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Minireview

PPARγ: An Essential Regulator of Adipogenesis and Modulator of Fat Cell Function

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Introduction

The most important function of adipose tissue is its ability to store and release fat during periods of feeding and fasting. This allows food intake to be intermittent, freeing up time for other activities, and provides a life-saving buffer against protracted periods of famine. That humans are capable of surviving months of total starvation reflects the awesome storage capacity and survival advantage of adipose tissue. However, modern times with the abundance of food and decreased opportunity for physical activity have exposed a significant disadvantage to energy storage in adipose tissue, namely obesity, with its associated morbidities of heart disease, non-insulin-dependent diabetes mellitus (NIDDM) and high blood pressure. With this in mind, there has been intense interest in elucidating the mechanisms controlling adipose tissue development. Three recent papers in the October issue of *Molecular Cell* highlight the critical role played by PPARγ (peroxisome proliferator-activated receptor-γ) in this process (Barak et al., 1999; Kubota et al., 1999; Rosen et al., 1999).

Insight from Adipogenic Cell Lines

Approximately 25 years ago, mouse fibroblast cell lines were established that could differentiate into adipocytes under certain tissue culture conditions, such as the addition of glucocorticoids, agents that increase cAMP, agonists of the IGF-1 receptor, and fetal bovine serum. Using these cell lines, 3T3-L1 and 3T3-F442A preadipocytes, it was found that differentiated adipocytes express many mRNA transcripts that are not found in preadipocytes. "Adipocyte-specific" genes were cloned and their promoter/enhancer regions explored. One of these genes, the adipocyte-specific fatty acid–binding protein gene (*aP2*), has been well characterized, and its promoter has binding sites for each of the two major adipogenic transcription factors, C/EBPα (CCAAT/enhancer binding protein-α) and PPARγ.

C/EBPα belongs to a large family of transcription factors possessing basic leucine zipper (bZIP) domains. C/EBPα is not expressed in preadipocytes but is turned on during differentiation, prior to the expression of most adipocyte genes, many of which contain C/EBP-binding sites (reviewed in Mandrup and Lane, 1997). Two other members of the C/EBP family are also expressed during adipogenesis, C/EBPβ and C/EBPδ, but at a time preceding the expression of C/EBPα (Yeh et al., 1995; Mandrup and Lane, 1997). It was proposed that early induction of C/EBPβ and C/EBPδ relays hormonal stimulation of adipocyte differentiation to downstream effectors, possibly C/EBPα and PPARγ, whose gene promoters bear binding sites for members of the C/EBP family (Yeh et al., 1995; reviewed in Mandrup and Lane, 1997).

PPARγ, a member of the PPAR subfamily of nuclear hormone receptors, heterodimerizes with the retinoid X receptor α (RXRα) to promote gene expression. Like C/EBPα, PPARγ is not expressed in preadipocytes (or is expressed at low levels) and is turned on during differentiation, prior to the expression of most adipocyte genes, many of which contain PPAR-binding sites (reviewed in Spiegelman, 1998; Auwerx, 1999). It is interesting to note that many of these genes also contain C/EBP-binding sites, providing a "double-dose" of transcription factor–binding sites.

Like most members of the nuclear receptor family, PPARγ activity is regulated by ligands. Endogenous ligands identified to date have relatively low affinity (2–50 μM) and are fatty acid–like compounds such as prostanoid derivatives of arachadonic acid (15-deoxy$\Delta^{12,14}$PGJ2), polyunsaturated fatty acids (linoleic acid), and oxidized forms of linoleic acid (9- and 13-hydroxyoctadecadienoic acid) (Forman et al., 1995; Kliewer et al., 1995; Nagy et al., 1998). Whether other endogenous ligands with higher affinity exist remains to be established. Pharmaceutical ligands include the thiazolidinediones (TZDs) such as troglitazone, BRL49653 (rosiglitazone), and pioglitazone. This clinically useful class of compounds was developed independently as insulin sensitizers for the treatment of insulin resistance in patients with NIDDM. Only subsequently was it discovered that the molecular target of TZDs is PPARγ (Forman et al., 1995; reviewed in Kliewer et al., 1995). The mechanism by which TZD-mediated stimulation of PPARγ improves insulin resistance is an important, active area of investigation.

C/EBPs, PPARγ, and Adipogenesis: Gain-of-Function Experiments

Unlike 3T3-L1 and 3T3-F442A adipocytes, NIH-3T3 fibroblasts are relatively resistant to adipogenesis and do not express C/EBPα or PPARγ, and only low levels of C/EBPβ and C/EBPδ. Forced ectopic expression of either C/EBPα, C/EBPβ, or PPARγ alone stimulates adipogenesis, while C/EBPδ works synergistically with C/EBPβ (Freytag, 1994; Tontonoz, 1995; Yeh, 1995; Wu, 1996). Also, marked synergy is observed when C/EBPα and PPARγ are coexpressed. These studies provided important insight into the existence of cascades (C/EBPβ and C/EBPδ inducing PPARγ expression), positive cross-regulation loops (PPARγ inducing C/EBPα expression and vice versa), and possibly autoregulatory loops (C/EBPα and PPARγ inducing their own expression) that all promote and maintain the differentiated state.

C/EBPs, PPARγ, and Adipogenesis: Loss-of-Function Experiments

C/EBPβ and C/EBPδ. C/EBPβ and C/EBPδ are induced during early stages of differentiation and this is thought to induce expression of PPARγ and C/EBPα, culminating in the terminal differentiation of adipocytes. As a test of this, Tanaka et al. (1997) generated gene knockout mice lacking either C/EBPβ, C/EBPδ, or both C/EBPβ and C/EBPδ. Neonatal lethality occurred in 35% of C/EBPβ −/− mice and 85% of C/EBPβ/δ double knockout mice. Animals surviving past the first 24 hr

were viable well into adulthood. White adipose tissue depots were nearly normal in the single knockout mice, but were reduced in weight by 70% in the C/EBPβ/δ double knockout mice. Remarkably, mRNA levels of *C/EBPα*, *PPARγ*, and adipocyte-specific genes such as *aP2* were normal in the small depots of double knockout mice. This finding suggests that other pathways must be capable of triggering PPARγ and C/EBPα expression, and hence adipocyte differentiation. ADD-1/SREBP-1, a key transcription factor in sterol regulation, is one candidate mediator of a C/EBPβ and C/EBPδ-independent pathway since it binds to the PPARγ promoter and induces its expression (Fajas et al., 1999). Also, gene expression induced by ADD-1/SREBP-1 leads to the production of PPARγ ligands (Kim et al., 1998).

C/EBPα. The first loss-of-function experiment was expression of antisense *C/EBPα* mRNA in 3T3-L1 adipocytes. This maneuver reduced expression of endogenous *C/EBPα* mRNA and protein, resulting in markedly reduced expression of *aP2* mRNA and protein, and decreased triglyceride accumulation (reviewed in Mandrup and Lane, 1997). This observation was confirmed and extended in gene knockout mice lacking C/EBPα that fail to develop normal depots of adipose tissue (Wang et al., 1995). Adipocytes are present in C/EBPα-deficient mice, but are relatively devoid of triglyceride, indicating that C/EBPα is not required for adipogenesis, per se, but plays an important role in the terminal maturation of fat cells. Additional support for this view comes from fibroblast cells isolated from C/EBPα +/+ and −/− embryos. Virally mediated ectopic expression of PPARγ plus treatment with the agonist troglitazone stimulated adipogenesis in greater than 70% of +/+ and −/− cells. However, −/− cells accumulated less triglyceride, showed impaired insulin-stimulated glucose uptake, and did not induce endogenous *PPARγ* mRNA, confirming that C/EBPα is not required for adipogenesis, but that C/EBPα and induction of PPARγ by C/EBPα are required to develop the fully differentiated state (Wu, 1999).

PPARγ. The field has eagerly awaited the results of gene knockout efforts aimed at proving the role of PPARγ in adipogenesis and uncovering its possible physiologic role in regulating insulin sensitivity. Such assessments, however, have been delayed due to an unexpected requirement of PPARγ for placental development (Barak et al., 1999; Kubota et al., 1999). Indeed, PPARγ, specifically the γ1 isoform, is expressed in the diploid trophoblast lineages, and its deficiency leads to placental dysfunction and death by E10. Prior to death, placental deficiency causes severe myocardial thinning, presumably due to impaired supply of fuel and/or oxygen. Interestingly, qualitatively similar placental and cardiac abnormalities are seen in RXRα knockout and RXRα/RXRβ double knockout mice, suggesting that these phenotypes may be due to impaired PPARγ-mediated signaling in the placenta.

To circumvent placental lethality, Barak et al. (1999) isolated PPARγ −/− 6- to 8-cell embryos and aggregated these with tetraploid PPARγ +/+ preimplantation embryos. In such chimeras, tetraploid cells (in this case PPARγ +/+ cells) are unable to contribute to the embryo proper, but their contribution to extraembryonic tissues tends to be extensive. By such efforts, Barak et al. were able to generate two E12.5 PPARγ −/− embryos, one E15.5 PPARγ −/− embryo, and one live born PPARγ −/− mouse. The cardiac phenotype was rescued in the embryos demonstrating that it was secondary to placental dysfunction. Of note, the E15.5 −/− embryo, similar to the +/− embryo, showed expression of a β-galactosidase reporter cassette, which had been inserted in-frame into the PPARγ coding region of the gene-targeting construct. β-galactosidase activity was noted in an anatomical location where brown fat subsequently appears in normal but not the −/− live-born mouse, raising the interesting possibility that brown adipocyte precursors begin to develop, but fail to elaborate into brown adipose tissue in the absence of PPARγ. Histological analysis will be required to better understand the nature of these E15.5 β-galactosidase-positive cells. The live-born PPARγ −/− mouse was a runt weighing 70% of a similarly created PPARγ +/− littermate. The health of the runt deteriorated at day P5, and at day P6.5 was examined along with the +/− littermate. The PPARγ-deficient mouse was notable for total absence of white and brown adipose tissue (complete lipodystrophy) and fatty liver (secondary to lipodystrophy). Although the number is small, the conclusion is profound: PPARγ is required for adipogenesis in vivo.

A similarly profound conclusion was provided in the study by Rosen et al. (1999) in which chimeric mice of a more conventional kind were generated by injecting ES cells homozygous for PPARγ deficiency into PPARγ +/+ blastocysts. The authors observed that PPARγ −/− ES cells contributed significantly to the composition of a number of tissues, including heart, spleen, small intestine, and skeletal muscle, but contributed poorly to adipose depots (which consist of adipocytes plus other cells) and hardly at all to mature adipocytes within the depot. In addition, the authors observed that PPARγ null ES cells are unable to contribute to the sebaceous glands of the skin, suggesting an important role of PPARγ in their development as well.

In vitro fat cell differentiation assays also confirmed the important role of PPARγ. Using fibroblasts isolated from PPARγ +/+, +/−, and −/− embryos (Kubota et al., 1999) or ES cells of similar genotypes (Rosen et al., 1999), it was observed that PPARγ null cells (fibroblasts or ES cells) expressed normal levels of C/EBPβ and C/EBPδ in response to the differentiation protocol, but compared to wild-type cells had reduced or very low levels of C/EBPα and completely failed to induce expression of aP2 and leptin, or to accumulate lipid. Heterozygous fibroblasts and ES cells showed an intermediate impairment of adipogenesis. These in vitro loss-of-function experiments strongly support the view that PPARγ is downstream of C/EBPβ and C/EBPδ, but upstream of C/EBPα and terminal fat cell differentiation. A schematic model reflecting this view is shown in Figure 1.

Roles of PPARγ and C/EBPα in Fully Differentiated, Mature Adipocytes

Since the adipocyte "phenotype" is a byproduct of the genes it expresses, and since the expression of these "adipocyte-specific" genes is controlled primarily by PPARγ and C/EBPα, it stands to reason that these transcription factors regulate function of mature adipocytes as well as adipogenesis. However, this aspect of PPARγ and C/EBPα has been relatively unexplored. A case in

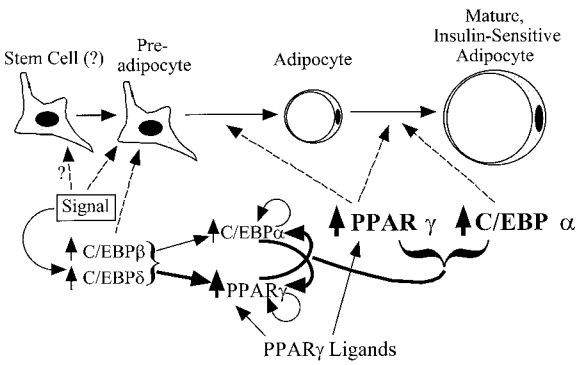

Figure 1. Molecular Control of Adipogenesis

Signals (in vitro differentiation signals = glucocorticoids, cAMP, IGF-1 agonists, and fetal bovine serum; in vivo signals = unknown) interact with stem cells and/or preadipocytes increasing expression of C/EBPβ, C/EBPδ, and possibly other transcription factors, leading to modest induction of C/EBPα and potent induction of PPARγ. Ligands, possibly fatty acids, increase activity of PPARγ. PPARγ further induces C/EBPα and also increases its own expression. Similarly, C/EBPα induces PPARγ expression as well as its own expression. These positive cross-regulation loops ensure that high levels of PPARγ and C/EBPα are maintained. PPARγ then stimulates adipocyte differentiation. Combined high levels of PPARγ and C/EBPα promote acquisition of the mature adipocyte phenotype. In the absence of PPARγ, adipocytes fail to develop. In PPARγ heterozygous mice, adipocytes develop but tend to store less lipid. In the absence of C/EBPα, adipocytes develop but store less lipid and fail to become insulin sensitive. In the absence of C/EBPβ and C/EBPδ, adipogenesis is markedly decreased; however, adipocytes that do develop appear to be normal.

point may be leptin, the adipocyte-derived circulating product of the *obese* gene which is deficient in *ob/ob* mice. The function of leptin is to communicate the status of fat stores to the brain, and accordingly, *leptin* mRNA levels in mature fat cells rise and fall with feeding and fasting. Similarly, leptin expression and secretion is proportional to adipocyte size. The cause of this important regulation is unknown but may involve C/EBPα and PPARγ, as these transcription factors have been shown to regulate leptin gene expression. C/EBPα directly and potently transactivates the proximal *leptin* promoter (He, 1995) while TZD-mediated activation of PPARγ inhibits leptin gene expression, apparently without binding to *leptin* gene regulatory sequences (Hollenberg, 1997). Such negative regulation by activated PPARγ may be analagous to the ability of liganded retinoic acid receptor (RAR) to inhibit the transcriptional activity of C/EBPβ and C/EBPα, possibly by competing for common, limiting cofactors (Schwarz et al., 1997).

To investigate the role of PPARγ in modulating mature adipocyte function, Kubota et al. (1999) studied mice heterozygous for PPARγ deficiency. While these animals are apparently normal on a regular diet, they are partially resistant to high fat diet–induced obesity and markedly resistant to the insulin resistance that normally accompanies such obesity. On the surface, this protection from obesity and insulin resistance could be viewed as being due to impaired adipogenesis secondary to decreased PPARγ levels. However, if such a mechanism occurred, it would worsen insulin resistance due to excess deposition of lipid in tissues other than fat, such as occurs in models of lipodystrophy. Along these lines, it is important to note that high fat diet–induced fatty liver was present in wild-type mice, but was prevented in PPARγ +/− mice, a condition that would worsen if adipocyte lipid storage was limiting. As an alternative mechanism, Kubota et al. (1999) report that *leptin* mRNA and circulating protein levels are elevated in high fat–fed PPARγ +/− mice, possibly due to decreased PPARγ-mediated inhibition of *leptin* gene expression. In support of this, the authors show that differentiated embryo fibroblasts heterozygous for PPARγ, compared to wild-type cells, have increased *leptin* mRNA levels and increased leptin secretion. The increase in circulating leptin decreases food intake and increases energy expenditure, protecting the mice from high fat diet–induced obesity and insulin resistance. In addition, the elevated leptin may modulate insulin sensitivity independently of its ability to decrease adipose tissue fat stores (Shimomura et al., 1999).

These findings present the following paradox: both PPARγ "overactivity" due to TZD stimulation and PPARγ "underactivity" due to haploinsufficiency protect against obesity-induced insulin resistance, leading to the somewhat perverse, but provocative inference that "normal" amounts of PPARγ activity, under certain circumstances, promote disease, and that both agonists and antagonists of PPARγ could be clinically useful. In this regard, *PPARγ* could be viewed as a "thrifty gene," promoting fat storage to survive starvation when food is scarce, while producing excessive fat storage leading to disease when food is plentiful. The paradox also suggests that the two effects are likely to have different explanations. While the mechanism by which TZD-mediated stimulation of PPARγ improves insulin resistance is not definitively established (Spiegelman, 1998), it seems likely that it is related to PPARγ's ability to increase the number of small adipocytes, by stimulating adipogenesis, and decrease the number of large adipocytes (reviewed in Kubota et al., 1999), which are known to produce excess amounts of tumor necrosis factor-α and free fatty acids. PPARγ haploinsufficiency, while not affecting adipogenesis, limits further adipocyte hypertrophy, possibly by increasing leptin expression. Although the mechanisms are distinct, with TZDs promoting adipogenesis on the one hand and reduced *PPARγ* gene dosage decreasing hypertrophy of mature adipocytes on the other hand, the end result is the same—a decreased number of large adipocytes, thus protecting against obesity-induced insulin resistance. The field anxiously awaits additional information, both in mice and humans, on the very intriguing "insulin-sensitizing" effects of PPARγ haploinsufficiency.

Adipogenesis: Unanswered Questions

As outlined above, recent years have witnessed a phenomenal increase in knowledge regarding the molecular control of adipogenesis. These findings now lead to the next level of questions. Does an "excess" supply of fuel to preadipocytes directly stimulate adipogenesis or does this induce production in mature adipocytes of a paracrine signal that secondarily promotes adipogenesis in neighboring cells? Of particular interest is the role of dietary fats to serve as precursors to PPARγ ligands. Alternatively, does positive caloric balance alter systemic hormones, insulin being one example, which

then stimulate adipogenesis? Do the candidate signals mentioned above directly engage C/EBPβ and/or C/EBPδ, or are other transcription factors involved? Finally, and importantly, what cell within a fat cell depot, in vivo, is the adipocyte precursor? Given the identification of markers such as C/EBPβ, C/EBPδ, C/EBPα, and PPARγ, this important question should now be addressable. Based upon recent progress, it is likely that answers to these questions will be forthcoming.

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