus disease 2019.\textsuperscript{124} Changes in the function, RNA stability, and target gene expression of ROR\textalpha have been reported owing to various polymorphisms in the structure, 3’-UTR, and response sequences of ROR\textalpha, respectively. Mutations in these nucleotide sequences may also affect individual differences in human diseases.

7. CONCLUSION

In summary, ROR\textalpha is involved in the regulation of several physiological processes, including development, metabolism, and circadian rhythm. Furthermore, ROR\textalpha is an important regulator of plasma cholesterol levels and is involved in lipid homeostasis. For example, apolipoprotein expression increases high-density lipoprotein (HDL) levels and CYP expression activates oxysterol metabolism by ROR\textalpha activation. ROR\textalpha-deficient mice are more likely to develop atherosclerosis through lowered HDL levels, increased inflammatory cytokine expression, and U/R-induced damage. The transcriptional activity of ROR\textalpha is promoted by the endogenous ligand cholesterol derivatives, which promote the formation of transcription initiation complexes. However, ROR\textalpha transcriptional activation is attenuated when intracellular cholesterol is reduced by lipid-lowering drugs that inhibit cholesterol synthesis, such as statins. Research has been conducted with the aim of developing a new treatment for atherosclerosis by understanding the ROR\textalpha transcriptional network, which has an anti-atherosclerotic effect. Thus, the recent characterization of endogenous ligands, mediators, and initiation complexes associated with the transcriptional regulation of these nuclear receptors has facilitated the development of synthetic ligands and highlighted the potential of application of ROR\textalpha as a therapeutic target for several diseases. However, how nuclear receptors sense differences in the concentrations of multiple metabolite ligands and interact with each other to activate or suppress target gene expression in vivo remains to be determined. This review sheds light on the current literature related to the structure and function of ROR\textalpha, its genetic inter-individual differences, and its potential as a therapeutic target in atherosclerosis.

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Conflict of Interest The authors declare no conflict of interest.

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