Expert Opinion

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Nuclear Receptors as Drug Targets: New Developments in Coregulators, Orphan Receptors and Major Therapeutic Areas

19 – 21 May 2003, Philadelphia, Pennsylvania, USA

Frances M Sladek
University of California, Riverside, Riverside, CA 92521, USA

Nuclear receptors (NRs) are ideal targets for drug discovery. Not only do they control a myriad of biological and disease processes, but they are also regulated by small lipophilic molecules that can be easily exchanged with a drug of choice. All 48 of the NR genes in the human genome have been identified, many of their structures have been solved and their ligands identified. Their mechanism of action has been elucidated and many of their target genes have been identified. Nonetheless, presentations at the recent conference sponsored by IBC Life Sciences indicated that while many NRs already have marketable drugs, the latest tools in robotics, genomics, proteomics, and informatics are helping to identify more selective drugs.

Keywords: bioinformatics, cancer, cofactors, drug discovery, human disease, nuclear receptors (NRs), partial agonists, promoter selectivity, structure–activity relationship

Expert Opin. Ther. Targets (2003) 7(5):

1. Introduction

Nuclear receptors (NRs) constitute a large superfamily of ligand-dependent transcription factors that are involved in nearly every aspect of vertebrate development and are linked to a wide range of human diseases [1] (see Table 1). NRs bind specific DNA elements in the regulatory regions of genes via a highly-conserved DNA-binding domain (DBD) and specific ligands via another highly-conserved domain, the ligand-binding domain (LBD), which consists of a series of approximately 12 helices that form a hydrophobic pocket. The binding of ligands in the pocket induces conformational changes in the receptor that affect the recruitment of coregulatory molecules (cofactors) that stimulate (co-activators) or repress (corepressors) transcription, typically via modification of the chromatin and interaction with the basal transcription machinery [2,3]. A detailed understanding of this mechanism of action is what is driving drug discovery for NRs, as discussed in the recent conference on 'Nuclear Receptors: New Developments in Coregulators, Orphan Receptors and Major Therapeutic Areas,' the highlights of which are summarised in this report.

2. Why nuclear receptors are good drug targets

In addition to ligands that act as pure agonists and antagonists, NRs can also bind partial (or mixed) agonists (or antagonists), which is what makes NRs really good drug targets. These compounds typically bind with a lower affinity than pure agonists and induce unique conformational changes in the receptor such that a specific cohort of cofactors are recruited, resulting in certain biological responses, but not others. The ability to selectively modulate the receptors has led to the search

 ISSN 1472-8222

Table 1. Nuclear receptors as drug targets.

Abbreviation	Full name	Disease/function
TRα,β	Thyroid hormone receptor	Hypothyroidism, obesity
RARα,β,γ	Retinoic acid receptor	Inflammatory skin disorders, leukaemia
PPARα,δ,γ	Peroxisome proliferator-activated receptor	Diabetes, coronary heart disease, obesity
RORα,β,γ	Retinoic acid-related orphan receptor	Atherosclerosis, immunological disorders, neurological disorders, osteoporosis
LXRα,β	Liver X receptor	Atherosclerosis
FXR	Farnesoid X receptor	Dyslipidaemia, liver disease
VDR	Vitamin D receptor	Osteoporosis, calcium homeostasis, cancer prevention
PXR	Pregnane X receptor	Xenobiotic metabolism
CAR	Constitutive androstane receptor	Xenobiotic metabolism
HNF4α,γ	Hepatocyte nuclear factor 4	Diabetes, haemophilia, lipid metabolism
RXRα,β,γ	Retinoid X receptor	Leukaemia, coronary heart disease
ERα,β	Oestrogen receptor	Breast cancer, osteoporosis, atherosclerosis, CNS
ERRα,β,γ	Oestrogen-related receptor	Early embryo development
GR	Glucocortioic receptor	Immunological disorders, metabolic disorders
MR	Mineralcorticoid receptor	Hypertension, myocardial hypertrophy
PR	Progesterone receptor	Breast cancer, infertility, pregnancy maintenance
AR	Androgen receptor	Prostate cancer, X-linked androgen insensitivity, spinal/muscular atrophy
NGFI-B, Nurr1	Nerve growth factor-induced-B	Neurological disorders, immunological disorders, cancer
GCNF	Germ cell nuclear factor	Fertility/contraception

Bold: NRs with marketed drugs.

Sampling of NRs as drug targets ordered by subfamilies, modified from the information presented by T Burris (Eli Lilly).

NR: Nuclear receptor.

for selective oestrogen receptor (ER) modulators (SERMs), selective androgen receptor (AR) modulators (SARMs), selective liver X receptor (LXR) modulators (SeLRMs), selective peroxisome proliferator-activated receptor (PPAR) modulators (SPARMs) etc. (globally referred to as selective NR modulators [SNuRMs]). For example, the ideal SERM would activate ER in the bone to fight osteoporosis, but not in breast or endometrial tissue, which might lead to cancer.

M Koegl (PheneX Pharmaceuticals AG, Heidelberg, Germany) noted that the key to identifying SNuRMs is to identify all of the cofactors that bind NRs and to determine their tissue distribution and expression profile during development. To this end, his company has screened the LBDs of all NRs using the yeast two-hybrid system. They found typically 50 – 100 different interacting proteins for each NR, with certain cofactors, such as steroid receptor co-activator (SRC)-1, binding nearly all NRs, whilst others, such as peroxisome proliferator-activated receptor-γ co-activator (PGC)-1, prefer just a few. This finding was also reflected in a computer-assisted review of the published literature [4]. The concept of each ligand-bound NR recruiting a different profile of cofactors is also the basis of the Molecular Braille Technology presented by R Carlson and R Evans-Storm (Karo Bio, Durham, NC, USA). This powerful new technique uses peptides identified in phage display to detect changes in NR surface conformation in response to ligand binding. The binding by a set of 4 – 16 peptides to ER, for example, can determine whether a test compound induces a conformational change similar to that of given reference compounds, such as oestradiol or tamoxifen, thereby allowing a quick, and relatively inexpensive, classification of the test compound.

The other key to developing SNuRMs is promoter selectivity or predictivity, i.e., finding a drug that will activate a given NR on certain target genes but not others. Much of the selectivity is thought to be derived from the fact that, like ligands, the specific DNA sequence of a response element can also induce allosteric changes in the NR [5]. Although much less well-documented than ligand-induced changes, this notion of DNA sequence affecting the profile of cofactors recruited by a NR to a given promoter permeated the talks at the conference and is expected to be broadly applicable to all NRs. Specific examples were provided by B Haendler (Schering AG, Berlin, Germany) and L Freedman (Merck, West Point, PA, USA) for the AR and vitamin D receptor (VDR), respectively. The author's and colleagues' own findings on the target genes of orphan receptor HNF4 also indicate that there are at least 165 unique DNA sequences to which HNF4 binds in the human genome, indicating an incredible amount of heterogeneity in response elements and therefore a great potential for gene-specific responses. All told, combining the ability of both partial agonists and specific DNA response elements to induce specific conformational changes in NRs enhances tremendously the possibility of finding a drug that has the appropriate desired effects without negative side effects.

3. New drugs for classical nuclear receptors

Whereas most of the classical NRs have had drugs for some time, nearly all of those drugs have unwanted side effects. For

example, glucocortioic receptor (GR) has long been a target for anti-inflammatory drugs but chronic use leads to bone loss, diabetes, myopathy, and hypertension. To avoid these and other negative effects of GR drugs, L Buckbinder (Pfizer, Groton, CT, USA) screened for dissociated agonists (DAGRs) that regulate certain anti-inflammatory genes, but not others, and found two compounds (DAGR1 and DAGR2) that do not promote the differentiation of osteoblasts nor stimulate gluconeogenic enzymes in cell-based assays. J Baxter (University of California San Francisco, San Francisco, CA, USA) reported on a new mode of action for thyroid hormone receptor (TR) agonists. Like previous agonists, these compounds (GC-24II, GC-1, KB-000141) decrease obesity, cholesterol and triglyceride levels. However, unlike traditional agonists, they bind not in the ligand-binding pocket but in the dimer interface and seem to have fewer unwanted effects compared with traditional TR agonists, such as tachycardia, arrhythmia and heart failure. Freedman (Merck) also presented data on 20-epi VitD3 analogues (MC-1627 and MC-1288) that may avoid the extreme hypercalcaemic effects of the more traditional 1,25-dihydroxy VitD3 by causing VDR to selectively bind certain co-activators.

T Mirzadegan (Roche Biosciences, Palo Alto, CA, USA) presented an elegant example of how computational chemistry can be used to design a drug specific to a given NR isoform. Mirzadegan and colleagues compared the structure of RARγ, which is implicated in emphysema, to that of RARα and RARβ and used molecular dynamic simulations (SANDER, FlexX and Locally Enhanced Sampling [LES]) to identify first a unique residue in RARγ that contacts the ligand and then a new ligand specific for RARγ. Ro-3300074 has a different ligand scaffold and fewer side effects than the traditional all-*trans*-retinoic acid. It promotes the regeneration of lung tissue, decreases emphysema and restores lung function. and is soon to enter Phase II trials.

Finally, B Cheskis (Wyeth Women's Health Research Institute, Collegeville, PA, USA) presented exciting data on a potentially new drug target that links the ER to another well-characterised family of regulators, the Src kinases. Cheskis identified modulator of non-genomic activity of oestrogen receptor (MNAR) in a liver cancer cell line, which increases the ability of ER to activate transcription by forming a complex with ER and Src via ten LXXLL motifs and three PXXP (SH3) motifs, respectively. MNAR is found in many tissues but it is amplified in breast cancer cells [6].

4. Orphan receptors as drug targets

Much attention at the conference was paid to several of the receptors that are involved in intermediary metabolism and for which ligands have recently been identified [7]. Chief among these was LXR, which binds oxysterols. It plays a role in lipid transport by increasing cholesterol efflux via activation of ABCA1, Cyp7A and ApoE genes, and decreases inflammation by inhibiting cytokine gene expression in macrophages. However, while LXR agonists can reduce plaque formation associated with atherosclerosis, they also tend to increase triglyceride levels and induce adipogenesis, both of which are pro-atherogenic. D Lala (Pharmacia, St Louis, MO, USA) identified a SeLRM (PHA-769956) that increases the activity of LXRα but not that of LXRβ. Whereas it is much less potent than a previously identified LXR ligand (T-0901317), it does not increase the expression of SREBP1c or FAS, both of which are associated with the negative effects of LXR agonists. R Heyman (X-Ceptor Therapeutics, San Diego, CA, USA) presented *in vivo* data about another exciting SeLRM (XCT-628) that decreased the arterial lesions in a mouse model of atherosclerosis by 50%. The compound not only prevented the lesions from getting worse, it actually made them regress from the baseline, something that has never before been seen with the current drugs on the market. A decrease in macrophage foam cell content and an increase in plaque stability were also observed. T Burris (Eli Lilly, Indianapolis, IN, USA) presented a potential new role for LXR in diabetes, showing that it repressed the expression of gluconeogenic enzymes such as PEPCK and G6P in the liver. He also showed that LXR and PPARα shared the same 'chemical space', in that they both bind fenofibrates, although LXR prefers the ester form, which act as antagonists, whilst PPARα prefers the acid form, which act as agonists. Therefore, it appears that fenofibrates that are used clinically to lower triglyceride levels may be acting via LXR, in addition to PPARα. This would make fenofibrates the first clinically used compound to act via LXR.

5. Use of X-ray structure analysis to de-orphanise receptors

Another common theme of the conference was the use of X-ray crystallographic structure analysis and mass spectometry to de-orphanise receptors and, in conjunction with molecular modelling, to rationally design drugs to increase the specificity or affinity of known ligands. M Geiser (Novartis Biomedical Research Institute, Basel, Switzerland) presented the structure of the LBD of RORα which, when expressed in insect cells, was found to contain cholesterol in the ligand-binding pocket [8]. Additional space in the pocket accommodated derivatives of cholesterol such as cholesterol sulfate, epicholesterol and 7-dehydroxycholesterol, although it is not yet clear whether the cholesterol acts as a structural cofactor or as a traditional ligand that induces an activated form of the receptor. Nonetheless, since RORα has been linked to bone metabolism, this raises the issue of whether drugs used to treat high cholesterol, such as statins, might also increase osteoporosis.

Another orphan receptor also linked to lipid metabolism and diabetes, HNF4, was similarly de-orphanised by T Willson and colleagues (GlaxoSmithKline, Research Triangle Park, NC, USA) when the structure of its LBD was solved [9,10]. In its ligand-binding pocket were a mixture of fatty acids but, surprisingly, the fatty acids could not be removed from the

native protein, suggesting that HNF4 may not be a good drug target. Upon crystallisation of the *Drosophila* orthologue of RXR, ultraspiracle protein (USP), D Moras (IGBMC, Illkirch, France) found that it also unexpectedly, and irreversibly, bound phospholipids [11]. Moras went on to show that not all NR LBDs expressed in heterologous systems bind ligands, which may or may not be fortuitous. The agonist structure of the ERRγ LBD revealed an empty ligand-binding pocket, consistent with the fact that known ERRγ ligands, such as 4-hydroxy tamoxifen (4-OHT) and diethylstilbesterol (DES), are antagonists and are not found in bacteria [12].

6. Non ligand-based drugs

The structure of yet another NR LBD showed that not all NRs have ligand-binding pockets. Willson's group showed that the space where one would expect to see the pocket in NGFI-B and its *Drosophila* orthologue, DHR-38, was filled with phenylalanines, leaving a volume of < 30 Å^3 compared to a typical volume of ~300 Å^3 for receptors that have ligands [13]. Since NGFI-B is linked to manic depression and schizophrenia, this finding, like that of the non-exchangeable ligands, is very disappointing. Nonetheless, P Ordentlich (X-Ceptor Therapeutics, San Diego, CA, USA) showed that the activity of the related Nurr1, which also plays a role in the CNS as well as in immunity and cancer, could be modulated by something other than a ligand. Upon screening a library of 500,000 compounds, they found one compound, 6-mercaptopurine (6-MP), that stimulated the transcriptional activity of Nurr1 via the N-terminal portion of the protein, far from the LBD [14,15]. 6-MP has been used since the 1950s to treat leukaemia and more recently to treat inflammatory diseases such as Crohn's disease.

Finally, one of the most exciting new drugs that was presented at the conference does not even have a NR as a target, although its identification was made possible by research on NRs. Activated NRs recruit co-activators which often contain histone acetylase transferase (HAT) activity, while inactive NRs form complexes with corepressors which recruit histone deacetylase (HDAC) activity. V Richon (Aton Pharmaceuticals, Tarrytown, NY, USA) reported that the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) is being used in an oral formula to treat advanced malignancies of both solid tumours and lymphomas and leukaemias. SAHA induces apoptosis and differentiation, and blocks proliferation of transformed cells by increasing the expression of cell cycle inhibitor p21 and decreasing the expression of cyclin D1. The effects are presumably due to increased acetylation of histones H3 and H4, which can be tracked in the blood of patients. The toxic effects (anorexia, fatigue, diarrhoea) are manageable and reversible with some patients being on the drug for up to 2 years [16].

7. Other developments and future directions

Another area that was addressed that will become increasingly important to drug discovery is the interplay between herbals, xenobiotics and drug metabolism. Not only do NRs such as HNF4, LXR, FXR, PPAR and RXR play a pivotal role in regulating the expression of the Phase I and II genes that regulate xenobiotic and drug metabolism, NRs PXR and constitutive androstane receptor (CAR) do as well, and they respond directly to many of those same compounds. JT Moore (GlaxoSmithKline, Research Triangle Park, NC, USA) and M Redinbo (University of North Carolina, Chapel Hill, NC, USA) both discussed the role of PXR in drug–drug interactions. Hyperforin, the active ingredient in St John's Wort, which is popularly used to ward off mild depression, is a ligand for PXR and stimulates its activity on the Cyp3A gene, which metabolises the majority of prescription drugs [17]. Therefore, transplant patients taking cyclosporin for immune suppression or women taking ethyloestradiol for birth control can drastically affect the metabolism of their medication if they also take St John's Wort. Since PXR has a very large ligand-binding pocket (1300 Å^3) it can accommodate many different types of compounds, such as the antibiotic rifampacin and the phyto-oestrogen coumestrol found in soy products, alfalfa and infant formula, increasing greatly the potential for unwanted drug interactions. Similar effects are possible with CAR, which is stimulated by phenobarbital, although its mechanism of action is different from that of PXR (Moore; M Negishi [NIEHS, Research Triangle Park, NC, USA]) [18].

The conference also discussed the issue of how to deal with the deluge of data coming not just from genomics experiments with DNA microarrays containing tens of thousands of genes, but also from automated cell- and in vitro-based assays that spew out hundreds of thousands of data points per assay per day. As PY Yim (Rosetta Inpharmatics, Kirkland, WA, USA) pointed out in the Pre-Conference Workshop on the topic, there are programmes to keep track of experiments, to pull out specific data points upon request and to visualise 500,000 data points in one presentation (e.g., Spotfire). This allows for mega analyses across different doses, time points, tissues, and species as well as compounds that can provide new insights into mechanisms that cannot be gained by more traditional methods of experimentation or data analysis.

Finally, DR Artis (Plexxikon, Inc., Berkeley, CA, USA) provided a superb example of how structure–activity relationships, automation and informatics will send drug discovery into overdrive in the near future. Artis described high throughput crystallisation that resulted in an amazing 1300 co-crystals, including 250 unique structures solved, and gave examples of two anti-diabetic compounds acting on PPARγ (PLX-101203, PLX-101204) that were identified in the process, all accomplished during the course of 1 year.

8. Conclusion and expert opinion

The parameters for rational drug discovery for NRs have been identified – the receptors, their structure and mechanism of action, the cofactors they recruit, and the target genes they regulate. However, whereas we have detailed

information about the first two, we have only begun to uncover the tip of the proverbial iceberg of the latter two. What is needed now is a systematic effort to identify and characterise all cofactors, all DNA response elements and all target genes for each NR in the human body, as well as all of their isoforms and post translational modifications, topics barely touched upon in the conference. Such information, which would provide an indispensable atlas not just for drug discovery but also for basic research, should be a public resource.

The second area that needs more attention is the development of new ways to organise and analyse the mountains of data being generated on a regular basis. Nearly every presentation in the conference relied on flow diagrams to keep track of the numerous target genes, many with contradictory effects, for just one NR. In the author's laboratory, more than 4000 genes have been identified in the human genome that contain verified HNF4-binding sites in their regulatory regions. Even if only one quarter of those genes are true targets, that still leaves two orders of magnitude more target genes than we are used to thinking about. Factor in potentially hundreds of different cofactors, dozens of developmental time points, several tissues and countless environmental conditions, and then consider that any one gene is also regulated by multiple transcription factors, many of which regulate each other's expression [19]. A complex biological web quickly emerges, rivalling the intricacy of the worldwide web. Clearly, we need to develop new methods to handle, organise, analyse and even present all the information that is being produced, not just by the prodigious drug discovery efforts of pharma, but by all the biological experiments in the post-genomic age.

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Affiliation

Frances M Sladek PhD
Department of Cell Biology and Neuroscience,
5429 Boyce Hall, University of California,
Riverside, CA 92521-0314, USA
Tel: +1 909 787 2264; Fax: +1 909 787 3087;
E-mail: frances.sladek@ucr.edu