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Minireview: PPARγ as the target of obesogens☆

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ABSTRACT

The peroxisome proliferator-activated receptor gamma (PPARγ) is a key regulator of adipogenesis and is medically important for its connections to obesity and the treatment of type II diabetes. Activation of this receptor by certain natural or xenobiotic compounds has been shown to stimulate adipogenesis in vitro and in vivo. Obesogens are chemicals that ultimately increase obesity through a variety of potential mechanisms, including activation of PPARγ. The first obesogen for which a definitive mechanism of action has been elucidated is the PPARγ and RXR activator tributyltin; however, not all chemicals that activate PPARγ are adipogenic or correlated with obesity in humans. There are multiple mechanisms through which obesogens can target PPARγ that may not involve direct activation of the receptor. Ligand-independent mechanisms could act through obesogen-mediated post-translational modification of PPARγ which cause receptor de-repression or activation. PPARγ is active in multipotent stem cells committing to the adipocyte fate during fat cell development. By modifying chromatin structure early in development, obesogens have the opportunity to influence the promoter activity of PPARγ, or the ability of PPARγ to bind to its target genes, ultimately biasing the progenitor pool towards the fat lineage. Obesogens that act by directly or indirectly activating PPARγ, by increasing the levels of PPARγ protein, or enhancing its recruitment to promoters of key genes in the adipogenic pathway may ultimately play an important role in adipogenesis and obesity.

1. Introduction

A major advance in the study of adipocyte development was the discovery of genes specifically expressed in mature adipocytes. Among the first to be identified was the p422 protein [1,2], later called aP2 (adipocyte protein 2) and now termed fatty acid binding protein (FABP4). Using FABP4 as a marker, it became possible to study how the differentiation of pre-adipocytes into adipocytes was regulated. Indeed, FABP4 expression is considered to be indicative of a cell committed to the adipocyte lineage [3]. An enhancer complex, termed adipocyte regulatory factor 6 (ARF6), was soon found to drive expression of FABP4 [4]. With biochemical and mass spectrometric methods, ARF6 was characterized as a heterodimer of the nuclear receptors PPARγ2 and RXR [5]. The RXR–PPARγ heterodimer is a key regulator of the adipogenic program and numerous PPARγ target genes have been identified [reviewed in [6–8]]. These include lipoprotein lipase (LPL), which generates non-esterified fatty acids (used in triglyceride synthesis) from lipoproteins, and aquaporin 7, which facilitates the transportation of glycerol, the backbone of triglycerides, into adipocytes [9]. During adipogenesis, PPARγ expression is positively reinforced by CCAATT enhancer binding protein alpha (C/EBPα) [10], the activity of which is modulated by PPARγ itself [10], glucocorticoid signaling [11], insulin signaling [12], as well as cAMP levels [13]. After the adipogenic program is initiated, insulin stimulates PPARγ- and C/EBPα-expressing cells to accumulate/store the lipid that they produce [14].

As is the case for nearly all nuclear hormone receptors, PPARγ can be perturbed by environmental chemicals. PPARγ is perhaps even more susceptible than most nuclear receptors because its ligand-binding pocket is large and can accommodate a diversity of chemical structures [15]. Since PPARγ is a master regulator of adipogenesis, a logical hypothesis is that inappropriate activation of the receptor contributes to obesity. Obesogens are chemicals, natural or xenobiotic, that promote obesity by increasing the number of fat cells, up-regulating fat storage into existing fat cells, changing the amount of calories burned at rest, shifting energy balance to favor storage of calories or altering the mechanisms through which the body regulates appetite and satiety. The first obesogen for which a definitive mechanism of action has been elucidated is the PPARγ and RXR activator, tributyltin [16,17]. The most well known pharmaceutical obesogens, which are also agonists of

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PPARγ, are the thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone, used to treat type 2 diabetes. TZDs are linked to weight gain in humans [18] and increased adipogenesis in cell culture [19]. Activation of PPARγ by TZDs increases proliferation of new fat cells, thereby reducing adipocyte hypertrophy, which has been associated with inflammation, oxidative stress, and insulin resistance [20]. Whether these newly generated adipocytes "crave" to be filled with lipid is uncertain. However, it is well established that obese humans have a higher than normal fat cell number [21]; thus the hypothesis that increased adipocyte number leads to obesity is plausible and needs to be tested.

Considering the existence of pharmaceutical obesogens such as TZDs and xenobiotic obesogens such as organotins, it is highly likely that other compounds, which can inappropriately activate PPARγ, will be obesogenic. The topic of obesogens and their potential mechanisms of action has been extensively reviewed in recent years [22–25]. PPARs as the targets of environmental chemicals, particularly phthalates has also been recently reviewed [26–28]. Therefore, this minireview focuses on recent evidence linking endocrine disrupting chemicals to PPARγ in particular and examines the molecular mechanisms through which they might act.

2. Characterizing relationships among chemicals, obesogenicity, and PPARγ

It is currently an open question whether most or all chemicals that activate PPARγ will ultimately be shown to be obesogenic. The ability of pharmaceutical drugs, such as the TZDs, and xenobiotic chemicals such as tributyltin, to activate PPARγ and induce adipogenesis in vitro and in vivo is well documented [reviewed in [22–25]] and it was recently shown that PPARγ activation is required for the obesogenic effects of TBT [29]. Of the other known xenobiotic obesogens, phthalates are the most likely to act through PPARγ to cause increased adipocyte conversion [27,30–32]. Phthalates are of particular concern since they are omnipresent organic chemicals that give plastics, like polyvinyl chloride (PVC), more flexibility and durability [27]. Phthalate metabolites in human urine are correlated with increased waist diameter and body mass index in adult males [33,34] and some phthalates are PPARγ activators [32]. However, there are currently no in vivo animal studies that address whether phthalates cause adipogenesis and obesity through PPARγ.

Aside from organotins and phthalates, there are no other confirmed endocrine disrupting chemical ligands for PPARγ, notwithstanding its large ligand-binding pocket. Numerous classes of chemicals that activate PPARγ have been identified in drug discovery efforts aimed at developing new anti-diabetes drugs. An increasing number of natural products that activate PPARγ have also been identified. Some of these activate PPARγ and induce adipogenesis in cell models such as 3T3-L1 cells; e.g., flavanone [35], bixin [36], and emodin [37]. Others, such as the flavonoid, pseudobaptigenin (found in red clover) [38], elderberry extracts [39], the putative active component of ginseng, ginsenoside 20S-protopanaxatriol [40], carvacol (a component of thyme oil) [41] and pucinic acid [42] all activate PPARγ in reporter gene assays but have not been linked to adipogenesis and obesity. While the evidence from pharmaceutical and xenobiotic obesogens might be taken to suggest that these compounds will be obesogenic, the situation is not so simple.

Selective PPAR modulators have recently been described that are either receptor-selective or behave as agonists in some cell types but not others due to differential recruitment of coregulators to PPAR target genes [reviewed in [43–45]]. Two such drug candidates, INT-131 and telmisartran, activate PPARγ, yet do not appear to lead to adipogenesis or obesity (although clinical data are currently scant) [44,45]. An even more extreme example is mycophenolic acid. Mycophenolic acid was shown to inhibit adipogenesis in 3T3-L1 pre-adipocytes through a non-PPARγ-dependent pathway; however, it was also shown to be a PPARγ activator in the same cells [46]. Complicating the issue even further, Spiegelman and colleagues have demonstrated that a functional PPARγ ligand-binding domain may not be required for adipogenesis in murine 3T3-L1 cells [47]; although PPARγ activation is definitely required for adipose-derived multipotent stromal cells to differentiate into adipocytes [29]. Thus, it is not sufficient simply to test chemicals for activity on PPARγ as a surrogate to predict adipogenic potential. Rather, biological assays in relevant cell types (e.g., multipotent stromal cells or 3T3-L1 cells) are the minimum required to infer potential for obesogenicity, irrespective of PPARγ activation.

3. Obesogens and ligand-independent mechanisms

One simple way for a chemical to increase the potential for adipogenesis is to increase the steady-state level of PPARγ mRNA. For example, sildenafil (known as Viagra) promotes adipogenesis by increasing the expression of adipogenic genes, including PPARγ through a protein kinase G-dependent mechanism [48]. While it might be possible to conclude that sildenafil is an obesogen that works by increasing PPARγ expression, all chemicals that increase adipogenesis will inevitably result in a concomitant increase in PPARγ expression. Thus, while a rise in PPARγ transcript/protein levels is an auspicious event for adipogenesis, since adipogenesis does not occur without PPARγ [49,50], it may reflect the outcome, rather than the mechanism of obesogen action. PPARγ can be targeted by obesogens at the transcriptional level via modification of chromatin structure, thereby facilitating the expression of PPARγ during adipogenesis (see below).

The classical model of nuclear hormone receptor activation is that in the absence of ligand, co-repressors are bound, chromatin is condensed and transcription is minimal at target genes. Ligand binding triggers a conformational change in the receptor that favors binding of co-activators and release of co-repressors, chromatin decondensation and transcriptional activation. However, nuclear receptors can also be de-repressed or activated through various post-translational modifications (PTMs) causing active release of co-repressors in the absence of PPARγ ligands [reviewed in [51]]. Indeed, PPARγ is already known to be post-translationally phosphorylated, SUMOylated and ubiquitinated [reviewed in [52], and may function as an unliganded receptor (or might be activated by an endogenous ligand) during adipogenesis in 3T3-L1 cells [47]]. Recently, it was shown that a specific phosphorylation mark on PPARγ, established by cyclin-dependent kinase 5 (CDK5), is associated with genes that are misregulated in diabetes in response to a high fat diet [53]. The presence or absence of PTMs on PPARγ, such as phosphorylation, could be obesogenic if they stabilize PPARγ protein, increase the ability of the receptor to activate transcription of adipogenic genes or regulate the ability of the receptor to interact with the transcriptional machinery. The effects of specific PTMs (e.g., phosphorylation at S112) can be cell-type specific and the physiological effects of PPARγ PTMs are currently poorly understood; thus, this is a high priority area for future research. Whether obesogens exist that target one of the mechanisms underlying PPARγ PTMs remains to be demonstrated, but we consider this possibility quite plausible.

4. Targeting PPARγ early during lineage commitment of adipocytes

Obesogens that are studied in the 3T3-L1 cell culture model necessarily reflect the actions of PPARγ in the context of adipocyte

conversion from pre-adipocytes, since 3T3-L1 cells are already committed to the adipocyte lineage and can no longer differentiate into other tissues such as bone, cartilage, muscle, or brown fat [7]. Mature adipocytes are thought to be generated from white adipocyte precursors that are committed to the adipocyte lineage [7,54–56]. This adipocyte precursor is probably derived from multipotent stromal cells (MSCs) found in almost all fetal and adult tissues [57]. Most evidence supports the theory that MSCs are the progeny of perivascular cells that surround blood vessel walls [58,59]. A subpopulation of these stem cells expressing PPARγ [60] and CD24 [61] resides within the stromal-vascular niche of adipose tissue. These adipose progenitors have lost expression of MSC markers [61], and exhibit increased DNA methylation at the promoters of non-adipogenic genes, and decreased methylation at adipogenic genes, thereby fostering lineage restriction [62,63]. While these cells still have the capacity to become bone, cartilage, and muscle in vitro [61], they differentiate almost exclusively into mature adipocytes when transplanted to nude or lipodystrophic mice [60,61]. Interestingly, these adipose progenitors are present in newborn mice [60] (which have very little fat) thus indicating that there is a population of cells, expressing PPARγ, which are programmed towards the adipogenic lineage early in life. In adulthood, these progenitors are predicted to regenerate existing fat cells [7] about every 10 years [21].

Taken together, these findings show that PPARγ is active early in development during the commitment phase of adipogenesis and might influence fate decisions of stem cells. As a result, PPARγ becomes a vulnerable target of obesogens during prenatal events. The organotin compound, tributyltin (TBT), when administered to pregnant mice, will cause newborn offspring exposed, *in utero*, to develop adipocytes prematurely in the liver, testis, mammary gland and inguinal adipose tissue [17]. Adipose-derived MSCs harvested from such animals showed pronounced commitment to the adipocyte lineage compared to controls, when induced to differentiate [29]. Furthermore, adipogenesis occurred at the expense of osteogenesis, suggesting that prenatal exposure to TBT biased the stem cells towards the adipogenic lineage [29]. There are other chemicals, such as organophosphates and 4-tertoctylphenol, that alter lineage commitment in cell culture, to thwart the bone differentiation capacity of MSCs [64]. Whether their effects involve PPARγ remains to be determined.

5. Chromatin remodeling surrounding PPARγ: towards the adipogenic lineage

Recent research has pointed to the influence of histone methylation on lineage programming in stem cells, including MSCs. Like embryonic stem cells [65,66], naïve T cells [67], and neural progenitors [68], MSCs also exhibit bivalent chromatin marks on histone H3 proteins associated with promoters of lineage specific genes [69]. For example, tri-methylation of H3 at lysine 4 (H3K4me3) is linked to activation, whereas H3K27me3 is linked to repression [65,66,70]. These opposing histone modifications are predicted to prime genes such that they can be up-regulated promptly when differentiation is induced, simply by demethylating H3K27 [65].

In adipose-derived MSCs, these bivalent marks are exhibited on the PPARγ promoter. When differentiation is stimulated, H3K27 is demethylated completely, leaving only the activating mark on the PPARγ promoter [69]. An obesogen could alter early programming events when these lineage-specific histone modifications are established. Moreover, the demethylases that remove the H3K27me3 marks, JMJD3 and UTX [71], could be inappropriately stimulated temporally or spatially, thereby activating PPARγ and the adipogenic program prematurely. Perhaps the most convincing evidence that xenobiotic chemicals can alter early chromatin remodeling events is derived from rat expression microarray analysis using the common fungicide vinclozolin. Several DNA, RNA, and histone methyltransferases were shown to be altered significantly in the testes of offspring prenatally exposed to vinclozolin, compared to controls [72]. A subset of these genes remained altered in subsequent generations, despite the fact that vinclozolin exposure only occurred in F0 [72]. Whether obesogenic compounds have the same effect is currently being explored in our laboratory and elsewhere.

Modification of histones surrounding the PPARγ promoter will poise PPARγ for activation upon differentiation. However, PPARγ promotes more than one differentiation process. For example, PPARγ is expressed in monoblasts [73] and promotes macrophage differentiation [74]. While adipocytes and macrophages diverge from a common origin early in development, both share their requirement for PPARγ. An additional layer of regulation, differential recruitment of PPARγ to enhancer elements, is required for PPARγ to promote a cell-specific transcriptional program. Similar to the estrogen receptor, PPARγ has an affinity for distal and intronic regulatory regions [75]. The PPARγ/RXR heterodimer binds to direct repeats separated by one nucleotide (DR1) with PPARγ binding 5′ to RXR [76,77]. A DR1 element itself is not indicative of a biologically relevant binding site, but the presence of enhancer-specific histone methylation increases the likelihood that a particular DR1 element may be a functional PPARγ response element [78]. Unlike the aforementioned promoter marks, active enhancers possess distinctive methylation patterns associated with mono- and di-methylated histone H3, at lysine 4 (H3K4me1, H3K4me2) [79,80]. In macrophages, there are activating H3K4me1 marks on histones associated with the enhancers of cytokine and immunity genes, linked to nearby binding of PPARγ and the ets-factor PU1 [81,82]. These same enhancers are repressed in adipocytes, while highly induced genes are associated with adjacent PPARγ and C/EBPα binding [83]. It is currently unknown whether PPARγ recruits methyltransferases to the DNA, or instead whether the presence of these histone marks increases the likelihood of PPARγ binding to the DR1 consensus. In this case, obesogens could be acting to alter the chromatin landscape such that PPARγ preferentially is recruited to the enhancers of adipogenic genes.

6. Conclusions and future directions

There is compelling evidence to suggest that chemicals in our environment are a contributing factor in the obesity epidemic; although, the full extent to which they influence obesity in humans is unknown at present. Obesogens that act early in development and demonstrate the potential to predispose humans to obesity later in life are of particular interest in this emerging field. Since PPARγ is a master regulator of adipocyte development, chemicals that act through PPARγ, have been, and will continue to be a major focus of investigations into environmental obesogens. Organotins (such as tributyltin) and phthalates (such as monoethylhexylphthalate) are two classes of obesogenic compounds that target PPARγ. There may be numerous other obesogenic chemicals that remain to be identified and this is the subject of active investigation around the world. The role of environmental chemicals in the development of obesity and diabetes has attracted sufficient interest to be the topic of an upcoming workshop aimed at summarizing the state of the art and planning the way forward that will be hosted by the National Toxicology Program in January 2011.

Although it is clear that activation of PPARγ can lead to adipogenesis and obesity, activation of PPARγ, per se, is insufficient to classify a compound as an obesogen since at least a few PPARγ activators have been identified that may not be linked with obesity. Even when an obesogenic chemical is demonstrated to be a PPARγ

activator, it may not be immediately apparent how the ligand acts to increase fat cell number and lipid storage in humans and to what extent PPARγ activation is involved or required for the obesogenic phenotype. It will be important in the future to fully understand how prenatal and early life exposure to obesogenic chemicals can program exposed individuals to gain weight and what role modulation of PPARγ expression or activity early in life plays in this process.

In addition to direct effects of ligands on PPARγ activation, we explored the idea of non-ligand mediated effects on PPARγ. While it is well known that PPARγ can be modified post-translationally, the connection to obesity is not yet fully elucidated. PPARγ is regulated at the epigenetic level in MSCs or in precursor cells occupying the white adipose vascular niche and only recently has the role for PPARγ in the commitment stage of adipogenesis been addressed. More and more evidence supports the existence of a group of cells expressing PPARγ and possessing characteristic epigenetic marks that are primed to become adipocytes. Yet, we do not fully understand how obesogens perturb this process and bias stem cells towards the adipogenic lineage. Future efforts will explore whether obesogens preferentially mark PPARγ for activation or make chromatin more accessible to PPARγ. Ultimately, it will be important to understand how perturbation of PPARγ by obesogens in stem cells ultimately leads to obesity in humans.

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