Nuclear Receptor Resource
Research Article
Nuclear Receptors as "nutriable" targets
J.P.Vanden Heuvel, INDIGO Biosciences Inc., State College PA

Nuclear receptors are "nutriable" targets

1 Definition of druggable and nutriable targets

A druggable target, or more appropriately a druggable domain, is a functional region of a protein for which a significant fraction of family members have been successfully targeted by drugs (1). Rhodopsin-like GPCRs, certain ion-channel domains and nuclear receptor ligand-binding domains are clear historical examples of druggable domains. The drug molecule that is designed to interact with a druggable domain has certain physiochemical properties. Historical approaches for predicting 'druglikeness' of new chemical entities have often relied on Lipinski's 'Rule of Five' and related measures, which state that an potential drug should have a molecular weight less than 500, a partition coefficient log P less than 5, no more than 5 H-bond donors or more than 10 H-bond acceptors (2). The general approach of drug discovery is to identify a druggable target associated with a particular disease state and to screen a library of compounds for their ability to appropriately regulate this protein. Over the past decade, there has been a significant decrease in the rate that new drug candidates are being translated into effective therapies in the clinic. In particular, there has been a worrying rise in late-stage attrition in phase 2 and phase 3 (3). Currently, the two single most important reasons for attrition in clinical development are (i) lack of efficacy and (ii) clinical safety or toxicology, which

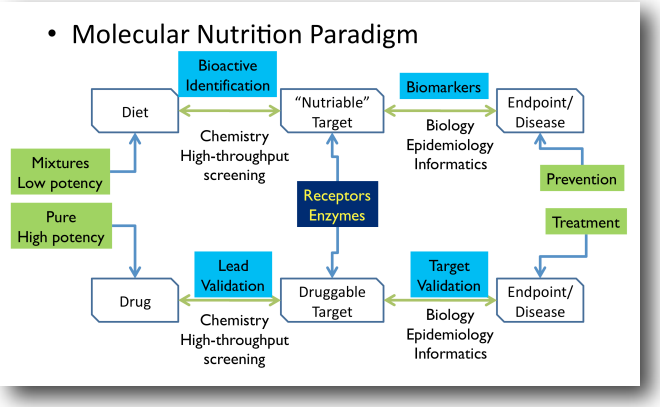

Figure 1. Nutriable versus druggable targets

each account for 30% of failures. Thus, the current philosophy of rational drug design, or more specifically, the 'one gene, one drug, one disease' paradigm has been significantly challenged (3). "Polypharmacology" or the binding of a drug to multiple target proteins, with clinical effects being mediated through the modulation of the set of protein target, is gaining favor (1).

What makes the protein druggable is the fact that a domain on this protein responds to small chemicals including intracellular metabolites, xenobiotics such as drugs as well as dietary agents. Thus a druggable target is responsive to components of the diet and hence nutriable. Nutriable targets include GPCR, nuclear receptors and various kinase families. Differences in drug discovery versus the molecular nutrition approach to disease treatment is highlighted in Figure 1. In the subsequent discussion, the interaction with dietary lipids with nuclear receptors will be explored

2. Nuclear receptors as sensors of dietary lipids.

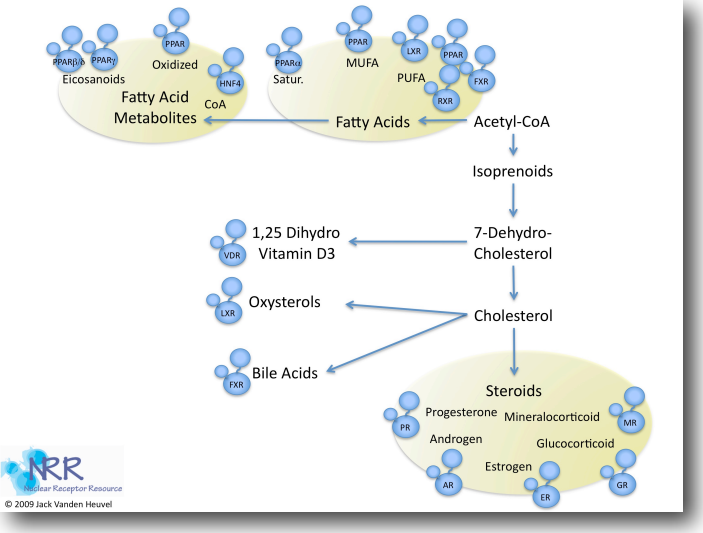

Figure 2. Nuclear receptors as targets of intermediary metabolism

A family of proteins that may act as lipid sensors are the nuclear receptors (NR). Members of the NR superfamily act as intracellular transcription factors that directly regulate gene expression in response to lipophilic molecules. They affect a wide variety of functions, including fatty acid metabolism, reproductive development, and detoxification of foreign substances. To date, over 300 NRs have been cloned, many with unknown endogenous ligands (orphan receptors), with 48 human nuclear receptor genes. Phylogenic analysis has shown six subfamilies (NR1-6) with various groups and individual genes (4). Several NRs respond to metabolites of intermediary metabolism (Figure 2) as well as dietary constituents (Figure 3)

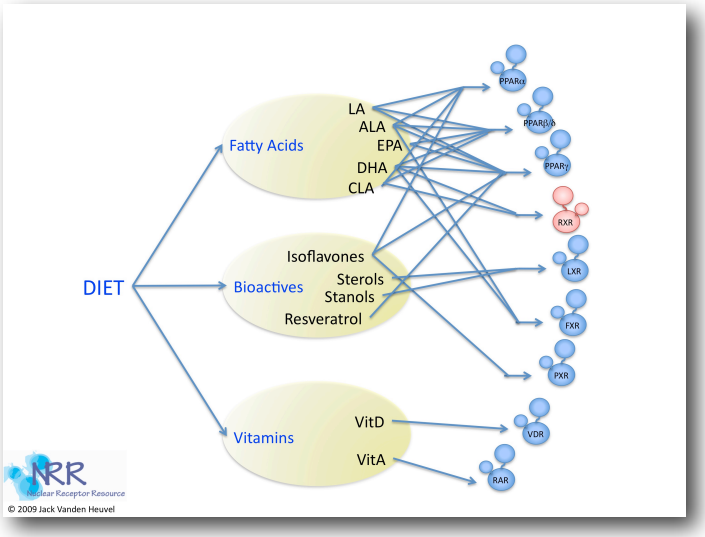

Figure 3. Dietary components that regulate gene expression via nuclear receptors

and include the fatty acid receptors peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), retinoid X receptor (RXR) and hepatocyte nuclear factor 4 α (HNF4α) (5). More recently, farnesoid X receptor (FXR) has been added to this list of NRs activated by fatty acids (6) while LXR is now questioned for such categorization [9]. The receptors shown in Figure 1 may be considered constituents of a large group of NRs, the "metabolic nuclear receptors" which act as overall sensors of metabolic intermediates, xenobiotics and compounds in the diet and allow cells to respond to environmental changes by inducing the appropriate metabolic genes and pathways (7,8).

Most NRs regulate gene expression in predominantly the same fashion. Prior to activation, NRs often exist in multiprotein complexes that vary depending on the family of receptor under question. When a ligand binds to its cognate receptor, a conformational change occurs ("activation") that changes the protein-protein interfaces of the molecule. As a result, the activated receptor interacts with a NR response element (NRE) within the regulatory region of a target gene; upon recruitment of various transcriptional coactivators and subsequent RNA polymerase II (polII), initiation of transcription of the target gene occurs.

In the following sections, the four subfamilies of NRs that respond to dietary fatty acids (and other bioactive molecules), PPAR, RXR, LXR and FXR will be described. The dietary and metabolic intermediates that activate these receptors are summarized in Figure 2 and 3. (Please see (9) for more details)

2.1 Peroxisome Proliferator-Activated Receptors (PPARs)

Of the several identified fatty acid receptors, perhaps the family that can best explain the effects of ω3-PUFAs and the conjugated linoleic acids (CLAs) are the PPARs. The PPAR family of receptors was originally named based on their ability to respond to xenobiotics (peroxisome proliferators); however they were also the first to be examined as a fatty acid receptor. It has now been well established that PPAR is a ligand-activated transcription factor involved in gene expression in a tissue-, sex- and species- dependent manner. The PPARs exist as three subtypes (α, β and γ) that vary in expression, ligand recognition and biological function.

PPARα was the first transcription factor identified as a potential fatty acid receptor. Based on numerous studies from the PPARα knockout mice ($PPAR\alpha^{-/-}$), this receptor plays a role in the regulation of an extensive network of genes involved in glucose and lipid metabolism. In particular, PPARα regulates fatty acid transport, fatty acid binding proteins, fatty acyl CoA synthesis, microsomal, peroxisomal and mitochondrial fatty acid oxidation, ketogenesis and fatty acid desaturation.

Several groups have implicated saturated and unsaturated fatty acids as natural ligands for PPARα. Natural PPARα ligands in human serum include palmitic acid, oleic acid, LA and AA. Notably, PPARα is the only PPAR subtype that binds to a wide range of saturated fatty acids. The 9z 11e CLA isomer is a potent PPARα ligand with a K_D in the low nM range and it affects PPAR-responsive enzymes including acyl-CoA oxidase (ACO), liver fatty acid binding protein (L-FABP) and cytochrome P450 4A1 (CYP4A1). Omega-3 PUFAs such as ALA, EPA and DHA activate PPARα with a similar potency and efficacy as LA

PPARγ is expressed in many tissues including adipose, muscle, vascular cells, macrophages and epithelial cells of the mammary gland, prostate and colon. Activated PPARγ induces LPL and fatty acid transporters (CD36), and enhances adipocyte differentiation, as well as inhibits cytokine and cylcooxygenase 2 (COX-2) expression, perhaps by modulating NFκB function. The PPARγ null mouse is non-viable, implicating an important role for this protein in ontogeny, and also making the examination of a role for this receptor in gene expression difficult.

Clinically relevant anti-diabetic agents such as pioglitazone and rosiglitazone are potent PPARγ agonists (K_d in low nM range). A number of fatty acids and eicosanoid

derivatives bind and activate PPARγ in the micromolar range. Unlike the PPARα subtype, PPARγ has a clear preference for PUFAs. The fatty acids LA, ALA, AA, and EPA bind PPARγ within the range of concentrations of free fatty acids found in human serum. Although fatty acids are not particularly efficacious activators of PPARγ, intracellular conversion of fatty acids to eicosanoids, through enhanced expression of 15-lipoxygenase, greatly increased PPARγ mediated transactivation.

PPARβ (also called FAAR, NUC1 or PPARδ) is the least understood of the three subtypes in many respects, including the identification of target genes as well as endogenous and dietary ligands. This receptor is ubiquitously expressed and is often found in higher abundance than PPARα or γ. Examination of the PPARβ/δ null mice has shown a role for this NR in development, myelination of the corpus callosum, lipid metabolism, and epidermal cell proliferation. There has been some indication that PPARβ/δ is involved in adipogenesis , although this has been refuted. Few high affinity ligands for PPARβ/δ are known, either xenobiotic or endogenous. Omega-3 PUFAs and CLA are efficacious PPARβ/γ activators; in fact this NR is the most highly regulated of those tested, albeit at higher doses of fatty acids. Prostaglandin A1 (PGA1), PGD2 and PGD1 can activate PPARβ/δ in reporter assays . 15-HETE and the toxic lipid 4-hydroxynonenol (4-HNE) are also a PPARβ/δ activators.

2.2 Retinoid X receptors (RXRs)

RXRs are involved in the transduction of retinoid signaling pathway although their role in regulation of gene expression induced by ω3-PUFAs has garnered increasing attention. RXRs (α, β or γ) can form homodimers or they may serve as a dimerization partner for other NRs including retinoic acid receptors (RAR), thyroid hormone receptor (TR), vitamin D_3 receptor (VDR) and PPARs. As a heterodimerization partner, RXR is involved in regulation of multiple cellular pathways. RXRα and β have ubiquitous distribution, whereas RXRγ is expressed in certain organs such as heart, skeletal muscle and central nervous system structures.

Although intensely studied for synthetic ligands, little is known of the natural activators of this receptor. RXR is activated *in vitro* by the vitamin A metabolite 9-*cis* retinoic acid (9-*cis* RA), but the levels of this molecule *in vivo* are extremely low. Through reporter assays it was observed that DHA is an RXR ligand and docosatetraenoic acid, a structurally related compound, activates RXR at a much higher concentration. DHA's effect was not observed in other NRs such as RAR, thyroid hormone receptor and Vitamin D receptor, although as stated above, this fatty acid activates PPARα, β/δ and γ. Several fatty acids including unsaturated, mono-saturated and PUFAs such as AA and DHA have been identified as ligands of RXR, thus confirming the activation observed in reporter assays. The 9E11E CLA isomer was the most potent of the CLA isomers at activating RXRα and was comparable to the efficacy seen with 9cis-RA. Phytanic acid, a branched chain fatty acid derived from chlorophyll has also been reported to activate RXR *albeit* weakly. Phytanic acid is capable of adipocyte differentiation and induces aP2 mRNA in 3T3-L1 preadipocytes and may act as a natural rexinoid in 3T3-L1 cells.

2.3 Liver X receptors (LXRs)

LXRα and LXRβ are transcription factors commonly known as cholesterol sensors (8). Although they are important regulators of transport and metabolism of sterols and fatty acids, whether they are direct sensors of ω3-PUFAs has been questioned. Expression of LXRα is restricted, whereas LXRβ is ubiquitously present. LXRα is present in certain organs namely liver, kidney, intestine, adipose tissue and adrenals. LXRα and β share a high degree of amino acid similarity (~80%) and are considered paralogues; as a result there are very few subtype specific agonists. Oxysterols including 24(S), 25-epoxycholesterol, 22R-hydroxycholesterol, and 24(S)-hydroxycholesterol, are natural ligands of LXRs. PUFAs competitively blocked activation of LXR by oxysterols. This offers a potential mechanism for the ability of dietary PUFAs to decrease the synthesis and secretion of fatty acids and triglycerides in liver. This suppressive effect can be eliminated by deletion and mutation of LXR responsive element (LXREs) located in the promoter region of SREBP-1c. However, others have shown that the unsaturated fatty acid suppression of SREBP-1 and its targeted lipogenic genes is independent of LXRα. Perhaps the effects of fatty acids on LXR-mediated events are being mediated by a direct interaction between PPARα and LXRα. In fact, several xenobiotic PPARα ligands antagonize LXR's transcriptional activity.

2.4 Farnesoid (or farnesol) X receptor (FXR)

FXR (or NR1H4) is a member of the same subclass of NR as LXRα and β, as well as the ecdysone receptor (ECR). FXR heterodimerizes to RXR and binds to DNA sequences consisting of an inverted repeat spaced by one nucleotide (IR-1). FXR is mainly expressed in the liver, gut, kidney, and adrenal cortex. FXR binds and is activated by several bile acids, including chenodeoxycholic acid, lithocholic acid (LCA), and deoxycholic acid. FXR received its name due to the fact that is activated by

a large variety of endogenous isoprenoids, including farnesol. Other endogenous activators include all-trans-retinoic acid, and as mentioned above, fatty acids. This nuclear receptor binds to AA, ALA and DHA with (K_i's in the low µM range) but has little or no affinity for palmitic or stearic acid.

3. Conclusions

Diets high in ω3 fatty acids have long been associated with decreased risk of cardiovascular disease (CVD) and metabolic diseases. EPA and DHA are found in high concentrations in fish oils and are thought to improve heart health through decreasing thrombosis, inflammation and plaque formation in arteries. The mechanism of these effects may be the result of regulation of gene expression via NRs, several of which are known to be "fatty acid receptors". PPARα and PPARβ/δ are receptors for unsaturated, mono-unsaturated and PUFAs as well as several AA metabolites. Activation of PPARα is associated with increased fatty acid catabolism, decreasing inflammation and stimulating the reverse cholesterol pathway. PPARγ has a clear preference for PUFAs and is also the target of AA metabolites. This receptor is involved in storage of lipids in adipocytes as well as decreasing inflammation and stimulating the reverse cholesterol pathway. RXR is an important heterodimerization partner for NRs and hence can affect numerous metabolic pathways. DHA and several other PUFAs bind to and activate this central NR. LXR's role as a sensor of fatty acids is somewhat controversial, although it is clearly an oxysterol receptor. Several studies have shown that fatty acids (unsaturated and saturated) antagonize LXR activity. This receptor is involved in fatty acid synthesis, bile acid synthesis and reverses cholesterol transport and synthetic agonists are being touted as anti-atherosclerosis agents. FXR is the newest identified member of the fatty acid receptor group and is activated by PUFAs. This NR is involved hepatic bile acid clearance and evidence is growing that it may be a potential target in other tissues, notably in the endothelial wall and in macrophages. Taken together, these NRs represent potential targets for ω3-PUFAs that can help explain their mechanism of action in preventing CVD.

Citations

1. Overington, J. P., Al-Lazikani, B., and Hopkins, A. L. (2006) How many drug targets are there?, *Nat Rev Drug Discov 5*, 993-996.
2. Billingsley, M. L. (2008) Druggable targets and targeted drugs: enhancing the development of new therapeutics, *Pharmacology 82*, 239-244.
3. Hopkins, A. L. (2008) Network pharmacology: the next paradigm in drug discovery, *Nat Chem Biol 4*, 682-690.
4. Nuclear Receptors Nomenclature Committee (1999) A unified nomenclature system for the nuclear receptor superfamily, *Cell 97*, 161-163.
5. Khan, S. A., and Vanden Heuvel, J. P. (2003) Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review), *J Nutr Biochem 14*, 554-567.
6. Zhao, A., Yu, J., Lew, J. L., Huang, L., Wright, S. D., and Cui, J. (2004) Polyunsaturated fatty acids are FXR ligands and differentially regulate expression of FXR targets, *DNA Cell Biol 23*, 519-526.
7. Nagao, K., and Yanagita, T. (2008) Bioactive lipids in metabolic syndrome, *Prog Lipid Res 47*, 127-146.
8. Francis, G. A., Fayard, E., Picard, F., and Auwerx, J. (2003) Nuclear receptors and the control of metabolism, *Annu Rev Physiol 65*, 261-311.
9. Vanden Heuvel, J. P. (2009) Cardiovascular Disease-related Genes and Regulation by Diet, *Curr Atheroscler Rep 11*, 448-455.