The Orphan Nuclear Receptors:
Unrecognized Targets of Botanical Medicines?

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These notes are designed as a primer for the lecture and do not necessarily represent lecture content.

Orphan Nuclear receptors

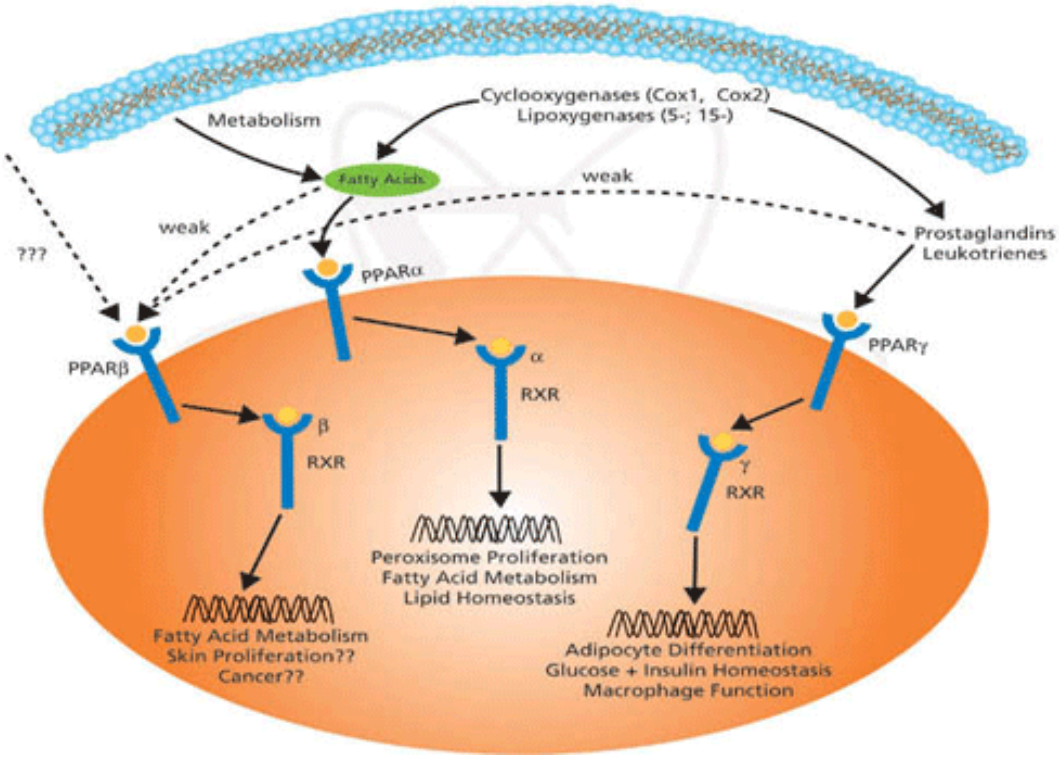
At this point in time, 48 nuclear receptors have been identified in the human genome.[1, 2] These 48 proteins make up a superfamily of nuclear hormone receptors/transcription factors. In controlling the expression of various genes these receptors play key roles in regulating a myriad of biological functions during embryonic development and in adult tissues, as well as in many disease states.

Members of this receptor family are related to each other in terms of their amino acid sequence and their function within cells. They therefore have structural features in common. These include a central highly conserved DNA binding domain and generally greater than 60% homology in the ligand binding domain (LBD). The quintessential characteristic of nuclear receptors, and the basis for the name of the family, is that their transcriptional activities can be regulated by small molecules, usually comprised of hydrophobic compounds binding to the LBD. In essence the LBD acts as a molecular switch that recruits co-activator proteins and activates the transcription of target genes when flipped into the active conformation by endogenous and exogenous compounds.[3]

Intriguingly, most of the receptors in the nuclear hormone receptor family are ‘orphan receptors’, in that they are awaiting the recognition of specific ligands and functions. The identification of members of the nuclear receptor superfamily as intracellular receptors for both dietary lipids and their metabolic derivatives has focused attention on them as key regulators of metabolism.[4] Growing evidence demonstrates that orphan and adopted orphan nuclear receptors, such as peroxisome proliferator-activated receptors (PPAR), estrogen receptor-related receptors (more commonly called the estrogen-related receptors ERRs), liver x receptors (LXR), the farnesoid x receptor (FXR), NR4As, retinoid x receptors (RXR), and the pregnane x receptor (PXR), regulate the inflammatory and metabolic profiles in a ligand-dependent or -independent manner in mammals.[5] Key to this discussion and presentation will be the PPARs and the ERRs.

Peroxisome Proliferator-Activated Receptors (PPARs)

The peroxisome proliferator-activated receptors (PPARs) constitute a set of three receptor sub-types which are members of the nuclear receptor superfamily of ligand-activated transcription factors, which includes steroid hormone receptors. They are encoded by distinct genes that function as lipid sensors that regulate gene expression in many metabolically active tissues.[6] The PPARs, so named because in early research it was found that PPAR δ stimulation resulted in the increase in peroxisomes, have a significant role in cellular energy balance, fuel utilization, the metabolism of fatty acids and other lipids, the generation and remodeling of adipose tissue and fibrotic and hypertrophic responses in the heart and vascular wall. Many of these actions are via interactions with nuclear factors such as NF-κB and activator protein 1 (AP-1), thus modulating expression of pro-inflammatory cytokines and adhesion molecules, and altering cell signaling pathways.[7] Thus, PPARs, in response to stressors, play a role in the complex orchestration of adaptive cellular physiology working in a concerted mode with the vitamin D receptor and the retinoic acid receptor (RXR). Central to this proposal, PPARs have been found to be involved in inflammation and carcinogenesis, and other immune activity. Thus, every large pharmaceutical company is involved in PPAR research in areas such as diabetes, obesity, cardiovascular disease and immunology with drug development in mind.



The endogenous ligands to the PPAR sites were originally unknown, earning the PPARs the name “orphan nuclear receptors”. However, many ligands are now known: PPAR-α is known to bind polyunsaturated fatty acids such as docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA), oxidized phospholipids, lipoprotein lipolytic products as well as the lipid lowering

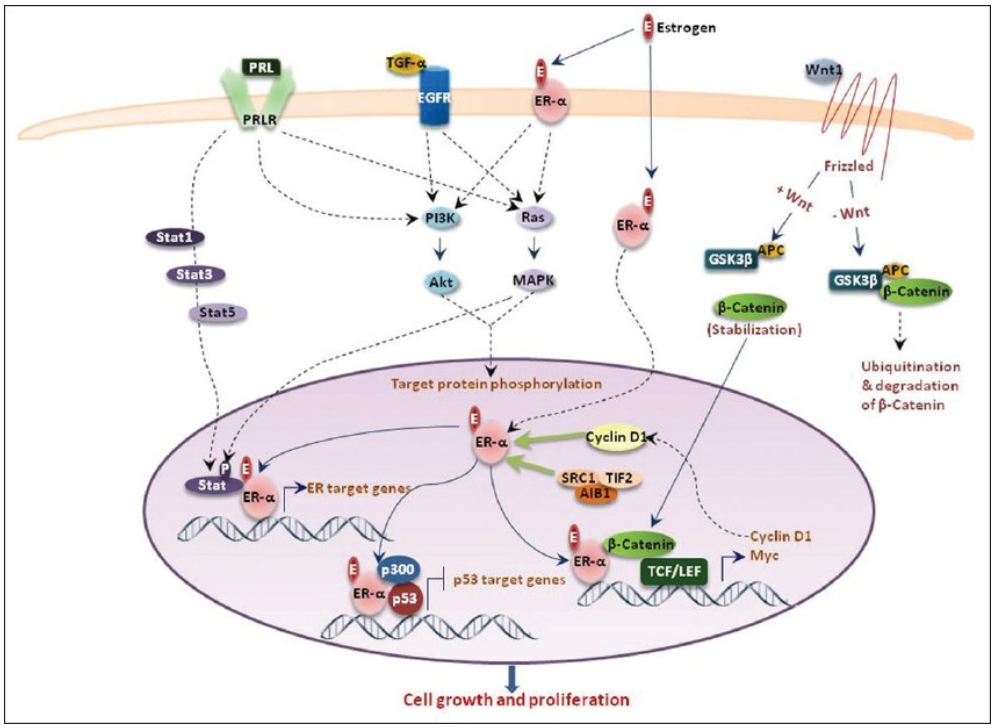
fibrates (fenofibrate and gemfibrozil).[7, 8] PPAR-δ ligands include polyunsaturated fatty acids, prostaglandins and is postulated to be biochemically related to lipid levels, wound healing and colon cancer but the evidence for a connection to all of these pathways is limited.[9] PPAR-γ binds fatty acid derivatives, such as hydroxyoctadecadienoic acid (HODEs), prostaglandin derivatives, such as 15-deoxy-δ-(12,14)-prostaglandin J2 (15d-PgJ2), and thiazolidinedione (TZDs) drugs, such as pioglitazone and rosiglitazone.[7] Nevertheless, the "true" endogenous ligands are still debated due to these receptors demonstrating a significant amount of promiscuity in ligand binding.

PPARs exists as a heterodimer with the retinoid X receptor (RXR). In the resting state, corepressors associate with PPAR/RXR.[10] Upon ligand activation, the corepressors dissociate from PPAR/RXR and the heterodimer is liberated to bind to PPAR response elements (PPREs), located in the regulatory regions of various target genes.[11] The expression of the target genes is thus regulated by the PPAR/RXR complex, either alone or in conjunction with other transcription factors.[7]

Plants that modulate PPAR isoforms are listed in Table 1 (*in vivo*) and Table 2 (*in vitro*). In some cases the compounds activating PPAR isoforms are known, in some cases they are not known.

The Estrogen-Related Receptors (ERRs)

The ERRs were the first orphan nuclear receptors identified on the basis of their sequence similarity to the estrogen receptors. However, it should be kept in mind that they do NOT bind



From http://www.carcinogenesis.com/articles/2011/10/1/images/JCarcinog_2011_10_1_35_91116_u1.jpg

estrogen even though there is a significant homology between the LBD of the estrogen receptor and the estrogen related receptor. The ERRs consist of three receptor sub-types, ERRα, ERRβ and ERRγ, which are ligand-activated transcription factors.

ERRs role in physiology

These receptors/transcription factors regulate gene expression in a wide distribution of tissues. ERRs are most highly expressed in metabolically active tissues, including tissues associated with lipid metabolism and high energy demand. As such, they are believed to play a central role in regulating energy metabolism.[12] At least one ERR isoform has been reported in every tissue examined to date. Of particular interest to brain health, is the identification of high levels of ERRα and ERRγ in the central nervous system.[13]

ERRα and ERRγ are now known to serve as regulators of β-oxidation and mitochondrial biogenesis in energy dependent tissues such as slow-twitch skeletal muscle and brown fat. Tissue- and cofactor-specific

functions are also observed for the ERRs as seen in white adipose tissue.[4] Consistent with a regulatory role in these processes, ERRα and ERRγ are highly expressed in tissues dependent on fatty acid oxidation for energy, such as heart, brown fat, and slow-twitch skeletal muscle. Moreover, ERRα expression is induced upon exposure to energy stresses such as cold, fasting, or exercise. ERRβ has more limited expression in adult tissues, and its function to date is less well characterized.[4] To date, no endogenous ligand has been identified.

Recently ERRα and ERRγ expression in human glioma cells has been shown.[14] Preliminary screens have shown that targeting ERRα and ERRγ appears to down regulate gene expression of key genes necessary for growth and maintenance of gliomal cancer cells (unpublished data, Laboratory of Clinical Investigation, NIA). In addition, there is a positive correlation between CA-125 (a ovarian cancer marker) and ERRα expression. Survival of those ovarian cancer patients that have ERRα positive tumors is reduced ($p = 0.015$), while those with ERRγ positive tumors have a longer progression-free survival ($p=0.020$) (Sun et al., 2005).

Recently the class of molecules known as flavonoids have been reported to function as ligands for the ERRs. The flavonoids are ubiquitous polyphenolic plant products that can be found in a variety of fruits, vegetables, spices and medicinal plants. Daily human consumption of polyphenols has been estimated to be 780 – 1058 mg/day, with 20-25% of this as flavonoids.[15] Thus the daily intake of flavonoids routinely exceeds those of vitamin E and β-carotene.[16] The touted health effects of the flavonoid class of phytochemicals are numerous and include antimicrobial, antiviral, anti-ulcerogenic, cytotoxic, antineoplastic, mutagenic, antioxidant, antihepatotoxic, antihypertensive, hypolipidemic, antiplatelet and anti-inflammatory activities.[17] In addition, the flavonoids are well established to be hormonally active and function as selective estrogen receptor modulators (SERM).[18]

Besides SERM activity, several flavonoids, such as the isoflavones genistein, daidzein, biochanin A and flavone 6, 3´,4´-trihydroxyflavone, have previously been reported to function as ERRα and ERRγ agonists based on virtual ligand screening and reporter-based assays (Tables

4 & 5). Other flavonoids, apigenin, kaempferol, luteolin have demonstrated the ability to induce or inhibit the activity of ERRγ. These ERR active flavonoids are not rare, as seen in Table 3 they occur in such commonly used plants as soy (*Glycine max*), red clover (*Trifolium pratense*), oregano (*Origanum vulgare*), chamomile (*Matricaria recutita*), garlic (*Allium sativum*), spearmint (*Mentha spicata*) and bay leaf (*Laurus nobilis*).[19, 20] Recent *in vitro* works shows a profound reduction in ovarian cancer cell proliferation after exposure to the flavonoid rich plant *Trifolium pratense.*

This opens the door to the possibility that many of the ubiquitous phytochemicals present in the diet of ancient humans, and modern humans who have diets rich in plant based foods, may be protective against certain diseases. In the case of the flavonoids shown to interface with ERRs, it may be that consistent low level exposure to the plants containing these compounds (Table 3) may provide a protective effect against various cancers. Moreover, it may suggest that humans that have very little flavonoid exposure from poor eating habits, may be missing an important metabolic regulation and protective effect against various diseases including cancer. At the least, it suggests that the flavonoids and more specifically the isoflavones, should be studied further for actions on the ERRs and the PPARs may be particularly ‘attentive’ to a broad array of phytochemicals.