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Previews

Fatty Acid Synthesis and PPARα Hand in Hand

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How can an ex-orphan be adopted? Is it possible to do so by attributing to it a key endogenous ligand that regulates its central functions? In the recent issue of *Cell*, Chakravarthy et al. attempted to answer this question by characterizing a new physiologically relevant ligand for the ex-orphan receptor peroxisome proliferator activated receptor alpha (PPARα).

PPARs form a subfamily of nuclear hormone receptors comprising three isotypes: PPARα, PPARβ/δ, and PPARγ. These transcription factors are drug targets in the context of the widespread human diseases diabetes (PPARγ) and dyslipidemia (PPARα), and their main physiological roles are to modulate energy metabolism and inflammatory pathways in response to dietary and endogenous signals.

To understand the concept of the orphan receptor, one has to look back a couple of decades to when the different members of the nuclear hormone receptor family were cloned. The first to be identified were those mediating responses to circulating steroid and thyroid hormones. Mainly based on sequence homology screens, the gene family expanded up to 48 members in humans. In most cases, no natural ligand was known to bind to the newly identified receptors. Therefore, they were categorized as orphan receptors. This was the case for PPARs in the early 1990s (Issemann and Green, 1990; Dreyer et al., 1992).

Then started the difficult quest for endogenous ligands for PPARs. The adoption of orphan nuclear receptors is not easy. The ideal candidate ligand must be able to occupy the ligand-binding pocket of the receptor. By doing so, it triggers a conformational change in the receptor that switches its association with co-repressors to co-activators. Ultimately, this culminates in the recruitment and activation of the transcriptional machinery at the promoter of target genes. In the past decade, a large number of physiologically relevant molecules have been proposed to be endogenous PPAR ligands. They are mostly unsaturated or polyunsaturated fatty acids and eicosanoids, and are often required at micromolar concentrations to achieve PPAR activation (Michalik et al., 2006). This low affinity is compatible with the serum levels of these ligands, but cellular concentrations have not been clearly established and, therefore, it is still not clear how many endogenous molecules are available in sufficient amounts in the cell nucleus to trigger PPAR-dependent transcription. Recently, a few reports have pointed to natural molecules with a nanomolar affinity for PPARs (Davies et al., 2001; Lin et al., 1999; Shaw et al., 2003; Schopfer et al., 2005). However, these findings have not shed new light on how PPARs, and especially PPARα, respond to dietary stimuli to maintain energy homeostasis, which is believed to be one of the major functions of PPARs, with obvious clinical implications.

Chakravarthy et al. (2009) have advanced the field by identifying an endogenous ligand with a nanomolar affinity for PPARα. It controls hepatic PPARα functions and its production is influenced by the diet. How this new ligand was identified is probably as remarkable as the finding itself. The authors tested the hypothesis raised by their previous work according to which fatty acid synthase (FAS) is implicated in PPARα-ligand production in the liver (Chakravarthy et al., 2005). They produced mice expressing a FLAG-tagged version of PPARα in the liver, pulled down the receptor, and identified lipid species bound to it by mass spectrometry. They compared the results obtained when the PPARα-associated lipids were purified from normal liver with those obtained from liver depleted of FAS (FASKOL mice). They identified the phospholipid 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (16:0/18:1 GPC) as the sole species to be associated with PPARα in a FAS-dependent manner. It binds to PPARα and is displaced by a potent synthetic PPARα agonist, which suggests interaction with the ligand-binding pocket. However, information is lacking on the atomic interactions of the phospholipid within the PPARα ligand-binding domain. The best evidence that 16:0/18:1 GPC functions as a PPARα ligand in vivo is that it activates transcription in a PPARα-dependent manner when perfused into the liver through the portal vein.

The way in which the availability of the phospholipid ligand for PPARα might be regulated is important. FAS yields palmitate, following which 16:0/18:1 GPC is thought to be generated through the diacylglycerol intermediate and choline-ethanolamine phosphotransferase-1 (CEPT1) activity in the endoplasmic reticulum and the nucleus (Figure 1). In support of this proposition, the depletion of CEPT1 either in hepatoma cell lines or in the whole liver results in decreased PPARα target gene transcription, which is restored to a normal level in cells treated with 16:0/18:1 GPC. In brief, the key finding of the *Cell* paper is that liver FAS and CEPT1 regulate PPARα functions by producing a potent PPARα agonist. This opens new perspectives but, in parallel, also poses new questions.

PPARα is known to promote gluconeogenesis. Importantly, it is now even more tightly linked to carbohydrate signaling, since carbohydrates indirectly stimulate PPARα activity by upregulating FAS and thus influence lipid metabolism. Therefore, in addition to lipid-sensing at the initiation of PPARα activity, there is carbohydrate-sensing as well, which is rather unexpected. One may argue that the mechanism of PPARα activation by 16:0/18:1 GPC is marginal and only operates when food is rich in sugars and poor in

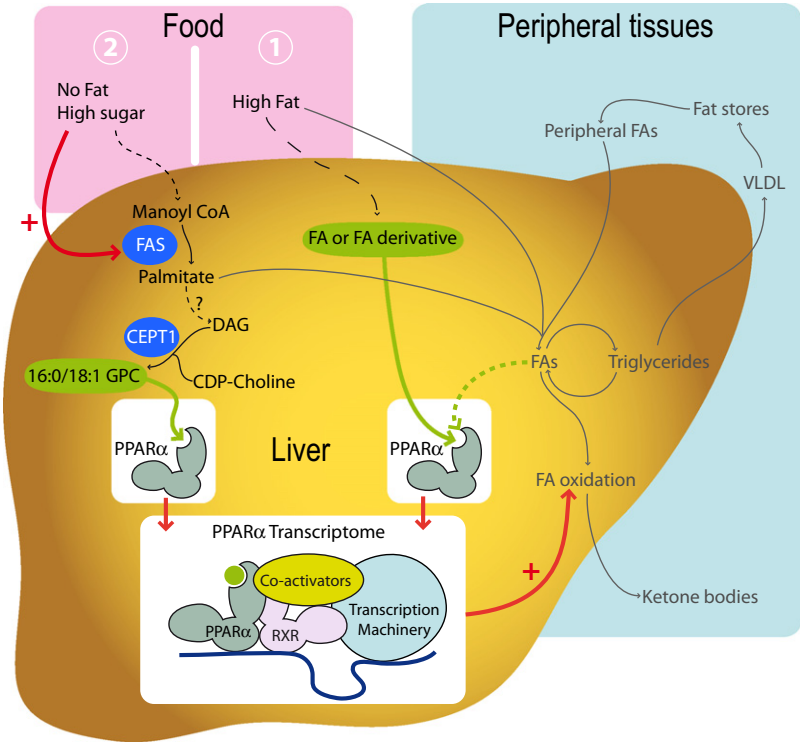

Figure 1. Routes to PPARα Activation in the Liver
Simplified pathways leading to PPARα ligand (in green) production are presented.
(1) Dietary fatty acids activate PPARα.
(2) On no fat diet, FAS and CEPT1 control the production of 16:0/18:1 GPC that activates PPARα.
Activation of PPARα results in the transcriptional regulation of numerous genes, the PPARα transcriptome, which contributes to maintaining the energy balance in part through the promotion of mitochondrial and peroxisomal beta-oxidation of fatty acids.
CEPT1, choline-ethanolamine phosphotransferase 1; DAG, diacyglycerol; FA, fatty acids; VLDL, very low density lipoprotein.

lipids. In line with this thought, FASKOL mice develop a fatty liver phenotype on a fat-free diet (no 16:0/18:1 GPC and no FA available as ligands), but not on a normal fat-containing diet (Chakravarthy et al., 2005). This would make sense if, as has been proposed, free FA released by peripheral tissues during fasting, which enter the liver by the artery, are not able to activate PPARα. Only "new fat," either from the diet or from FAS-dependent neosynthesis, would produce PPARα ligands, not the "old fat" recruited from peripheral stores (Chakravarthy et al., 2005). If this is true, FAS-mediated production of 16:0/18:1 GPC would stimulate PPARα-dependent pathways in the absence of sufficient ligands provided by the diet. This would ensure energy balance under fat-poor dietary conditions (Figure 1).

Another interesting avenue may open as our knowledge of the synthesis of endogenous PPAR ligands grows. It should become possible to activate PPARs selectively in an organ- and isotype-specific manner, which should enable therapeutic strategies to minimize the side effects of broad PPAR activation. Enhancing FAS, for instance, is likely to influence the PPARα transcriptome selectively, as 16:0/18:1 GPC does not bind to PPARγ and only weakly to PPARβ/δ. Finally, since Chakravarthy et al. (2009) identified numerous lipids that are associated with PPARα, but in a FAS-independent manner, the possibility exists, although not likely, that several lipids might bind simultaneously to the same PPARα molecule in a so far unknown way. Could some kind of integration of these signals take place at the level of combinatorial ligand binding?

One important question remains. The production of 16:0/18:1 GPC is controlled by FAS and thus its interaction with PPARα increases when FAS is upregulated by a carbohydrate-rich diet, which necessarily requires feeding. However, the expression of PPARα is circadian (Lemberger et al., 1996), with little PPARα in the fed state, and an increase during fasting, coinciding with the need for FA oxidation to maintain the energy balance. Since 16:0/18:1 GPC production and peak of PPARα activity are antiphasic, one could postulate that if FAS is essential for producing the PPARα ligand, additional regulation is required to present the ligand to PPARα when needed. Alternatively, 16:0/18:1 GPC and other already known PPARα ligands may operate under different temporal and physiological conditions.

In conclusion, much remains to be discovered in the field of PPAR-ligand production, and future studies will contribute both to a better appreciation of the functions of PPARs and their use as drug targets in different metabolic deregulations.

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