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Advances in Orphan Nuclear Receptor Pharmacology: A New Era in Drug Discovery

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ABSTRACT: Over the past decade, advances in biophysical chemistry, genomic analysis, and structural biology have resulted in the exponential growth of knowledge and critical insight into the function and regulation of orphan nuclear receptors. This article summarizes the current progress in illuminating the structure, function, and regulation of orphan nuclear receptors and their involvement in the physiology, development and molecular mechanism of different pathological conditions. Moreover, current strategies for discovering endogenous ligands, downstream NR-regulated target genes, and new drugs for future therapeutics will be discussed.

Nuclear receptors (NRs) consist of 48 transcription factors in humans. Of these, 10 are considered orphan NRs (ONRs) because the natural ligand has not yet been identified, while 26 are former orphans but are now labeled as adopted NRs after the discovery of their respective natural ligands. Although adopted NRs bind endogenous ligands, it remains unclear whether the NR function is ligand-regulated. The remaining 12 NRs employ endocrine hormones as endogenous ligands and are classified as endocrine NRs (Figure 1).[1] Ligand binding to NRs typically induce a conformational change that enable binding of NRs to target DNA motifs across the genome called nuclear receptor response elements (NRREs) and recruitment of co-regulator proteins that modulate transcription of target genes. Because ONRs could potentially be ligand-regulated or druggable, they are attractive therapeutic targets using small molecule compounds.[1,2]

Endocrine NRs			
GR	ERα	THRα	RARα
MR	ERβ	THRβ	RARβ
AR	PR	VDR	RARγ
Orphan NRs			
EAR-2	TLX	DAX1	COUP-TFI
TR2	PNR	GCNF	COUP-TFII
		NOR-1	SHP
Adopted NRs			
CAR	REV-ERBα	RXRα	PPARα
FXR	REV-ERBβ	RXRβ	PPARδ
Nur77	SF1	RXRγ	PPARγ
Nurr1	TR4	LXRα	ERRα
HNF4α	RORα	LXRβ	ERRβ
HNF4γ	RORβ	PXR	ERRγ
LRH-1	RORγ		

Figure 1. Human nuclear receptors (NRs) classified into endocrine NRs, which employ endocrine hormones as endogenous ligands, orphan NRs, which currently have no known natural ligand, and adopted NRs, which are former orphans but with natural ligands recently identified.

Research on ONRs over the past 30 years has been focused on their physiological roles and the molecular mechanism of their link to diseases.[3] Because NR LBDs exhibit structural plasticity and malleability, deorphanizing the remaining ONRs could be challenging. Drug discovery advances targeting ONRs herald a new era that may illuminate unexpected ligands with corresponding novel regulatory pathways and pharmacological applications. Here, advances on elucidating orphan NR structure, identifying regulatory regions bound by NRs, and determining NR role in the physiology, progression, and molecular mechanism of disease will be presented, as well as strategies for identifying endogenous regulators and establishing NR druggability for novel therapeutics.

■ ORPHAN NR STRUCTURE

ONRs have four major domains that exemplify classical NR architecture:[4] a disordered N-terminal domain encompassing activation function 1 (AF1), a DNA-binding domain (DBD) that bind specifically to NRREs, a ligand-binding domain (LBD), and a hinge region usually targeted by post-translational modifications. The size of the LBD pocket (LBP) can vary greatly among ONRs, and the AF2 region responsible for coactivator recruitment may or may not be present.[2]

While the overall domain architecture of the NR LBD is highly conserved, the current collection of NR X-ray crystal structures has demonstrated structural peculiarities that likely confer ligand selectivity and ability to form macromolecular complexes.[2,4] Orphan NR LBD regions range from a collapsed pocket to large binding cavities, suggesting that ligand-mediated activation may not be required for all ONRs.[2,5] However, several recently adopted NRs undergo large conformational changes to accommodate a bulky ligand, such as REV-ERBs. The collapsed pocket of REV-ERBβ dramatically expanded by 600 Å^3 to bind the porphyrin heme, which was identified as the endogenous ligand.[2] Thus, there is a possibility that ONRs previously thought to be ligand-

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Table 1. Orphan and Recently Adopted Nuclear Receptors, Probable Ligands, and Implications to Diseases

nuclear receptor	probable ligands	implications to disease
Nur77/NR4A1	6-mercaptopurine; cytosporone B; unsaturated fatty acids	activated in pancreas, colon, and liver cancers; potential target for the treatment of neurodegenerative diseases, such as Parkinson's; aberrant expression in metabolic disorders
Nurr1/NR4A2	6-mercaptopurine; cytosporone B; unsaturated fatty acids	activated in bladder and lung cancers; implicated as a therapeutic target for treating Parkinson's disease and schizophrenia; aberrant expression in metabolic disorders
NOR1/NR4A3	6-mercaptopurine; cytosporone B	inactivated in nasopharyngeal carcinoma; aberrant expression in metabolic disorders
DAX-1/NR0B1	unknown	associated with X-linked adrenal hypoplasia congenita and hyopogonadotropic hypogonadism; promotes cervical cancer cell growth; highly expressed in epithelial ovarian tumors
SHP/NR0B2	unknown	increased expression in hepatocellular carcinomas and intestinal precancerous lesions
TLX/NR4E1	unknown	overexpressed in brain cancer cells such as neurocytomas and gliomas
PNR/NR2E3	unknown	higher expression correlated with good clinical outcome for breast cancer; increased PNR autoantibodies observed in pancreatic cancer patients
COUP-TFI/NR2F1	unknown	expressed in a number of prostate tumors but correlations between expression levels and disease parameters not observed
COUP-TFII/NR2F2	unknown	higher expression in prostate tumors and indicator of earlier recurrence of prostate cancer
EAR2/NR2F6	unknown	highly expressed in lymphomas and colorectal tumors
TR2/NR2C1	unknown	interaction with TRA16 results in ERβ suppression, thereby inhibiting the development of lung tumors; higher expression in breast cancer cells
RORα/NR1F1	sterol derivatives SR1001; melatonin; CGP52608	inactivated in breast and prostate cancers; target for treating autoimmune disorders
RORβ/NR1F1	all-*trans* retinoic acid; melatonin	implicated in the early development of colorectal cancer
RORγ/NR1F3	sterol derivatives SR1001; melatonin; 3CI-AHPC; CGP 52608	absence of RORγ promotes T-cell lymphomas; activated in gastric tumors; target for treating autoimmune disorders
ERRα/NR3B1	isoflavones; octochlorocamphene; chlordane; XCT790 diethylstilbestrol	increased expression in ER breast cancer correlated with poor clinical outcomes
ERRβ/NR3B2	isoflavones; 4-hydroxytamoxifen; diethylstilbestrol; GSK4716	reduced expression in prostate cancer
ERRγ/NR3B3	isoflavones; 4-hydroxytamoxifen; diethylstilbestrol; GSK4716	increased expression inhibits growth of prostate cancer cells
LRH-1/NR5A2	phospholipids; GSK8470	activated in pancreatic and breast cancers; target for treating diabetes and metabolic disease; implicated in colorectal cancer and inflammatory bowel disease
GCNF/NR6A1	unknown	expression lower in both ER^+ and ER^- breast tumors; low levels correlated with higher sensitivity to the anticancer drug trabectedin

independent could actually have endogenous metabolites and are druggable.

Ligands act as conformational switches that trigger movements of the C-terminal helix (H12) and define whether the LBD is in an active or inactive conformation.[4] When bound to a ligand acting as an agonist, H12 adopts an active conformation where the coactivator-binding groove (AF2) becomes accessible and permits docking of coactivators. Without a ligand, H12 can be observed in a continuum of positions including inactive conformations that block coactivator docking and permit co-repressor binding instead.[2] Binding of coactivators to the LBD enhance the transcription of target genes, while co-repressors do the opposite. Currently available structures of ONRs have challenged this classical model as some ONRs have a collapsed ligand binding pocket (LBP) where the binding cleft is filled with hydrophobic side chains.[2,5] Some NRs like the NR4As are proposed to bind ligands through their AF1 domain instead of LBP.[6] Moreover, some ONRs have an H12 in a constitutive agonist (or active) conformation even when a ligand is not present.

ORPHAN AND ADOPTED NUCLEAR RECEPTOR AS ATTRACTIVE DRUG TARGETS

A subset of receptors has been implicated in the molecular pathology of cancer, namely, ERRs, RORs, Nur77 subfamily, LRH-1, and the rest of the remaining ONRs.[6−8] Moreover, RORs and LRH-1 have been linked to metabolic diseases and autoimmunity,[9] while Nur77 and Nurr1 are implicated in neurodegenerative disorders and metabolic diseases.[5] Table 1 summarizes the probable NR ligands and possible implications of several orphan and adopted NRs to different pathologies.[5−10]

Recent pharmacological studies have zeroed in on receptors that bind oxysterols, fatty acids, bile acids, and other common metabolites because these NRs can be observed within the context of their metabolic milieu. Most NRs that bind such ligands were at a time orphans and their functional nexus to disease and related signaling pathways were once elusive. The discovery of natural ligands has not only illuminated their biological significance and link to disease development but also has provided hints on molecular scaffolds of candidate drugs that could target these NRs for therapeutic intervention. With approximately 14% of all U.S. FDA-approved small-molecule drugs targeting NRs, identification of drugs targeting ONRs could be routinely accomplished.[1] Finding drugs targeting ONRs that elicit the same physiological effects as current drugs but via alternative molecular mechanisms could potentially solve issues of drug resistance and undesirable side effects in existing NR targets.

To identify natural ligands and candidate drugs, several biophysical techniques are currently being employed for medium- to high-throughput screening and functional assays: ThermoFluor, fluorescence polarization, luciferase transactivation, surface plasmon resonance, fluorescence resonance energy transfer (FRET), and Alpha Screen.[7,11] Moreover, ligands are being identified via direct binding to NR immobilized on solid support. Here, cell lysates or compound mixtures are passed over the immobilized NR and subsequently washed. Drugs or metabolites bound to the target protein are later identified via liquid chromatography coupled to mass spectrometry (LC-MS).[5,11] Formation of the

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NR−ligand complex could also be confirmed thermodynamically via isothermal titration calorimetry (ITC) and structurally through NMR spectroscopy and X-ray crystallography.[5,7,11]

The future holds great promise for the reliable identification of true positive hits in ligand screening by translating biophysical methods to cellular environments. Techniques such as isothermal microcalorimetry and in-cell NMR strengthen the physiological relevance of biophysical measurements.[12] The rapid advancement of cell biophysical and single-molecule measurements will enhance our ability to observe relevant mechanisms that translate ligand−NR interactions to physiological effects. Moreover, advances in cryoelectron microscopy (cryo-EM) instrumentation[11] will enable solving the structure of full-length (i.e., LBD with DBD) and multimeric ONR−cofactor−nucleic acid complexes, which will be a significant step forward in understanding the role of ONRs in various diseases.

UNCOVERING THE ROLE OF ORPHAN NRS IN DISEASE DEVELOPMENT VIA GENOMIC ANALYSIS

Another area where exciting advances are being made is genomic analysis, which has elucidated the complex genomic network involved in the development of disease.[1] Before the advent of genomics, studies were focused on a single NR at a time within a single type of tissue. These studies have been helpful in shedding light to relationships between NR function and the development of disease, but they lack the comprehensive and stratified context conferred by genomic analysis. For instance, genomic studies could uncover mechanisms on how cells adapt and evade targeted therapeutics and how amino acid substitutions affect drug resistance mechanisms in hormone-dependent cancer.[1]

New techniques are being developed to further delineate regulatory regions bound by NRs. A shining example is ChIP-exonuclease that substantially improved resolution relative to that of ChiP-seq from about several hundred base pairs to a single nucleotide. New enhancer assays, like FIREWAch and STARR-seq, are being employed to elucidate the role of NR-regulated enhancers in basal, ligand-induced, and ligand-independent transcriptional responses. With new genes linked to different pathologies being discovered at a rapid pace, determining how NRs regulate these genes could possibly give novel therapies employing drugs that modulate NR function. For instance, several large-scale genome profiling projects, such as ENCODE and monENCODE, catalogued regulatory genome in different cell types and biological systems and have identified high-occupancy target (HOT) genomic regions, which are bound to a wide array of transcription factors, including NRs. HOT regions have been implicated in human diseases and different types of cancer. NRs that bind to HOT regions, such as those in the MCF7 ER^+ breast cancer cell line, are actively being investigated whether they bind individually or in large complexes and whether all of these NRs could mediate ligand-responsive transcriptional regulation of proximal target genes.[1] Thus, HOT-binding NRs, some of which are orphans, are attractive targets for the development of novel therapeutic approaches.

CONCLUDING REMARKS

While endogenous and synthetic small molecule ligands have been discovered for several "adopted" NRs, their regulation mechanism still remains elusive. In some cases, there is no consensus on the physiological significance of such ligands or the ligands do not modulate function at all. However, to prove whether ONRs are truly ligand-independent transcription factors is also almost impossible. Many ONRs display high basal transactivation activity and could possibly function without a ligand or they may be regulated via an alternative manner, such as post-translational modifications. Fortunately, we have a wide array of biophysical, structural, and genomic techniques at our behest to illuminate the regulation mechanism of ONRs. Structure elucidation of the remaining ONRs complexed with endogenous modulators, along with information from genomic analysis in disparate tissues and disease states will help improve our understanding of their regulation mechanism and role in disease, which could provide crucial hints on possible therapeutic strategies.

Technological advances in recent years hold great promise for the future of ONR pharmacology research and are aimed toward surmounting some of the challenges and limitations encountered at different stages of the drug discovery process. The expanding arsenal of biophysical, structural, and genomic techniques will usher in a new era in drug discovery that opens future opportunities for identifying novel ligands targeting orphan NRs, which will potentially reveal new regulatory pathways and pharmacological applications.

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Notes

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ABBREVIATIONS

NR: nuclear receptor; ONR: orphan nuclear receptor; LBD: ligand-binding domain; LBP: ligand-binding pocket; DBD: DNA-binding domain; H12: helix 12; AF: activation function; NRRE: nuclear receptor response element; ChIP: chromosome immunoprecipitation; FIREWACh: functional identification of regulatory elements within accessible chromatin; STARR-seq: self-transcribing active regulatory region sequencing; REV-ERB: reverse Erb; ERR: estrogen-related receptor; ROR: retinoic acid-related orphan; Nur77: neuron-derived clone 77; Nurr1: nuclear receptor-related 1; NOR1: neuron-derived orphan receptor 1; DAX-1: dosage-sensitive sex reversal adrenal hypoplasia critical region on chromosome X gene 1; SHP: short heterodimeric partner; TLX: tailless homologue orphan receptor; PNR: photoreceptor cell-specific nuclear receptor; COUP-TF: chicken ovalbumin upstream promoter-transcription factor; EAR2: V-Erb-A avian erythroblastic leukemia viral oncogene homologue-like 2; TR2: testicular receptor 2; LRH-1: liver receptor homologue-1; GCNF: germ cell nuclear factor

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