Review Article

Prostaglandins as PPARγ Modulators in Adipogenesis

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1. Introduction

Obesity is a major health concern worldwide [1] and is associated with the development of a number of pathological disorders such as type 2 diabetes, hypertension, and cardiovascular disease [2–4]. Excess adipose tissue can be the consequence of both an increased number (hyperplasia) and an enlarged size (hypertrophy) of adipose cells. A major role of adipocytes is to store large amounts of triglycerides during periods of energy excess and to mobilize these depots during periods of nutritional deprivation [2–4]. Moreover, adipocytes are highly specialized cells that secrete various adipocytokines, whose release largely reflects the amounts of stored triglyceride [2, 5–8].

The regulation of adipocyte differentiation (adipogenesis) is complex and this process includes alteration of the sensitivity to hormones and the expression of a number of genes in response to various stimuli including lipid mediators. Peroxisome proliferator-activated receptor (PPAR) γ and CCAAT/enhancer-binding proteins (C/EBPs) are the most important transcription factors involved in the activation of adipogenesis, and they induce the expression of a number of adipogenic and lipogenic genes that participate in the control of adipogenesis [9, 10]. PPARs are members of the nuclear receptor superfamily and play critical roles in the regulation of storage and catabolism of lipids [11, 12]. To date, three types of PPAR subtypes have been identified, that is, PPARα, PPARβ/δ, and PPARγ [11, 12]. PPARs increase the expression of a variety of genes in various cells through heterodimerization with retinoic acid receptors or retinoid X receptors in a ligand-dependent manner [12–16]. Among them, PPARγ is expressed predominantly in adipose tissue and macrophages, is closely related to the regulation of lipid and glucose metabolisms, and is associated with the control of obesity and related diseases [11, 12]. Until now, many natural and synthetic ligands for PPARγ have been identified [17–19]. 15-Deoxy-Δ12,14-prostaglandin (PG) J2 (15d-PGJ2) was the first identified endogenous ligand for PPARγ, and it activated adipogenesis in cultured cells [20, 21]. Moreover, fatty acids such as lauric acid (C12:0) and petroselinic acid (C18:1) of the saturated fatty acids [22], linolenic acid (C18:3), eicosapentaenoic acid (C20:5), and docosahexaenoic acid (C22:6) of the ω3 (n-3) family [23], arachidonic acid of the ω6 (n-6)
family [22, 23], and very-long chain fatty acids [24] were later identified as other endogenous PPARγ ligands that activate PPARγ functions. In addition, 9-hydroxy and 13-hydroxy octadecadienoic acids (HODE), the components of oxidized low-density lipoprotein (ox-LDL), were also identified as endogenous ligands for PPARγ [25, 26]. However, whether these natural molecules can function as physiological ligands of PPARγ in vivo remains unknown. In addition to natural ligands, many synthetic ligands have been identified. For example, thiazolidinediones (TZDs) such as Troglitazone, Rosiglitazone, Ciglitazone, and Pioglitazone are used for the treatment of type 2 diabetes mellitus; and these ligands affect insulin resistance and glucose homeostasis by activating PPARγ functions [12, 18]. However, these TZDs increase hepatic toxicity and cardiovascular risk. Finally, Troglitazone was withdrawn from the market [27]. It is still unknown whether the toxicities associated with TZDs are derived from the binding with PPARγ.

PGs are lipid mediators that play a number of physiological roles in a variety of cells. PGs are synthesized through the following three enzymatic steps (Figure 1). First, arachidonic acid is liberated from the membrane phospholipids by the action of cytosolic phospholipase A2 (cPLA2) [28]. Second, arachidonic acid is converted to PGH2, which is a common precursor of all prostanoids, by either cyclooxygenase- (COX-) 1 or COX-2 [29]. The activity of these enzymes is critical to determine the production rate of PGs. Third, PGH2 is metabolized to various PGs, that is, PGD2, PGE2, PGF2α, prostacyclin (PGI2), and thromboxane A2 (TXA2), by the action of specific PG synthases [29]. PGs exert a wide range of actions through their binding to specific PG receptors that belong to the G protein-coupled receptors (GPCRs) gene family [30]. GPCRs span cell membranes via seven transmembrane-spanning segments and are the most important therapeutic targets. In this decade, the functions of PGs in the regulation of adipogenesis have been extensively investigated. Elucidation of the molecular mechanisms underlying adipogenesis may provide strategies for reducing the prevalence of obesity. This paper focuses on the recent advances in our understanding of the function of PGs as modulators of PPARγ in the regulation of adipogenesis.

2. Roles of COXs in Adipocytes

COX consists of two isozymes, COX-1 and COX-2, and is the rate-limiting enzyme in the PG biosynthesis [29]. COX-1 is constitutively expressed in most cells including adipocytes, whereas COX-2 expression is induced by various stimuli [29] and transiently activated in the early phase of adipogenesis, followed by lowered expression during adipogenesis [31]. There have been a number of reports regarding the contribution of COX isozymes to the regulation of adipocyte differentiation. However, the roles that COX-2 plays during adipogenesis are still controversial.

In cell-based studies, Yan et al. demonstrated that inhibition of COX activities by their selective inhibitors, for example, SC-560 for COX-1, and NS-398 and Celecoxib for COX-2, enhances adipocyte differentiation via an increase in the mRNA levels of PPARγ and C/EBPα. Thus, both COX-1 and COX-2 participate in the regulation of adipogenesis [32]. Moreover, in 3T3-L1 cells stably expressing COX-2 in the antisense direction, lipid accumulation is enhanced during adipogenesis with elevated expression of adipogenic genes such as PPARγ and C/EBPα. In addition, this enhancement of lipid accumulation in antisense COX-2-expressing cells can be reversed by cotreatment with either antiadipogenic PGE2 or PGF2α [33].

In contrast, when 3T3-L1 cells are pretreated before the initiation of adipocyte differentiation or treated during the clonal expansion phase with SC-58236, a selective COX-2 inhibitor, and then caused to differentiate into adipocytes, lipid accumulation is reduced along with repressed expression of the adipogenic fatty acid-binding protein 4 (FABP4, also called aP2) gene [34]. In contrast, a selective COX-1 inhibitor, SC-58560 does not have any effect on adipogenesis. Additionally, when 3T3-L1 cells are caused to differentiate into adipocyte in a medium containing each of two selective COX-1 and COX-2 inhibitors that are added after the clonal expansion phase, adipogenesis is not affected. Thus, inhibition of COX-2 activity suppresses adipocyte differentiation by repressing the clonal expansion phase [34].

In in vivo studies, overexpression of COX-2 in white adipose tissue (WAT) increases de novo recruitment of brown adipose tissue (BAT) and energy expenditure, while suppressing the high fat diet-induced gain in body weight [35]. Also, Ghoshal et al. reported that in COX-2 gene-knock-out mice, their total body weight is significantly lower than that of wild-type mice, along with reduced expression of adipogenic genes such as those of PPARγ and lipoprotein lipase [36]. In addition, PGD2 and 15d-PGJ2 levels in cells prepared from adipose tissues of COX-2 gene-knock-out mice and placed in primary culture are reduced as compared with those in wild-type mice [36]. Thus, further studies are needed to elucidate the precise functions of COXs in the regulation of adipogenesis.

3. Repression of the Early Phase of Adipogenesis by PGF2α

PGF2α and PGE2 suppress the differentiation of adipocytes and exert their functions as antiadipogenic agents exert by acting through their specific FP [37–41] and EP4 [42, 43] receptors, respectively.

PGF2α is synthesized by a variety of PGF synthase (PGFS) activity-carrying enzymes [44], for example, aldoketo reductase (AKR) 1B3 [45], AKR1B7 [46], and prostamide/PGFS [47] in mice. In humans, AKR1C3 acts as a PGFS in adipocytes and is associated with the suppression of adipogenesis through inhibition of PPARγ function [48]. Although PGFS has never been identified in adipocytes, we and another group identified AKR1B3 [31] and AKR1B7 [49] as being PGFSs in adipocytes.

AKR1B3-produced PGF2α is detected in preadipocytes and its level is enhanced with a peak at 3 h after the initiation of adipogenesis and then decreases [50], indicating that PGF2α suppresses an early phase of adipogenesis.
Fluprostenol, an FP receptor agonist, clearly reduces the expression of PPARγ and its target genes [31, 50]. Moreover, this Fluprostenol-mediated suppression of the gene expression is cleared by cotreatment with AL8810, an FP receptor antagonist, indicating that PGF2α inhibits adipocyte differentiation of 3T3-L1 cells by acting through an FP receptor.

AKR1B7 gene-knock-out mice display excessive adiposity resulting from adipocyte hyperplasia/hypertrophy and exhibit high sensitivity to diet-induced obesity. Treatment of 3T3-L1 cells or AKR1B7 gene-knock-out mice with Cloprostenol, an FP receptor agonist, decreases adipocyte size and inhibits the expression of lipogenic genes [49].

The precise molecular mechanism of PGF2α-mediated suppression of adipogenesis has been investigated. PGF2α represses the function of PPARγ by causing its phosphorylation via FP receptors [50]. In addition, Fluprostenol enhances the expression of COX-2 via activation of the mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) 1/2 pathway. Moreover, promoter-luciferase and chromatin immunoprecipitation assays demonstrated that PGF2α-derived COX-2 expression is activated by the binding of cAMP-responsive element binding protein (CREB) to the promoter region of the COX-2 gene in 3T3-L1 cells [50]. Thus, the MEK/ERK-CREB cascade forms a positive feedback loop, one that probably plays a critical role in the suppression of the early phase of adipogenesis by elevating the de novo production of antiadipogenic PGF2α.

4. Suppression of the Early Phase of Adipogenesis by PGE2

PGE2 is also known to suppress adipogenesis through suppression of PPARγ function. PGE2 and an EP4 agonist, AE1-329, increase the intracellular cAMP levels in
preamipocytes in a dose-dependent manner [42]. Moreover, AE1-329 decreases the expression of adiopogenic genes such as PPARγ and C/EBPα [51]. The inhibitory effect of PGE₂, but not that of Fluprostanol, is reversed by the addition of an EP4 antagonist, AE3-208 [42], indicating that PGE₂ suppresses adipogenesis through the EP4 receptor. Although the functions of PGE₂ and the expression of the functions of PGF₂α and the expression of PGEs have been investigated in adipocytes [27, 52, 53], the PGE₂-producing enzyme in adipocytes has never been identified. To date, three major PGESs have been identified [54, 55]. Microsomal PGES-1 (mPGES-1) is a member of the membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) protein family [56] and produces PGE₂ in response to various stimuli [57]. Microsomal PGES-2 (mPGES-2) has also been identified and its expression is high in the heart and brain [58]. Cytosolic PGES (cPGES) is constitutively and ubiquitously expressed in various cells [59].

PGE₂ production is detected in preadipocytes and increases during the early phase of adipogenesis with a peak at 3 h after the initiation of adipogenesis; and mPGES-1 is expressed in these cells, with its mRNA and protein levels being consistently detected during adipogenesis. Finally, we found that mPGES-1 is responsible for the production of PGE₂ in adipocytes [60]. This result is consistent with results showing that treatment of mouse embryonic fibroblast (MEF) cells with PGE₂ for the first two days of adipocyte differentiation is enough to suppress adipocyte differentiation, with reduced expression of the PPARγ gene and reduced accumulation of intracellular lipids [43].

In wild-type mouse MEF cells, inhibition of endogenous PG synthesis by indomethacin enhances adipocyte differentiation, and this enhancement is reversed by the addition of PGE₂. In MEF cells prepared from EP4 receptor gene-knock-out mice, adipocyte differentiation is elevated, and no more enhancement of adipocyte differentiation is observed following treatment with indomethacin. Thus, PGE₂-EP4 receptor signaling suppresses the early phase of adipocyte differentiation in MEF cells [43].

5. Synergistic Suppression of Early Phase of Adipogenesis by PGF₂α and PGE₂

Both PGF₂α and PGE₂ suppress the early phase of adipogenesis, and so we investigated the synergistic regulation of these PGs in 3T3-L1 cells. The increased production of PGF₂α and PGE₂ in the early phase of adipogenesis is a consequence of the elevated expression of the COX-2 gene [61]. PGF₂α forms a positive feedback loop that coordinate suppresses the early phase of adipogenesis through the increased production of antiadipogenic PGF₂α and PGE₂, both of which inhibit PPARγ function. In addition, PGE₂ also enhances the production of PGF₂α and itself through the elevation of the expression of the COX-2 gene in an EP4 receptor-mediated fashion. Moreover, when the cells are caused to differentiate into adipocytes in medium containing both PGF₂α and PGE₂, the expression of the adiopogenic genes is decreased to a greater extent than when the cells are cultured in a medium containing either of them. Thus, PGE₂ and PGF₂α synergistically suppress the early phase of adipogenesis through a self-amplifying loop, triggered by PGF₂α-EP receptor and PGE₂-EP4 receptor couplings and activation of the COX-2 gene expression in 3T3-L1 cells [61].

However, Inazumi et al. demonstrated that the differentiation of MEF cells prepared from EP receptor gene-knock-out mice is almost the same as that in these cells from wild-type mice and still shows sensitivity to indomethacin, indicating that FP receptor-mediated suppression is not directly associated with the regulation of adipocyte differentiation in MEF cells [43]. Therefore, the regulation of suppression of adipogenesis by PGE₂ and PGF₂α might occur in a cell-type-dependent manner.

6. Acceleration of Adipocyte Differentiation by PGD₂ and Its Metabolites

PGD₂ acts as an allergic and inflammatory mediators and is produced in a variety of cells such as mast cells, macrophages, and adipose cells [62, 63]. PGD₂ is produced from PGH₂ by the action of PGD synthases (PGDSs), enzymes that catalyze the isomerization of the 9,11-endoperoxide group of PGH₂ to PGD₂. Two distinct types of PGDSs have been identified. One is hematopoietic PGDS (H-PGDS), which is abundantly expressed in mast cells and Th2 cells [64]. The other is L-PGDS, which is detected abundantly in the brain, male genital organs, and heart [62, 63].

PGD₂ has been considered a candidate for a molecule that acts as an endogenous inducer of adipogenesis, basically because 15d-PGJ₂, one of its metabolites, has been identified as a ligand for PPARγ and activates adipogenesis in vitro [20, 21]. PGD₂ is nonenzymatically metabolized to PGs of the J series, that is, PGJ₂, Δ¹²-PGJ₂, and 15d-PGJ₂. However, the concentrations of 15d-PGJ₂ required for the activation of PPARγ reported in most of the literature are much higher (2.5–100 μmol/L) than those of conventional PGs (pmol/L range); and 15d-PGJ₂ is present in vivo at a low level that is insufficient for activation of adipocyte differentiation [65], whose finding is consistent with our current results indicating that 15d-PGJ₂ is not detectable in adipocytes [60]. Recently, we identified Δ¹²-PGJ₂ as being the dominant PGD₂ metabolite in differentiated adipocytes [60], in good agreement with recent results showing that Δ¹²-PGJ₂ is produced in adipocytes and activates the expression of adiopogenic genes in 3T3-L1 cells [66].

PGD₂ is synthesized by the action of L-PGDS in adipocytes [67]. However, another PGDS, H-PGDS may not be involved in the production of PGD₂ in adipocytes, because the expression level of H-PGDS is very low during adipogenesis. Although the function of PGD₂ or L-PGDS in vitro has been extensively investigated, the in vivo function is still controversial. Ragolia et al. reported that adipose size is increased in L-PGDS gene-knock-out mice under normal and high-fat diet feeding. Moreover, L-PGDS gene-knock-out mice become glucose intolerant and insulinresistant. Also the serum adiponectin level is decreased in such
 Activation of Adipogenesis in Adipose-Precursor Cells by PGI₂

PGI₂ activates the protein kinase A (PKA) pathway by binding to its IP receptor and enhances the differentiation of adipose precursor cells [72, 73]. The activation of IP receptors upregulates the expression of C/EBPβ and C/EBPδ, both of which are critical for the progression of the early phase of adipogenesis and directly activate the expression of the PPARγ and C/EBPα genes for maturation of adipocytes [9, 10]. Moreover, IP receptor gene-knock-out mice fed a high-fat diet do not show any changes in body weight, fat mass, or adipose size [74, 75]. Therefore, PGI₂ activates the progression of adipogenesis in the adipose precursor cells through the enhancement of the expression C/EBPβ and C/EBPδ via the cAMP-PKA pathway.

8. Conclusion

PGs are involved in the regulation of adipogenesis and act as modulators of PPARγ functions. The regulation of adipogenesis by PGs is very complex, because PGs regulate adipogenesis both positively and negatively. In the early phase of adipogenesis, PGF₂α and PGE₂ suppress the progression of adipogenesis, and their receptor-mediated mechanisms leading to suppressed PPARγ function have been well elucidated. In contrast, PGD₂ and its metabolites activate the middle-late phase of adipogenesis (Figure 2). In addition, recently we found that PGD₂ and its metabolite Δ₁₂-PGJ₂ accelerate adipogenesis by acting through DP2 (CRTH2; chemoattractant receptor homologous molecule of Th2 cells) receptors and PPARγ, thus, indicating that when elucidating the function of a given PG, the roles of not only it but also those of its metabolites should be considered.

All PGs function through their specific G protein-coupled receptors and PPARγ. Although their receptor agonists and antagonists are functional in the cultured adipocytes (in vitro), in vivo studies do not show clear effects of PGs in the regulation of obesity. Moreover, PG receptor gene-knock-out mice are not affected like the cells observed in in vitro studies. The explanation of the problems is quite difficult. As PGs have a variety of physiological functions, studies using gene-knock-out mice might not be appropriate to elucidate the functions of PGs in obesity. The precise in vivo functions of PGs especially those of 15d-PGJ₂ required further clarification. Tissue- (cell-)specific gene-knock-out
mice might be a powerful tool to identify the in vivo function of PGs. Understanding of the mechanisms of PG-mediated regulation of adipogenesis may lead to a novel therapeutic strategy for the treatment of obesity.

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