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Peroxisome proliferator-activated receptor γ (PPARγ) ligands as bifunctional regulators of cell proliferation

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Abstract

Peroxisome proliferator-activated receptor γ (PPARγ), a member of the ligand-activated nuclear receptor superfamily, plays a key role in mediating differentiation of adipocytes and regulating fat metabolism. PPARγ has been implicated in the pathophysiology of atherosclerosis, inflammation, obesity, diabetes, immune response, and ageing. Recently, it has been shown that activation of PPARγ by J_2 series cyclopentenone prostaglandins (cyPGs), especially 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) or synthetic agents, such as antidiabetic thiazolidinediones, causes anti-proliferation, apoptosis, differentiation, and anti-inflammation of certain types of cancer cells. The anti-proliferative effects of PPARγ activators are associated with *de novo* synthesis of proteins involved in regulating the cell cycle and cell survival/death. Anti-inflammatory effects of 15d-PGJ_2 are associated with interruption of nuclear factor-κB and subsequent blockade of inflammatory gene expression. Furthermore, 15d-PGJ_2 at nontoxic doses induce expression of phase II detoxification or stress-responding enzymes, which may confer cellular resistance or adaptation to oxidative stress. The presence of a reactive α,β-unsaturated carbonyl moiety in the cyclopentenone ring of 15d-PGJ_2 is important for part of biological functions this cyPG has. Recently, attention has been focused on the anti-proliferative activity of nonsteroidal anti-inflammatory drugs (NSAIDs) in cancerous or transformed cells, which is mediated through interaction with PPARγ irrespective of their ability to inhibit COX-2. Despite the fact that abnormally elevated COX-2 is associated with resistance to cell death, induction of apoptosis by certain NSAIDs is accompanied by up-regulation of COX-2 expression. This commentary focuses on dual effects of the typical PPARγ agonist 15d-PGJ_2 on cell proliferation and growth, and its possible involvement in the NSAID-induced COX-2 expression and apoptosis.

Keywords: Apoptosis; Cyclopentenone prostaglandins; 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2; Peroxisome proliferator-activated receptor γ; Cyclooxygenase-2; Nonsteroidal anti-inflammatory drugs

1. Introduction

PGs are involved in mediating or regulating many important physiological processes, including cell division, blood flow control, immune responses, ovulation, bone development, wound healing, and water balance. According to both epidemiologic and experimental studies, PGs play roles in the pathophysiology of carcinogenesis as well as in the inflammatory process [1,2]. Abnormally elevated levels of certain PGs have been often observed in various types of human cancers [1]. PGs, in general, have been shown to stimulate cell proliferation. However, data from recent studies indicate that J_2 series cyPGs, especially those acting as PPARγ ligands, have not only anti-inflammatory and cytoprotective activities, but also pro-apoptotic and anti-proliferative properties in certain types of cells [3]. PPARγ has been implicated in the pathophysiology of atherosclerosis, inflammation, obesity, diabetes, immune response, and ageing [4,5]. In this context, considerable interest has been focused on PPARγ agonists and activators as potential therapeutic agents. This commentary summarizes the biological

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Abbreviations: ARE/EpRE, antioxidant/electrophile responsive elements; AP-1, activator protein 1; COX, cyclooxygenase; cyPGs, cyclopentenone prostaglandins; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; ERK, extracellular signal-regulated kinase; GCL, glutamate-cysteine ligase; GSH, reduced glutathione; GR, GST reductase; HSP, heat-shock protein; HO-1, heme oxygenase-1; IKK, IκB kinase; NF-κB, nuclear factor-κB; MAPKs, mitogen-activated protein kinases; Nrf2, NF-E2 related factor 2; NSAIDs, nonsteroidal anti-inflammatory drugs; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; PGs, prostaglandins; PI3-kinase, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; RXR, retinoid X receptor.

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functions of endogenous and synthetic PPARγ ligands and their involvement in regulating cell proliferation and death.

2. cyPGs: formation and their role as *bona fide* endogenous PPARγ ligands

The first step in PG synthesis is hydrolysis of membrane phospholipids to produce free arachidonate catalyzed by phospholipase A_2. In the next step, COX converts arachidonic acid to PGG_2 and subsequently PGH_2, which is further metabolized to the biologically active prostanoids, including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TXA_2) by specific isomerases (Fig. 1).

The PGs have a diverse range of physiological functions depending on their types and target cells by interacting with specific receptors such as EPs for PGE_2, FP for $PGF_{2\alpha}$, DP for PGD_2, TP for TXA_2, IP for PGI_2, etc. Each receptor is linked to a distinct signal transduction pathway, mediating activation or inhibition of cellular responses.

Of the aforementioned PGs, PGD_2 readily undergoes dehydration to yield biologically active J_2 series PGs, such as PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2 (structures shown in Fig. 2). Members of the J_2 series PGs are characterized by the presence of a reactive α,β-unsaturated carbonyl group in the cyclopentenone ring, and have a unique spectrum of biological effects, including antitumor activity, inhibition of cell cycle progression, suppression of viral replication, and stimulation of osteogenesis [3].

Certain cyPGs have been known to activate the PPARs that are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. There are

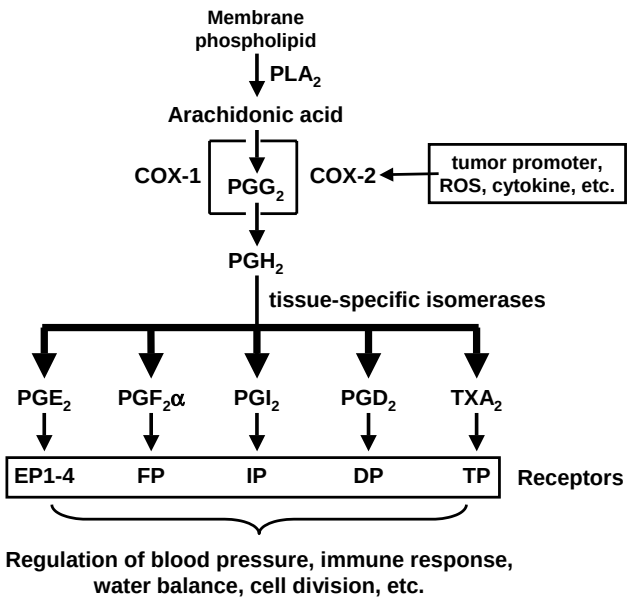

Fig. 1. General pathway for synthesis of PGs. Arachidonic acid is formed by the action of phospholipase A_2 (PLA_2) on membrane phospholipids, and is converted by COX to PGH_2 via PGG_2. PGH_2 is further metabolized to various PGs by specific isomerases. Each PG has its own coupled receptor on the cell surface.

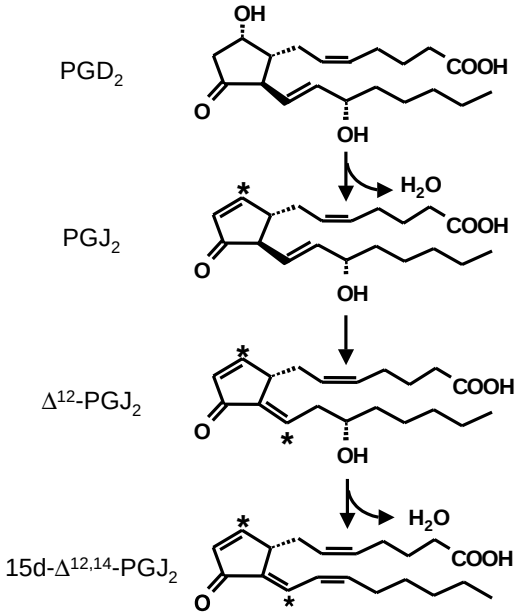

Fig. 2. Formation of cyPGs from the cyclopentane PGD_2 via dehydration. Asterisks (*) indicate the positions of chemically reactive electrophilic carbon atoms.

three isoforms of PPAR named α, β/δ, and γ which play central roles in regulation of fatty acid oxidation and glucose utilization [3,6]. Among these PPAR isoforms, PPARγ is preferentially expressed in adipose tissues and is involved in regulating fat metabolism and adipocyte differentiation [5,6]. Consistent with its potential role in lipogenesis, PPARγ has been implicated in atherosclerosis and obesity as well as inflammation [4]. Some synthetic PPARγ ligands (e.g. thiazolidinediones) have a therapeutic value for the treatment of metabolic disorders, such as hyperlipidaemia, atherosclerosis, diabetes, and obesity [4]. 15d-PGJ_2, one of the most well-defined cyPGs, has been recognized as a natural ligand with high affinity for PPARγ [3,4]. PPARγ is expressed in several human malignancies, such as breast [7], prostate [8], lung [9], gastric [10], colon [11], pancreatic [12], and hematologic [13] tumors. According to recent reports, synthetic PPARγ ligands as well as 15d-PGJ_2 can induce antiproliferation, apoptosis, and terminal differentiation of several types of cancerous and transformed cells, which may provide a promising therapeutic or preventive strategy for the management of certain types of human cancer [3,14].

3. Dual effects of PPARγ ligands in regulating cell growth

3.1. Antiproliferative or pro-apoptotic activity

3.1.1. Inhibition of tumor cell growth

There has been substantial accumulation of experimental data supporting that synthetic PPARγ ligands as well as 15d-PGJ_2 induce apoptosis in several types of cancer cells

[10,12,15–25]. Moreover, PPARγ ligands significantly inhibited growth of the transplantable tumors derived from breast and colon cancer cells in mice [18,26]. 15d-PGJ_2 attenuated the capability of the human breast cancer cell line (MDA-MB231) to induce tumors in nude mice [15]. Dietary administration of a synthetic PPARγ ligand GW7845 for 2 months significantly reduced the incidence, the multiplicity and the weight of mammary tumors in rats induced by nitrosomethylurea [27]. Furthermore, PPARγ agonists caused redifferentiation of human glioma cells as indicated by outgrowth of long processes and expression of the redifferentiation marker N-cadherin [20]. PPARγ agonists induced growth arrest and differentiation in CX-1 colonic tumor cells injected in Swiss nude mice [26]. Troglitazone, a synthetic PPARγ ligand, induced terminal adipocytic differentiation in patients with advanced liposarcoma [28].

3.1.2. Possible involvement of ROS

It has been known that antiproliferative and growth inhibitory effects of PPARγ ligands can be achieved through either PPARγ-dependent or independent mechanisms. Li *et al.* [17] have shown that 15d-PGJ_2-induced apoptosis of human myofibroblasts is unrelated to PPARγ, because PPARγ is not expressed in these cells. According to this study, 15d-PGJ_2 caused rapid production of ROS while inducing apoptosis. The pro-apoptotic effects of 15d-PGJ_2 were reproduced by H_2O_2 and blocked by antioxidants, such as *N*-acetyl-L-cysteine and pyrrolidine dithiocarbamate [17]. Likewise, cytotoxic effects of J_2 series cyPGs in the human neuroblastoma SH-SY5Y cell line were associated with intracellular production of ROS and redox alteration reflected by depletion of antioxidant defense molecules, such as GSH and GSH peroxidase and increased production of lipid peroxidation products (e.g. 4-hydroxy-2-nonenal and acrolein) [29]. The biochemical basis for the production of ROS by cyPGs remains to be clarified. Recently, cyPGs potentiated the inflammatory response to TNF-α through production of ROS and activation of ERK1/2 [30]. 15d-PGJ_2 caused transient activation of MAPKs and apoptosis signal-regulating kinase-1 (ASK1), which was prevented by *N*-acetyl-L-cysteine pretreatment in cultured primary astrocytes [31]. Moreover, a synthetic PPARγ agonist rosiglitazone failed to activate MAPKs and ASK1, whereas another thiazolidinedione ciglitazone activated them. These findings indicate that activation of MAPKs by 15d-PGJ_2 is mediated through PPARγ-independent mechanism which may involve ROS [31]. However, modest amounts of ROS can trigger the expression of antioxidative stress-responding enzymes by activating certain cellular signaling kinases, and this issue will be discussed later (see Section 3.2.4).

3.1.3. Genes involved in the PPARγ ligand-induced cell death

PPARγ activator-induced apoptosis was accompanied by caspase activation [16,23,24]. 15d-PGJ_2-induced apoptosis of hepatic myofibroblasts was blunted by the pan-caspase inhibitor *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (ZVAD-fmk) [17]. The caspase inhibition also led to abrogation of 15d-PGJ_2-mediated apoptosis in other cell types including human pancreatic cancer [12], MDA-MB-231 human mammary cancer [16], HT-29 colon cancer [22], JEG3 choriocarcinoma [23], and neuroblastoma SH-SY5Y [19,25] cells.

The anti-proliferative or pro-apoptotic properties of PPARγ activators are associated with their capability to regulate the expression of a wide array of genes involved in controlling the cell cycle and cell survival/death. Among the genes that can be induced/activated by PPARγ ligands, those involved in mediating death of malignant cells are of particular interest in consideration of their potential as therapeutic targets for the management of human malignancies. 15d-PGJ_2-mediated apoptosis in MDA-MB-231 human mammary cancer cells requires expression of genes which are critical to cell cycle arrest and apoptosis [16]. These include genes encoding the cyclin-dependent kinase inhibitor $p21^{waf/cip1}$, Bag-1, caspase-3, -4, and -8, AP-1 and c-Jun. 15d-PGJ_2 inhibited c-*myb* and cyclin D2 expression in the human leukemic HL-60 cell line, which led to induction of differentiation, inhibition of proliferation and induction of apoptosis [32]. Likewise, 15d-PGJ_2 and the synthetic PPARγ agonist ciglitazone inhibited growth of human pancreatic cancer cells, which appeared to be related to decrease in cyclin D1 expression and concomitant induction of $p21^{waf1}$ and $p27^{kip1}$ [24]. Interestingly, invasiveness of pancreatic cancer cells was significantly suppressed by 15d-PGJ_2, which was associated with reduced expression and activity of matrix metalloproteinase-2 and -9 [24]. Troglitazone-induced cell cycle arrest in human hepatoma cells was accompanied by induction of cell cycle-regulatory proteins, p21, p27, and $p18^{INK4c}$ [33]. In addition, both endogenous and synthetic PPARγ agonists induced apoptosis by regulating expression levels of the Bcl-2 family member proteins such as Bax and Bcl-2 [18,20,34,35]. Kondo *et al.* [19] reported that 15d-PGJ_2 caused elevated accumulation and phosphorylation of p53, which was accompanied by a preferential redistribution of p53 protein in the nuclei and a concomitant increase in its DNA binding activity. Moreover, the ectopic expression of the p53 antisense oligonucleotide blocked the apoptotic cell death, lending further support to the role of p53 in mediating 15d-PGJ_2-induced neuroblastoma cell death [19]. Baek *et al.* [36] have shown that troglitazone-induced apoptosis is accompanied by the elevated expression of early growth response-1 transcription factor (Egr-1) which has been linked to apoptosis, and that inhibition of ERK activation abolishes the Egr-1 induction by troglitazone. Egr-1 induction is a unique property of troglitazone compared with other PPARγ ligands and is independent of PPARγ activation [36]. Taken together, above findings indicate that apoptosis induced by PPARγ ligands involves *de novo* synthesis of distinct sets of genes encoding proteins that act as regulators and effectors of apoptotic cell death.

3.1.4. Intracellular events responsible for growth inhibitory effects of PPARγ ligands

Signaling pathways mediating the modulation of a milieu of genes by PPARγ ligands are poorly understood. PPARγ ligands, including the antidiabetic thiazolidinediones, certain fatty acids and metabolites of arachidonic acid, regulate gene expression by binding as heterodimers to RXR through specific PPRE in the promoter regions of target genes [6]. In the absence of a ligand, high affinity complexes are formed between the PPAR–RXR heterodimer and nuclear receptor corepressor proteins such as nuclear receptor corepressor (N-CoR) or silencing mediator for retinoid and thyroid hormone receptors (SMART), suppressing its interaction with DNA and coactivators including steroid receptor coactivator-1 (SRC-1), CREB-binding protein (CBP) and the CBP homologue p300 [6,14]. Upon ligand binding, the corepressor is displaced, and the receptor then associates with a coactivator molecule. The resulting complex binds to PPRE in the promoters of relevant target genes. The assembly of this complex on the promoter results in either activation or suppression of a specific gene. While troglitazone reversibly inhibited clonal growth of MCF7 cells, combination of this synthetic PPARγ activator with all-*trans*-retinoic acid synergistically and irreversibly inhibited growth and induced apoptosis, which was associated with a dramatic decrease in the Bcl-2 protein level [18].

Recently, Patel *et al.* [37] have provided compelling data demonstrating that the selective PPARγ ligand rosiglitazone up-regulates PTEN tumor suppressor gene in human colorectal carcinoma (Caco2) and mammary cancer (MCF7) cells. The up-regulation of PTEN expression correlated with suppression of proliferation in these cells. PPARγ ligand-mediated up-regulation of PTEN accompanied the reduced activity of PI3-kinase [37] or its downstream enzyme Akt [38]. In another study, treatment of HT-29 human colon cancer cells with wortmannin, an inhibitor of PI3-kinase potentiated the cell death induced by troglitazone [22], suggesting that growth inhibitory effects of PPARγ ligands are at least in part mediated through repression of the PI3-kinase pathway by PTEN. It can be hypothesized that activated PPARγ binds to the PTEN promoter, thereby increasing the transcription of the gene encoding PTEN. This possibility is schematically addressed in Fig. 3.

3.2. Proliferative, anti-inflammatory, and cytoprotective effects

3.2.1. Stimulation of tumor cell growth

Although the majority of the published studies imply the inhibitory effects of PPARγ ligands on tumor growth, there are some reports on the opposite effect of PPARγ ligands on the development of tumors. It was demonstrated that activation of PPARγ by troglitazone increased the frequency and the size of colon tumors in C57BL/6J-APC^{Min}/+ mice [39,40], a clinically relevant model for both human adenomatous polyposis and sporadic colon cancer. Chinery *et al.* [41] showed that PGJ_2 and 15d-PGJ_2 induced proliferation of COX-2-depleted colorectal cancer (HCA-7) cells at a nanomolar concentration. However, cell proliferation as determined by [^{3}H]thymidine incorporation was significantly reduced by these cyPGs at micromolar concentrations [41]. Therefore, the proliferative/antiproliferative effects of PPARγ ligands are likely to be dependent on their intracellular concentrations. It should be noted that PGs formed endogenously at submicromolar concentrations are biologically more relevant in terms of regulating cell proliferation. Hence, the roles of PPARγ in colon carcinogenesis are controversial.

The precise mechanisms responsible for differential effects (i.e. proapoptotic vs. proliferative) of PPARγ ligands remain incompletely clarified. Clay *et al.* [15] have pointed out that modest activation of PPARγ results in enhancement of cellular proliferation and tumorigenesis, whereas vigorous activation might induce differentiation and apoptosis, thereby inhibiting tumor growth. Whether PPARγ ligands induce apoptosis or stimulate cell proliferation may depend on the direction of propagation of intracellular signals involved, which reflects the concentration-dependent effect of PPARγ ligands on PPARγ activation. However, many other studies indicate that PPARγ ligands and agonists induce apoptosis without activating the receptor.

3.2.2. Anti-inflammatory effects

PPARγ ligands exert anti-inflammatory effects by blocking activation of NF-κB and AP-1 capable of binding to specific sequences in promoter element of the inflammatory response genes, thereby activating their transcriptional activity. In resting cells, NF-κB is sequestered in the cytoplasm by association with an inhibitory protein IκB. In response to signaling by proinflammatory cytokines, IKK is activated and phosphorylates IκB at specific serine residues. IκB is then ubiquitinated and degraded by the proteasomes, which allows functionally active NF-κB to migrate into the nucleus, regulating target gene expression.

It has been reported that 15d-PGJ_2 strongly inhibits NF-κB-dependent transcription by two distinct mechanisms [42,43]. First, 15d-PGJ_2 can interrupt NF-κB-dependent gene expression through covalent modifications of critical cysteine residues in IKK with subsequent prevention of IκB degradation and nuclear entry of NF-κB [42,43]. In this respect, the reactive α,β-unsaturated carbonyl group in the cyclopentenone ring is critical for IKK inactivation [43]. 15d-PGJ_2 bearing the α,β-unsaturated carbonyl moiety can act as a Michael acceptor, and hence reacts with cellular thiols [44]. In line with this notion, 15d-PGJ_2 exerted a strong inhibitory effect on IKK activity and IκB degradation in a HeLa cell line with low GSH *S*-transferase activity, whereas it was less effective in a HeLa derivative having high GSH *S*-transferase

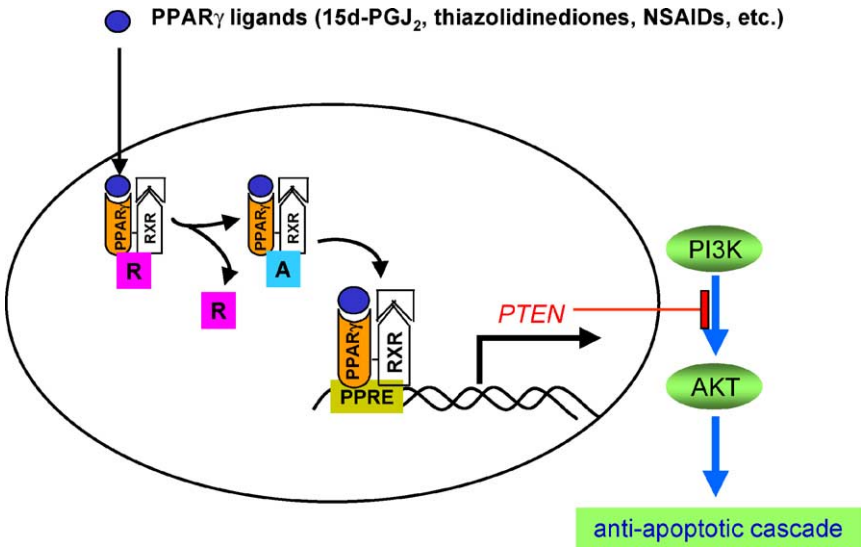

Fig. 3. A proposed mechanism of PPARγ ligands-mediated apoptosis. PPARγ forms a heterodimer with RXR, and ligation of both receptors is important to maximize the signaling. Upon binding to PPARγ ligands, the corepressor (R) is displaced, the ligand–receptor complex then associates with coactivator molecules (A), and binds to the PPRE located in the promoters of relevant target genes such as tumor suppressor PTEN. Induction of expression of PTEN may inhibit the anti-apoptotic PI3-kinase pathway.

activity [42]. The second mechanism by which 15d-PGJ_2 interferes with NF-κB activation involves direct inhibition of binding of NF-κB to target DNA without blocking IκB degradation and nuclear translocation of NF-κB. This is more likely to be achieved by alkylation of a conserved cysteine residue located in the DNA binding domain of Rel proteins (e.g. C38 in p65 and C62 in p50) [42]. Therefore, 15d-PGJ_2 may act as a negative feedback regulator of pro-inflammatory enzymes/molecules through NF-κB inactivation (Fig. 4).

15d-PGJ_2 suppressed the lipopolysaccharide (LPS)- and interleukin-1β (IL-1β)-induced expression of COX-2 and inducible nitric oxide synthase (iNOS) by interfering with the NF-κB signaling pathway [45,46]. In an animal model of *Helicobacter pylori*-induced gastritis, the *H. pylori*-derived LPS elicited gastric mucosal proinflammatory responses which were accompanied by massive epithelial cell apoptosis, up-regulation of COX-2 and iNOS [47]. Administration of the specific PPARγ ligand ciglitazone ameliorated the severity of inflammatory damage in gastric

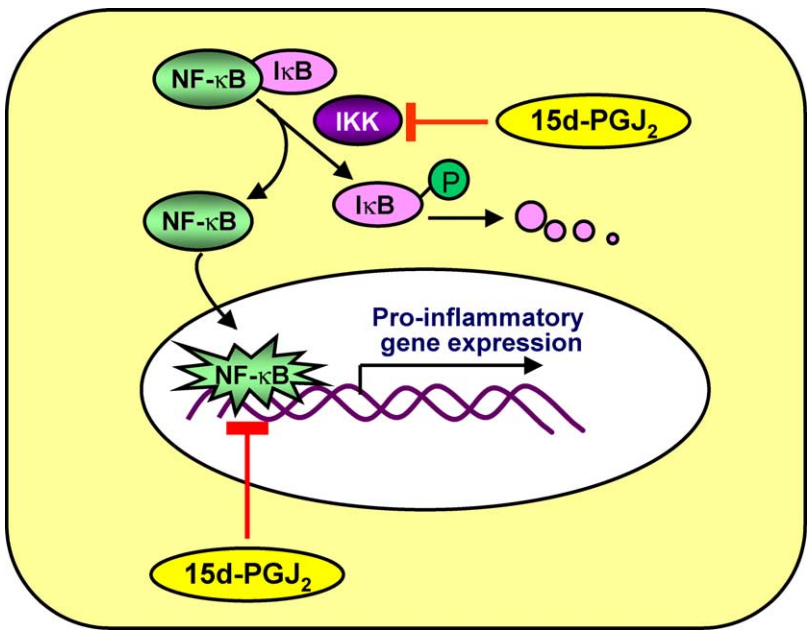

Fig. 4. Proposed mechanism responsible for the anti-inflammatory action of 15d-PGJ_2. 15d-PGJ_2 inhibits NF-κB-dependent gene expression either by functional inactivation of IKK, thereby preventing IκB degradation and nuclear entry of NF-κB, or direct interference with binding of NF-κB to a target DNA sequence. The α,β-unsaturated carbonyl group of 15d-PGJ_2 may cause the thiol modification in IKK and possibly the p50/p65 subunit of NF-κB, leading to suppression of NF-κB activation.

mucosa in a dose-dependent manner [47]. 15d-PGJ_2 also attenuated the ability of *H. pylori*-induced apoptosis in gastric epithelial cells by inhibiting the NF-κB activation [48].

Besides inactivating NF-κB, PPARγ ligands inhibit AP-1-mediated transcriptional activation of COX-2 through abrogation of c-Jun expression and competition with limiting amounts of coactivator CBP required for COX-2 expression by AP-1 [49]. Alternatively, ligands that activate PPARγ can cause anti-inflammatory responses by antagonizing PI3-kinase-mediated signaling cascades via up-regulation of PTEN in macrophages [37]. This leads to apoptosis of proinflammatory cells.

3.2.3. Cytoprotective effects

15d-PGJ_2 has cytoprotective as well as anti-inflammatory effects. It has been shown that 15d-PGJ_2 and related cyPGs act as general inhibitors of inflammatory responses by inducing the antioxidant enzyme HO-1 in activated microglial cells [50,51] and murine macrophages [52]. Besides up-regulation of HO-1, 15d-PGJ_2 induces the expression of genes involved in the heat shock response, including HSP70, HSP28, and HSP40 and those responsible for the redox regulation including GCL, thioredoxin reductase, GSH peroxidase, GR and GSH *S*-transferase [19,53,54]. It is likely that induction of above redox-regulating or stress-responding enzymes and/or potentiation of the cellular antioxidant capacity will help the cells acquire resistance to oxidative stress and build increased tolerance to additional insult.

Levonen *et al.* [53] have shown that 15d-PGJ_2 at low micromolar concentrations causes a robust increase in cellular GSH through transcriptional up-regulation of GCL, a rate-limiting enzyme of GSH synthesis as well as GR which confers resistance against oxidative death in human endothelial cells. Our preliminary studies also show that 15d-PGJ_2 at nontoxic doses (<5 μM) rescues rat pheochromocytoma (PC12) cells from oxidative or nitrosative stress.[1]

In contrast, a higher concentration (10 μM) of 15d-PGJ_2 induces endothelial cell apoptosis, which was aggravated by depletion of cellular GSH with buthionine sulfoximine pretreatment [53]. The proapoptotic and cytoprotective/antiapoptotic effects of 15d-PGJ_2 are independent of PPARγ, and dependent upon the dose and the type of target cells. It would be worthwhile elucidating the molecular mechanisms underlying differential effects of PPARγ ligands on cell survival/death.

3.2.4. Nrf2 as a transcriptional regulator of stress-responding gene expression

One of the important cellular signaling components responsible for mediating antioxidative stress-responding enzyme induction by 15d-PGJ_2 is the ARE/EpRE. Nrf2, a basic-leucine zipper transcription factor, has been reported to positively regulate the ARE/EpRE-mediated expression of phase II detoxification or cellular defensive enzyme genes in response to ROS and electrophiles [55–57]. Cells from Nrf2 knock-out mice are deficient in their ability to induce antioxidative stress genes, and are hence highly susceptible to oxidative stress [56,58]. Under normal physiological conditions, Nrf2 is sequestered by cytoskeleton-associated cytoplasmic 'Kelch-like ECH-associated protein 1 (Keap1)' which hampers the nuclear translocation of Nrf2 [59]. Upon stimulation by ROS or electrophiles, Nrf2 dissociates from its cytoplasmic docking protein Keap1 with subsequent nuclear translocation and transactivation of ARE/EpRE.

The actual mechanism of dissociation of Nrf2 from Keap1 is currently unknown, but it is thought to involve thiol modification or phosphorylation of Keap1, Nrf2, or both [59]. The thiol modification of Keap1 may result in dissociation of Nrf2 from Keap1. cyPGs such as 15d-PGJ_2 contain the α,β-unsaturated carbonyl moiety and readily undergo nucleophilic addition with thiols. Therefore, it is plausible that the critical cysteine residues of Keap1 can be modified through covalent interaction with cyPGs acting as a Michael acceptor, thereby activating Nrf2 as illustrated in Fig. 5. Alternatively, 15d-PGJ_2-induced oxidative stress activates several signal molecules such as MAPKs, protein kinase C or PI3-kinase, and these kinases cause release of Nrf2 from Keap1-Nrf2 complex, thereby allowing activated Nrf2 to translocate into the nucleus where it forms a heterodimer with small Maf protein [57]. The Nrf2–Maf complex through binding to a specific site of ARE/EpRE, regulates ARE/EpRE-driven gene expression [60]. The functional ARE/EpRE has been found in the promoter region of both GCL and GR [53,61], two of the most important enzymes involved in maintaining the cellular GSH level. Besides ARE/EpRE, other *cis*-acting elements may also play roles in transcriptional regulation of GCL. For instance, a catalytic subunit of rat GCL has consensus binding sites for NF-κB and AP-1 in its 5′-flanking region [62].

HO-1, a key enzyme in heme catabolism, is another prominent cellular defensive protein whose expression is regulated through Nrf2 binding to ARE/EpRE [58,63]. The enzyme is induced drastically in response to a variety of stresses including heavy metals, heat shock and UV irradiation. The enzyme is also up-regulated by cyPGs such as Δ^{12}-PGJ_2 [64] or 15d-PGJ_2 [50,51,65–67]. It is noteworthy that human neuroblastoma SH-SY5Y cells treated with 10 μM 15d-PGJ_2 displayed more than 70-fold increase in HO-1 mRNA as measured by an oligonucleotide microarray analysis [19]. 15d-PGJ_2 may promote inactivation of Nrf2-Keap1 complex by covalent thiol modification in Keap1, leading to increased Nrf2 binding to the ARE/EpRE-site in the HO-1 promoter (Fig. 5).

[1] S.-Y. Lim and Y.-J. Surh, unpublished observation.

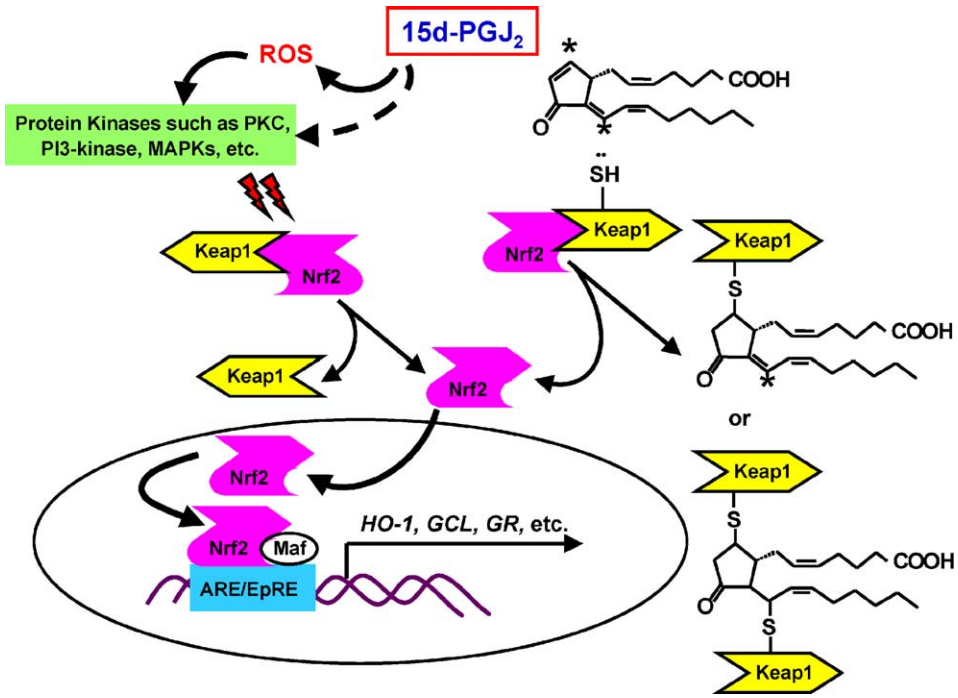

Fig. 5. A hypothetical mechanism responsible for induction of antioxidative stress-responding enzymes by 15d-PGJ_2. 15d-PGJ_2 activates several signal molecules such as MAPKs, protein kinase C or PI3-K via ROS generation or by unknown mechanisms. The activated kinases in turn phosphorylate and subsequently dissociate of Nrf2 from Keap1-Nrf2 complex, leading to translocation of Nrf2 to nucleus where it forms a heterodimer with small Maf protein. The Nrf2–Maf complex, through binding to a specific site of ARE/EpRE, regulates ARE/EpRE-driven gene expression. Alternatively, a critical cysteine residue of Keap1 can be modified through covalent modification by 15d-PGJ_2 that acts as a Michael acceptor, thereby activating Nrf2. Note that 15d-PGJ_2 has two electrophilic carbon centers as marked with asterisk: one in the cyclopentenone ring and the other in the side chain. Both electrophilic carbons in the corresponding α,β-unsaturated carbonyl group can participate in nucleophilic addition reactions with two thiols to form *bis* conjugate [54], but the predominant thiol conjugation is anticipated to occur in the electrophilic carbon in the ring [3,86,87].

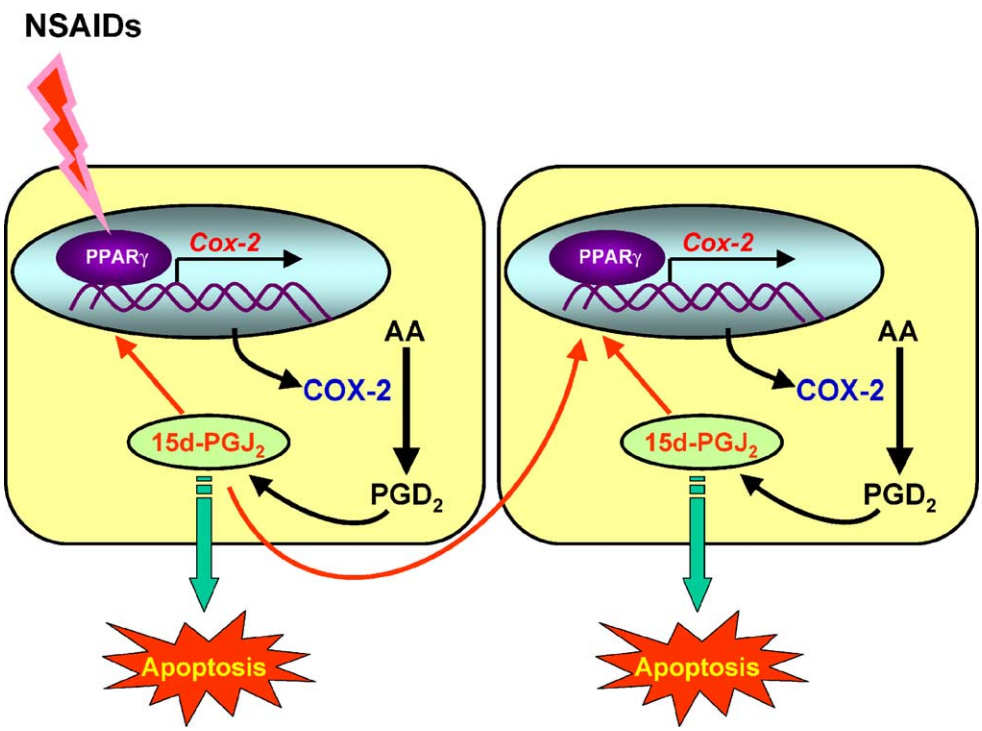

Fig. 6. Proposed pathways mediating the pro-apoptotic effect of NSAIDs in association with their COX-2 induction. NSAIDs may induce COX-2 expression through activation of PPARγ. The resulting accumulation of PGs, especially 15d-PGJ_2 could cause apoptosis in a paracrine and/or autocrine manner. AA, arachidonic acid.

4. NSAIDs as novel PPARγ agonists: implications for chemoprevention

COX catalyzes the first step in the formation of PGs from arachidonic acid (Fig. 1). Two isoforms of COX are known to exist: COX-1 and COX-2. In contrast to COX-1, COX-2 is barely detectable under normal physiological conditions, but is rapidly and transiently induced by proinflammatory cytokines, growth factors, ultraviolet and tumor promoters [68–70]. A substantial body of data suggests that COX-2 overexpression provides tumor cells with growth and survival advantages, including resistance to apoptosis and increased invasiveness or angiogenesis [71–73]. Moreover, many epidemiologic and clinical studies have revealed that the regular ingestion of NSAIDs, such as aspirin and sulindac, can reduce the risk of developing several malignant tumors including colorectal cancer [74–76]. Therefore, targeted inhibition of COX-2 is regarded as an effective and promising strategy for cancer prevention or treatment.

Pharmacological action of NSAIDs has been attributed to their inhibition of COX activity, particularly COX-2. However, a large number of studies have demonstrated that anti-carcinogenic efficacy of NSAIDs is independent of their inhibition of COX-2. Moreover, NSAIDs have opposing effects on COX-2 expression; they can inhibit cytokine-induced COX-2 expression, while NSAIDs alone can upregulate COX-2. Lehmann *et al.* [77] have reported that NSAIDs bind to and therby activate PPAR isoforms α and γ. Specifically, indomethacin, fenoprofen, flufenamic acid and ibuprofen act as PPARγ agonists. These NSAIDs induced COX-2 expression through the PPRE site [78,79].

Recent studies by Nikitakis *et al.* [80] have demonstrated that sulindac induces elevation of the protein and mRNA levels of both COX-2 and PPARγ, while inducing apoptosis in human oral squamous cell carcinoma cells. Antisense-mediated disruption of PPARγ expression abolished the growth inhibitory effect of sulindac, suggesting that induction of PPARγ expression and its activation is partially responsible for the antiproliferative effect of this NSAID [80]. Yamazaki *et al.* [81] showed that effects of different NSAIDs on proliferation and apoptosis in rheumatoid synovial cells were not associated with the activities and specificities of these drugs towards COX isozymes but correlated with their ability to stimulate the activation of PPARγ. Furthermore, the COX-2 selective NSAID NS-398 induced both apoptosis and COX-2 expression in colorectal carcinoma cells, which were mediated by sustained activation of the ERK pathway [82]. Another study has revealed that COX-2 plays a central role in growth inhibition of human hepatic myofibroblasts [83], which raised a question as to whether COX-2-derived PGs can induce apoptosis in these cells. Gilroy and Colville-Nash [84] demonstrated that induction of COX-2 was paradoxically associated with the onset of wound healing in carrageenan-induced pleurisy in rats. The authors considered a prostanoid product of COX-2 as one of the most likely candidates responsible for the anti-inflammatory effects. According to this report, COX-2 activation results in the production of the anti-inflammatory cyPGs, particularly 15d-PGJ_2, which may mediate the resolution phase of inflammatory damage [84].

Based on the findings reported in the literature, it is conceivable that inhibition of COX-2 does not necessarily contribute to the antiproliferative activities of NSAIDs. On the contrary, induction of COX-2 may confer pro-apoptotic and anti-inflammatory properties under certain conditions. However, the molecular mechanism underlying COX-2 induction during NSAIDs-mediated apoptosis remains to be elucidated. We have recently reported that up-regulation of COX-2 accompanies apoptotic death of the *ras*-transformed human mammary cells treated with the anti-cancer drug ET-18-O-CH_3 [85]. We speculate that NSAIDs induce COX-2 expression through activation of PPARγ, and the resulting accumulation of PGs, especially 15d-PGJ_2, in turn, could cause apoptosis which is mediated in a PPARγ-dependent or independent manner (Fig. 6). In this context, induction of COX-2 expression by NSAIDs may form a positive feedback loop of PPARγ activation in a paracrine and/or autocrine manner.

5. Conclusion

PPARγ has been implicated in the pathophysiology of diverse human disorders including atherosclerosis, inflammation, obesity, diabetes, immune response, and ageing. Recently, it has been shown that activation of PPARγ by J_2 series cyPGs, such as 15d-PGJ_2, which are endogenous ligands, causes anti-proliferation, apoptosis, differentiation, and anti-inflammation of certain types of cancer cells. However, 15d-PGJ_2 and structurally related cyPGs at nontoxic doses can induce expression of antioxidative stress-responding enzymes which is preferentially regulated by the basic-leucine zipper transcription factor Nrf2. The reactive α,β-unsaturated carbonyl moiety present in these cyPGs is important for the activation of this transcription factor through thiol modification of its inhibitory protein at critical cysteine residues.

There are multiple lines of compelling evidence indicating that COX-2 and its products are implicated in malignant transformation. With this knowledge, NSAIDs, especially the COX-2 selective inhibitors, have an emerging utility for the prevention and treatment of human cancer as well as for the management of inflammatory disorders. The antineoplastic effects of NSAIDs have traditionally been attributed to their ability to inhibit COX-2 activity without influencing expression of the enzyme. Numerous attempts have been made to elucidate the cellular and molecular basis of the tumoricidal activity of NSAIDs, but the specific molecules that NSAIDs target in exerting anticarcinogenic activity have not been identi-

fied yet. Recently, growing attention has been paid to the possible role of NSAIDs as potential PPARγ ligands in exerting the antiproliferative activity in transformed or cancerous cells, irrespective of their COX-2 inhibition. Moreover, induction of COX-2 expression may provide anti-inflammatory properties. A COX-2-derived PG, especially 15d-PGJ_2, is recognized as a possible mediator that links up-regulation of COX-2 expression by NSAIDs and their anti-proliferative effects. It is not determined yet whether the induction of COX-2 expression is causally linked to anticarcinogenic activities of NSAIDs. Molecular mechanisms underlying regulation of COX-2 expression by NSAIDs in relation to their regulation of cell proliferation merit further investigation.

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