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15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 as a potential endogenous regulator of redox-sensitive transcription factors

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Abbreviations:
AP-1, activator protein-1
ARE/EpRE, antioxidant/electrophile response element
COX, cyclooxygenase
cyPGs, cyclopentenone prostaglandins
15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2
γ-GCL, γ-glutamate cysteine ligase
GSTs, glutathione S-transferases
HIF, hypoxia inducible factor
HO-1, heme oxygenase-1
HRE, hypoxia response element
iNOS, inducible nitric oxide synthase
Keap1, Kelch-like ECH-associated protein 1

ABSTRACT

15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) has been known to display multifaceted cellular functions, including anti-inflammatory and cytoprotective effects. However, depending on the concentrations and intracellular microenvironment, this cyclopentenone prostaglandin can exert opposite effects. Because of the α,β-unsaturated carbonyl moiety present in its cyclopentenone ring structure, 15d-PGJ_2 can act as a Michael reaction acceptor and readily interacts with critical cellular nucleophiles, such as cysteine thiol groups in proteins. Many of the biological effects induced by 15d-PGJ_2 involve redox-transcription factors as the potential targets. Thus, 15d-PGJ_2 can modulate the transcriptional activities of nuclear factor-κB (NF-κB), activator protein-1 (AP-1), nuclear factor-erythroid 2p45 (NF-E2)-related factors (Nrf2), hypoxia inducible factor (HIF), etc. 15d-PGJ_2 is also well known as an endogenous ligand of peroxisome proliferator-activated receptor γ (PPARγ). However, the regulation of the aforementioned redox-sensitive transcription factors by 15d-PGJ_2 is not necessarily mediated via PPARγ activation, but rather involves covalent modification or oxidation of their critical cysteine residues acting as a redox-sensor. This commentary describes the biological and physiological functions of 15d-PGJ_2 and underlying biochemical and molecular mechanisms with emphasis on the modulation of redox-sensitive transcription factors and their regulators.

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MAPKs, mitogen-activated protein kinases
NF-κB, nuclear factor-κB
NQO1, NAD(P)H: quinone oxidoreductase 1
Nrf2, nuclear factor-erythroid 2p45 (NF-E2)-related factors
PPARγ, peroxisome proliferator-activated receptor γ
PPRE, PPAR response elements
RXR, receptor for 9 cis-retinoid
STAT, signal transducer and activator of transcription
TPA, 12-O-tetradecanoylphorbol-13-acetate

1. Introduction

Prostaglandins (PGs) are a family of biologically active autacoids synthesized from 20 carbon-containing polyunsaturated fatty acids, principally arachidonic acid generated from membrane phospholipids [1], and exert a vast variety of physiological functions [2]. Members of the J_2 series cyclopentenone PGs (cyPGs), characterized by the presence of an electrophilic carbonyl moiety in their cyclopentenone ring, have a unique spectrum of biological effects. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), one of the most-well defined cyPGs, functions as an endogenous ligand of peroxisome proliferator-activated receptor γ (PPARγ) and has not only anti-inflammatory and cytoprotective activities, but also pro-apoptotic and anti-proliferative properties depending on cell types and concentrations [2]. 15d-PGJ_2 is a dehydration derivative of PGD_2, and its synthesis initially depends upon the enzymatic machinery for PGD_2 generation [3]. Due to its electrophilic α,β-unsaturated carbonyl group in the cyclopentenone ring, 15d-PGJ_2 can form covalent adducts with cysteine thiols via Michael addition [4]. This may result in the alteration of cellular redox status and/or functions of target proteins, many of which play pivotal roles in fine-tuning of cellular signaling network.

A wide array of intracellular signal transduction cascades converge with distinct sets of transcription factors. Abnormal activation or improper silencing of transcription factors is implicated in many disorders, such as cancer [5]. Pro-oxidants and electrophiles can modulate redox-sensitive transcription factors, such as peroxisome proliferator-activated receptor (PPAR), nuclear factor-κB (NF-κB), activator protein-1 (AP-1), p53 and nuclear factor-erythroid 2p45 (NF-E2)-related factors (Nrf2). It is conceivable that 15d-PGJ_2 with both electrophilic and pro-oxidant properties can directly or indirectly interact with the aforementioned redox-sensitive transcription factors, thereby modulating their transcriptional activities. The purpose of this review is to summarize recent findings on the cellular functions of 15d-PGJ_2, particularly those exerted by targeting redox-sensitive transcription factors or their modulators.

2. Formation and chemical properties of 15d-PGJ_2

The first step in PG synthesis is the release of arachidonic acid from membrane phospholipids by phospholipase A_2. Arachidonic acid is then converted by cyclooxygenase (COX; also known as PGH synthase) to PGH_2. This unstable intermediate is converted enzymatically to a series of biologically active prostanoids, including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2, each of which has its own specific receptor. Among these, PGD_2 spontaneously undergoes chemical dehydration to form PGJ_2. PGJ_2 can undergo further dehydration by loss of the 15-hydroxyl group, which, coupled with migration of the 13,14-double bond, results in the formation of 15d-PGJ_2 (Fig. 1). These reactions are promoted by albumin but proceed at a relatively slow rate compared to the very rapid formation of PGs from PGH_2 by prostanoid synthases [4]. Recently, Brummond et al. [6] reported the total synthesis of 15d-PGJ_2 by utilizing an allenic Pauson-Khand-type reaction.

15d-PGJ_2 has multifaceted biological properties that are uniquely different from other components of the PG family. These include anti-neoplastic, anti-inflammatory, and anti-viral activities that are likely to be mediated by interaction with cellular signaling molecules, such as transcription factors [2]. Due to its characteristic α,β-unsaturated carbonyl moiety, 15d-PGJ_2 can readily react with cellular nucleophiles, such as cysteinyl thiol groups of proteins. Such reactions are termed Michael addition reactions and may occur in one or both of two electrophilic centers of 15d-PGJ_2 (Fig. 1; box). Several studies have shown that 15d-PGJ_2 has the most potent biological activity among cyPGs [7–9]. Kondo et al. [7] reported that the intracellular production of reactive oxygen species (ROS) was strongly induced by 15d-PGJ_2 in neuroblastoma cells. In addition, the reduced glutathione (GSH) levels and GSH peroxidase activity were significantly lowered by treatment with 15d-PGJ_2. Likewise, 15d-PGJ_2 has been reported to be the most potent inducer of endothelial apoptosis, which is attributed to its electrophilic cyclopentenone moiety [8]. Moreover, 15d-PGJ_2 induced the expression of heme oxygenase-1 (HO-1) to a greater extent than did PGA_2 and a simple

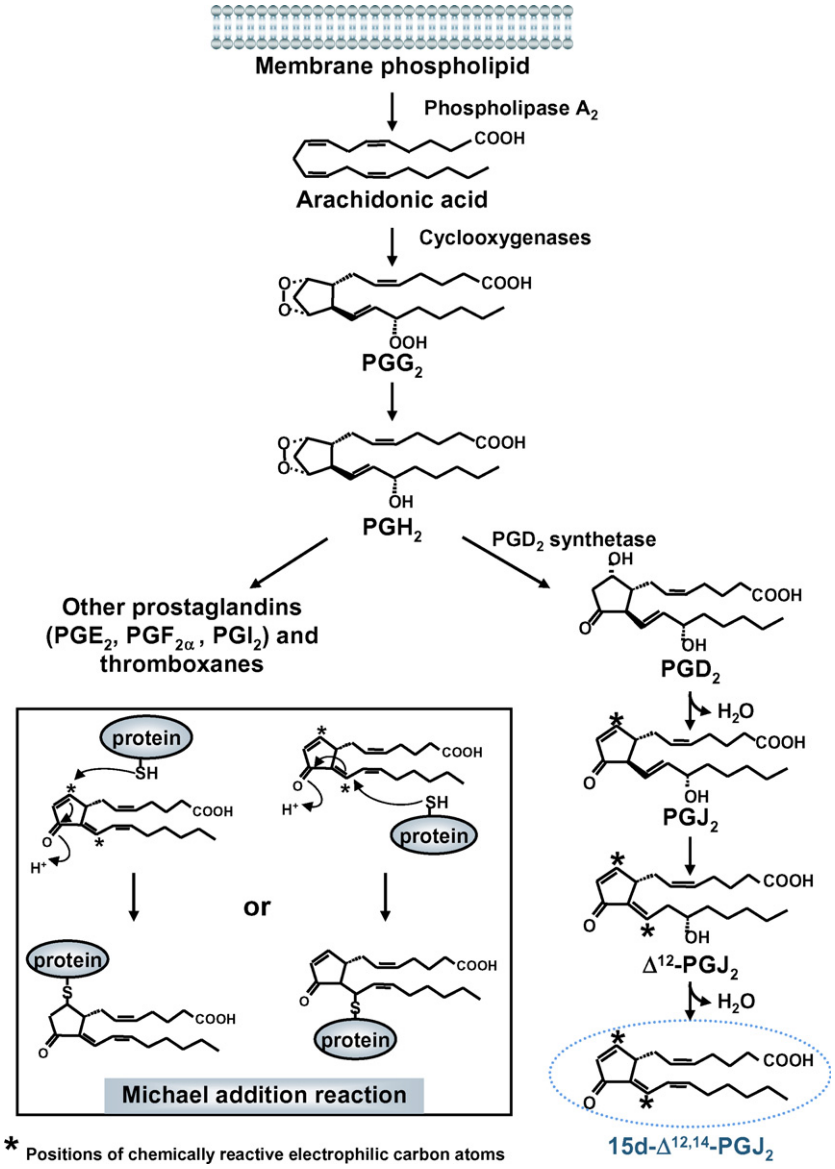

Fig. 1 – Formation of 15d-PGJ_2. Phospholipase A_2 catalyzes hydrolytic release of arachodonic acid (AA) from membrane phospholipids. Cyclooxygenases catalyze oxidative conversion of AA to prostaglandin H_2 (PGH_2). PGD_2, a precursor of 15d-PGJ_2, is formed by the action of PGD_2 synthase. Alternatively, other prostaglandins (PGE_2, $PGF_{2\alpha}$, PGI_2), and thromboxanes are formed. PGD_2 undergoes chemical dehydration to form the PGJ_2. PGJ_2 then undergoes further dehydration by loss of the 15-hydroxyl group, which, coupled with migration of the 13,14-double bond of PGJ_2, results in the formation of 15d-PGJ_2. Some of the effects of 15d-PGJ_2 on intracellular proteins are mediated by Michael addition reaction attributed to the reactive α,β-unsaturated carbonyl groups (box). Asterisks (*) indicate the positions of the chemically reactive or electrophilic carbon center.

cyclopentenone in human breast cancer cells [9], which was again largely due to its α,β-unsaturated ketone moiety. Such unique chemical structure of 15d-PGJ_2 enables this cyPG to bind to a broad spectrum of cellular proteins involved in intracellular signaling network. Multiple lines of evidence indicate that 15d-PGJ_2 modifies some critical cellular molecules including NF-κB [10,11] and AP-1 [12], IκB kinase (IKK) [13,14], thioredoxin [15], thioredoxin reductase [16], Kelch-like ECH-associated protein 1 (Keap1) [17,18], H-Ras [19], PPARγ [20,21], and cytoskeleton [22]. In particular, Stamatakis et al. [22] identified several proteins as potential targets of 15d-PGJ_2 in mesangial cells, such as heat-shock protein 90, nucleoside diphosphate kinase, cytoskeleton including actin, tubulin, vimentin, and tropomyosin. It is noteworthy that the majority of proteins identified as targets of 15d-PGJ_2 possess cysteine thiol residues that are susceptible to oxidative or covalent modification.

3. Biological/physiological functions of 15d-PGJ_2

15d-PGJ_2 has therapeutic/preventive potential against arthritis [23–29], ischemia–reperfusion injury [30–33], inflammatory

bowel disease [34,35], and Alzheimer's disease [36,37] in which inflammation plays an important pathophysiologic role. 15d-PGJ_2 exerts anti-inflammatory activities by several different mechanisms in aforementioned disorders. 15d-PGJ_2 inactivates transcription factors associated with inflammation, such as NF-κB, AP-1, and signal transducer and activator of transcription (STAT) [38], thereby down-regulating inducible pro-inflammatory genes (vide infra). Ricote et al. [38] demonstrated that 15d-PGJ_2 inhibited the expression of inducible nitric oxide synthase (iNOS), gelatinase B, and scavenger receptor A genes by blocking the activation of transcription factors AP-1, NF-κB, and STAT in a PPARγ-dependent manner in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated macropharges. The suppression of aforementioned inflammation-associated transcription factors by 15d-PGJ_2 appears to be mediated by modulating the upstream mitogen-activated protein kinases (MAPKs). In addition, 15d-PGJ_2 inhibited the production and secretion of pro-inflammatory mediators, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in TPA-treated monocytes [39] and IL-10 and IL-12 in lipopolysaccharide (LPS)-treated macrophages [40]. 15d-PGJ_2 down-regulates iNOS [33,41,42] and COX-2 [43–45], which are typical pro-inflammatory enzymes. However, Vichai et al. [46] showed the positive feedback regulation by 15d-PGJ_2 of COX-2 expression in mouse lung fibroblasts. Other studies also demonstrated the similar dual effect of 15d-PGJ_2 on COX-2 production [43]. The addition of 15d-PGJ_2 to human osteoarthritic chondrocytes reduced the IL-1β-induced COX-2 level, whereas in the absence of a COX-2 inducer, 15d-PGJ_2 up-regulated COX-2 expression without concomitant elevation of PGE_2 synthesis [43]. Although 15d-PGJ_2 is involved in the resolution of inflammation, its effects on the expression of COX-2 remain controversial.

15d-PGJ_2 has been reported to be a natural ligand for PPARγ. Some synthetic PPARγ agonists have been known to exert anti-diabetic effects through high-affinity binding to PPARγ [3]. However, the effect of 15d-PGJ_2 on the pathogenesis of diabetes is not fully understood. It has been reported that 15d-PGJ_2 potently induces adipogenesis, promotes differentiation of pre-adipocytes into mature, triglyceride-containing fat cells [47] and prevents precursors from being differentiated into osteoclasts [48]. Furthermore, 15d-PGJ_2 repressed leptin production and induced adipogenesis at concentrations capable of activating PPARγ [49]. Thus, 15d-PGJ_2 may play a pivotal role in controlling adipocyte development and glucose homeostasis (reviewed in [3]).

4. The redox-sensitive transcription factors as cellular targets of 15d-PGJ_2

It is becoming clear that 15d-PGJ_2 has the ability to modify multiple redox-sensitive transcription factors [50]. The docking studies in combination with comparative electrostatic potential analysis have revealed that 15d-PGJ_2 can covalently modify some cellular proteins such as p50, p65, p53, and c-Jun [51]. Table 1 lists the redox-sensitive transcription factors that are regulated by 15d-PGJ_2. 15d-PGJ_2 is also likely to generate ROS or to modulate the ROS sensitivity of the cell. Thus, addition of 15d-PGJ_2 to cells triggers a series of events dependent on the generation of ROS, which are prevented by addition of an antioxidant, such as *N*-acetyl-L-cysteine. Some transcription factors and related signaling molecules have cysteine residues that can serve as a redox sensor, and oxidation of such cysteine thiols may modulate their biological functions. Therefore, besides direct covalent modification, 15d-PGJ_2 may oxidize some critical residues of the redox-sensitive transcription factors and their regulators.

4.1. *PPARγ*

PPARs are key transcription factors that catalyze and coordinate distinct biochemical events regulated for maintaining lipid homeostasis, such as the differentiation of adipocytes, regulation of lipoprotein and lipid metabolism [52]. The PPAR family consists of three different subtypes, namely PPARα, PPARγ, and PPARβ/δ. Among these isoforms, PPARγ has been implicated in inflammation, immune response, and pathogenesis of some disorders including atherosclerosis, obesity, diabetes, Alzheimer's disease, cancer, etc. [4,52]. It was demonstrated for the first time in 1995 that 15d-PGJ_2 is an endogenous ligand for PPARγ [53,54]. 15d-PGJ_2 up-regulates the expression [27,31,36,55], transcriptional activity [25,45,56–61], and DNA binding activity [36,62] of PPARγ, and many of the cellular events mediated by 15d-PGJ_2 have been shown to be PPARγ-dependent [3,4,63]. Recently, the precise dynamic events that occur during ligand-binding and PPARγ-activation processes have been reported [20,21]. As a core structural moiety, the α,β-unsaturated carbonyl group of 15d-PGJ_2 is important in covalent binding of this cyPG to a cysteine residue (Cys285) in the PPARγ ligand binding domain (LBD). 15d-PGJ_2 binds PPARγ, releasing PPARγ from its corepressor. After 15d-PGJ_2 binding, PPARγ forms heterodimers with the receptor for 9 cis-retinoid (RXR) in the nucleus. PPARγ/RXR heterodimers interact with transcriptional coactivators and bind to sequence specific PPAR response elements (PPRE) located in target genes thereby stimulating their transcription (Fig. 2). The covalent modification of PPARγ by 15d-PGJ_2 was proposed to be a two-step reaction that employs a 'dock and lock' mechanism of ligand binding, in which 15d-PGJ_2 first approaches the ligand-binding pocket (dock), and then the covalent binding of the ligand occurs at a relatively low rate (lock) [21]. According to this supposition, the first (docking) step, transition from the free to the non-covalently bound form, may not be sufficient to activate PPARγ, but the second (locking) step, i.e., conversion from the non-covalently to the covalently bound form, appears to be critical for PPARγ-activation [21].

Shiraki et al. [20] have provided clear evidence that the structural moieties within a single molecule are used differently in PPARγ-dependent and -independent functions. In the case of 15d-PGJ_2, the electrophilic carbon (C9) within a cyclopentenone ring has been considered to react with the cysteine residue in NF-κB and other proteins [10,64]. On the other hand, the carbon at position 13 reacts preferentially with the sulfur atom of the cysteine residue in PPARγ [20]. Furthermore, the carboxyl group of 15d-PGJ_2 is required for the formation of a hydrogen bond with Tyr-473 in helix 12 of

Table 1 – Transcription factors modulated by 15d-PGJ$_2$

Redox-sensitive transcription factors	Mode of regulation	Consequences
PPARγ	• Covalent binding with cys285 in ligand binding domain [20,21] • ↑ PPARγ transcriptional activity [25,45,85,89,97,110–113] • ↑ PPARγ DNA binding activity [36,114] • ↑ PPARγ expression [27,31,36,100]	Anti-inflammation [25,28,45,89]; Cytoprotection [31]; Apoptosis [27,110,111,113]/Cell cycle arrest [36,112]; Anti-angiogenesis [114]; Induction of VEGF [97,100]
Nrf2/Keap1	• Covalent binding with a cysteine residue of Keap1 [71] • ↑ Nrf2 DNA binding activity [69] • ↑ Nuclear accumulation of Nrf2 [18,70,71]	Induction of antioxidant enzymes: HO-1 [18,69,71], peroxiredoxin I [18], γ-GCL [70], heat shock protein [69], NQO-1 [71], etc.
NF-κB	• ↓ NF-κB transcriptional activity [10,25,38,41,42] • ↓ NF-κB DNA binding activity [10,11,42,82,83] • Covalent binding with Cys179 of IKKβ [14], Cys62 of p50 [11], and Cys38 of p65 [10] • ↓ IκBα degradation, nuclear translocation of p65, and recruitment of p300 by p65 [13,42,82]	Anti-inflammation [10,11,13,38,41]; Inhibition of iNOS expression [13,41,42,83]; Inhibition of COX-2 expression [10,83]
AP-1	• ↓ AP-1 transcriptional activity [38] • ↓ AP-1 DNA binding activity [12,25,45,81–84] • ↓ JNK [81,85] • Covalent binding with Cys269 of c-Jun [12]	Anti-inflammation [25,38,81,82,84,85]; Inhibition of iNOS expression [82,83]; Inhibition of COX-2 expression [45,81–83]; Inhibition of eNOS expression [84]
STAT	• ↓ STAT1 transcriptional activity [38] • ↓ IFNγ-induced phosphorylation of STAT1 and STAT3 [88–90] • ↓ STAT1 DNA binding activity [88,89] • ↓ IL-6, IL-10-induced STAT3 phosphorylation [61,91]	Anti-inflammation [38,61,88–91]; Inhibition of iNOS expression [88,89]
p53	• ↑ p53 expression [27,57,93,94] • ↑ p53 nuclear accumulation [93,94] • ↑ p53 phosphorylation [93] • ↑ p53 DNA binding activity [93] • Covalent modification [16,95]	Apoptosis [27,57,93,94]
HIF-1	• ↓ HIF-1 activity [60] • ↓ HIF-1 transcriptional activity [16]	Inhibition of VEGF production [60]

PPARγ. 15d-PGJ$_2$ biotinylated at the carboxyl group failed to pull down PPARγ from cells, whereas it still bound to AP-1 and other proteins [12,64]. Thus, the mode of covalent binding of 15d-PGJ$_2$ to the PPARγ LBD appears to be different from that employed in PPARγ-independent actions.

4.2. *Nrf2*

Maintenance of the correct homeostatic redox status potential (i.e., the appropriate balance between oxidants and antioxidants) is essential for proper cellular functions but is perpetually threatened by extrinsic factors, such as increases in the levels of ROS during inflammation or exposure to xenobiotics that are metabolized to antioxidant-depleting electrophiles [65]. Genes encoding detoxifying/antioxidant enzymes have been known to be up-regulated by eletrophiles and ROS as part of adaptive cellular survival response. This coordinated response is regulated through a *cis*-acting element known as antioxidant/electrophile response element (ARE/EpRE) located in the promoter or enhancer region of many of the antioxidant genes. Nrf2 has emerged as the critical regulator of ARE/EpRE-dependent transcription. In the absence of oxidative stress signals, Nrf2 is sequestered in the cytoplasm because of its association with a cytoskeleton protein Keap1. Upon exposure of cells to oxidative stress or electrophiles, Nrf2 is released from its repressor protein Keap1 and translocates to the nucleus [66,67]. In the nucleus, Nrf2 interacts with ARE located in the promoter of Nrf2-responsive genes, such as HO-1, glutathione *S*-transferases (GSTs), NAD(P)H:quinone oxidoreductase 1 (NQO1), γ-glutamate cysteine ligase (γ-GCL), etc. (reviewed in [68]) and activates their transcription.

15d-PGJ$_2$ can activate Nrf2 by directly binding to Keap1 through covalent linkage, resulting in induction of some antioxidant proteins including HO-1 [69], peroxiredoxin I [18], γ-GCL [70] and heat shock protein 70 [69]. Particularly, cysteines of the linker region of Keap1 are essential for Keap1 binding of 15d-PGJ$_2$ [71]. 15d-PGJ$_2$-induced antioxidant gene expression is considered to be attributed to its characteristic α,β-unsaturated carbonyl moiety (Fig. 3). Recent studies from our laboratory have shown that 15d-PGJ$_2$ up-regulated HO-1 expression via the Nrf2-ARE signaling. Thus, 15d-PGJ$_2$ increased the levels of Nrf2 in the nucleus and its binding to AREs in MCF-7 cells. The elimination of the double bond present in the cyclopentenone ring of 15d-PGJ$_2$ abolishes its ability to activate Nrf2 and induce HO-1. These findings suggest that the modification (either covalent or oxidative) of

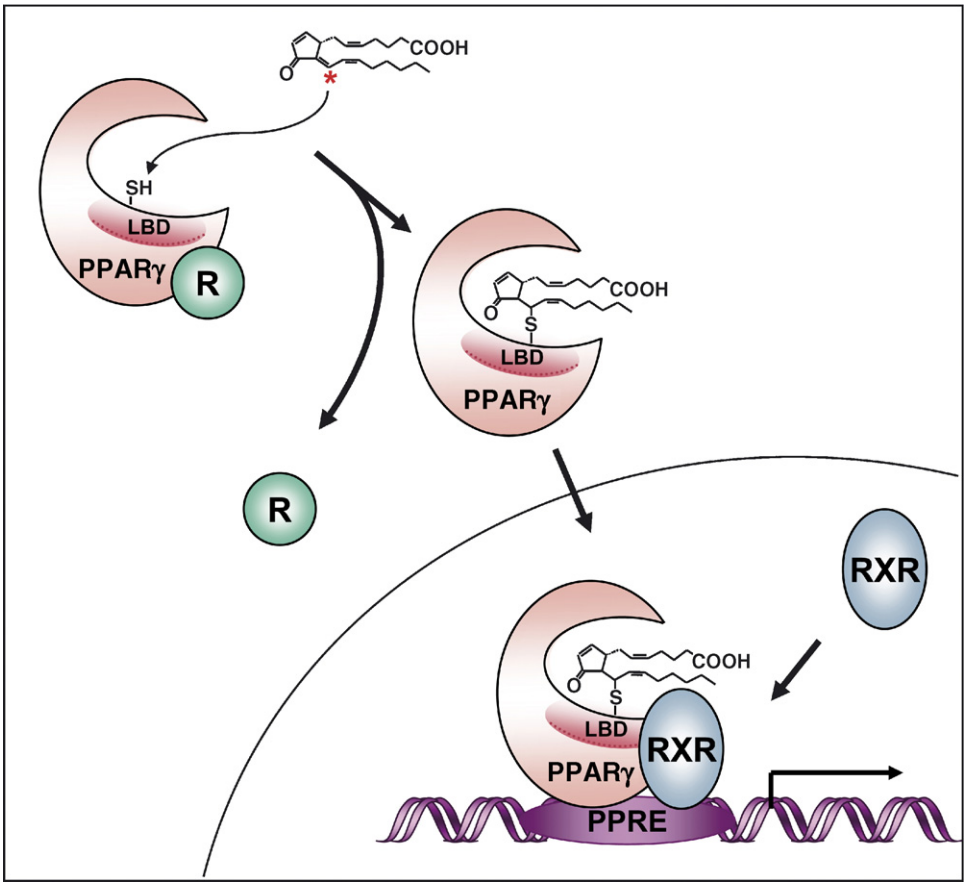

Fig. 2 – A proposed mechanism of 15d-PGJ$_2$-mediated activation of PPARγ. 15d-PGJ$_2$ binds PPARγ, releasing PPARγ from its corepressor (R). It is considered that the carbon at the position 13 (*) of 15d-PGJ$_2$ reacts with the Cys285 in ligand binding domain (LBD) of PPARγ. Once translocated to the nucleus, the 15d-PGJ$_2$-PPARγ complex forms a heterodimer with nuclear retinoid X receptor (RXR) to recognize PPAR-response elements (PPRE) in the promoter region of the target genes thereby stimulating their transcription.

Keap1 cysteine thiol by 15d-PGJ$_2$ is essential for translocation of Nrf2 to the nucleus.

4.3. *NF-κB*

The transcription factor NF-κB is one of the ubiquitous eukaryotic transcription factors that exerts pleiotropic effects via numerous intracellular signal transduction pathways involved in the induction of pro-inflammatory genes including iNOS, COX-2, adhesion molecules, and cytokines [72,73]. In many malignant tumors, constitutively elevated NF-κB activation is frequently observed, which is causally linked to enhanced proliferation, resistant to apoptosis, invasion, etc. (reviewed in [5]). Therefore, targeting abnormally elevated NF-κB activation in precancerous or malignant cells is considered to be an important strategy for cancer chemoprevention as well as therapy.

The five members of the mammalian NF-κB family, i.e., p65/RelA, RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2), exist in unstimulated cells as homo- or heterodimers. NF-κB proteins are characterized by the presence of a conserved 300-amino acid Rel homology domain located toward the N-terminus that is responsible for dimerization, interaction with IκB proteins, and binding to DNA [74]. The heterodimeric protein NF-κB remains sequestered in the cytoplasm as an inactive complex with its inhibitory counterpart IκB subunit, including IκBα, IκBβ, IκBγ, BCL-3, and IκBε. Upon oxidative stimulation, some of these IκB proteins are rapidly phosphorylated by IKKα and IKKβ and degraded via the ubiquitin-proteasome pathway [75,76]. The resulting free NF-κB dimers translocate to the nucleus and bind to specific consensus sequences of DNA [77].

Recently, several studies have demonstrated that 15d-PGJ$_2$ exerts a strong anti-inflammatory effect by attenuating the expression of pro-inflammatory mediators in activated monocytes/macrophages mainly through the inhibition of NF-κB-dependent transcription of inflammatory genes [10,11,13,41]. As a COX metabolite, 15d-PGJ$_2$ can act as a negative modulator of pro-inflammatory signaling by blocking the NF-κB activation pathway at multiple levels [38,41]. Several components of NF-κB can be covalently modified by 15d-PGJ$_2$ (Fig. 3). 15d-PGJ$_2$ inhibited phosphorylation of IκBα at the Ser32 residue [13], possibly by inactivating the IKK complex. The plausible mechanisms of IKK inhibition by 15d-PGJ$_2$ involve covalent modification of the critical cysteine residue (Cys179) in IKKβ [14]. In addition, 15d-PGJ$_2$ can inhibit DNA binding of NF-κB by

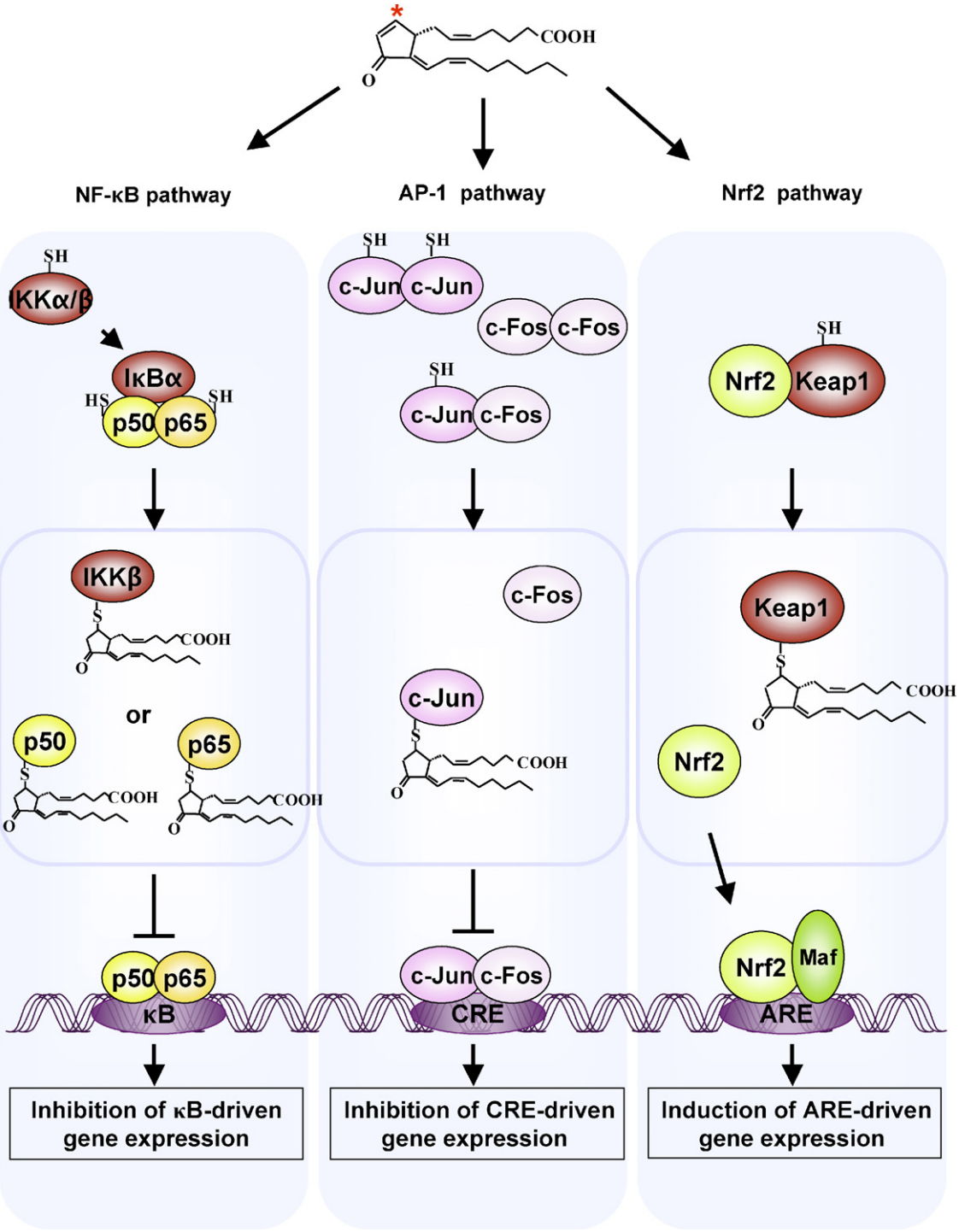

Fig. 3 – Proposed mechanisms responsible for the covalent modification by 15d-PGJ_2 of some signal-transducing cellular proteins. 15d-PGJ_2 binds specific cysteine residues in IKKβ (Cys179), IκBα (Ser32), p50 (Cys62), or p65 (Cys38) in the NF-κB signaling pathway, and c-Jun (Cys269) in the AP-1 pathway, respectively. These lead to functional inactivation of NF-κB or AP-1. Additionally, 15d-PGJ_2 can activate Nrf2 by direct binding to the cysteine residue of Keap1 that is the repressor protein of Nrf2, resulting in induction of some antioxidant enzymes. The modification of these proteins by 15d-PGJ_2 is caused preferentially by the electrophilic carbon of the α,β-unsaturated carbonyl group (*) present in the cyclopentenone ring.

direct modification of DNA-binding domains of the NF-κB subunits, i.e., Cys62 of p50 [11] or Cys38 of p65 [10]. Giri et al. [42] showed that 15d-PGJ_2 blunted NF-κB signaling by inhibiting degradation of IκB, nuclear translocation of p65, activation of PI3K-Akt, and recruitment of p300 by p65 in primary astrocytes. Maximal NF-κB transcriptional activity requires interaction with other components of transcriptional machinery, such as p300/CREB-binding protein (CBP) [78]. Phosphorylation of

p65 at Ser276 is critical for its interaction with p300/CBP, and mutation at Ser276 completely abolished the recruitment of p300/CBP [79]. Therefore, 15d-PGJ_2 may extend its anti-inflammatory activity by interfering with this pathway, thus impairing NF-κB-dependent transcriptional activity without affecting the nuclear translocation or DNA binding of NF-κB.

4.4. AP-1

AP-1 is another redox-regulated transcription factor that is involved in regulating a wide array of cellular functions [80]. AP-1 exists as either homo- or heterodimers consisting of the members of Jun (c-Jun, JunB and Jun D) and Fos (c-Fos, FosB, Fra-1 and Fra-2) families, which interact with each other via their basic leucine-zipper domain. To form the Jun-Fos-DNA complex, the leucine-zipper domain and the DNA binding domain containing clustered basic amino acids located upstream of leucine-zipper are important. Classical regulation of cellular AP-1 activity occurs via two mechanisms: one is an increase in the transcription of *c-fos* and *c-jun*, and the other is the phosphorylation of c-Fos and c-Jun proteins. AP-1 activity is also regulated by redox-dependent mechanisms (reviewed in [5]).

Besides NF-κB inactivation, 15d-PGJ_2 has been shown to disrupt AP-1 activation in several experimental systems. 15d-PGJ_2 inhibited AP-1 DNA binding activity in IL-1β-treated mesangial cells [81] and human chondrocytes [25,82] in a PPARγ-dependent [25] or independent fashion [81,82]. Simonin et al. [83] demonstrated that 15d-PGJ_2 inhibited LPS-induced DNA binding activity of AP-1 and NF-κB which was PPARγ-independent. Jozkowicz et al. [84] showed that 15d-PGJ_2 attenuated AP-1 DNA binding capacity via PPARγ-independent mechanisms. It has been reported that 15d-PGJ_2 inhibits AP-1 binding to the DNA by suppression of c-Jun NH_2-terminal kinase (JNK) [81,85]. In particular, *N*-acetyl-L-cysteine reversed the inhibition by 15d-PGJ_2 of AP-1 activity, suggesting that 15d-PGJ_2-mediated inactivation of AP-1 is attributable to its ability to induce oxidative stress [85]. The direct interaction of 15d-PGJ_2 with AP-1 proteins has been demonstrated by Perez-Sala et al. [12]. 15d-PGJ_2 covalently modifies Cys269 of c-Jun and directly inhibits the DNA binding activity of AP-1 (Fig. 3). In addition, 15d-PGJ_2 can promote the oligomerization of a fraction of c-Jun through the formation of intermolecular disulfide bonds or 15d-PGJ_2-bonded dimers [12], which may explain the nuclear accumulation of c-Jun by 15d-PGJ_2 [82]. The inhibition of AP-1 signaling by 15d-PGJ_2 may contribute to its anti-inflammatory and anti-proliferative properties as evidenced by down-regulation of AP-1-driven expression of pro-inflammatory enzymes, such as COX-2, iNOS, eNOS, vascular endothelial growth factor (VEGF), matrix metalloproteinases, etc. [25,81,82,84,85].

4.5. STAT

The mechanisms underlying the anti-inflammatory effects of 15d-PGJ_2 have been suggested to be mediated by antagonizing the activation of not only NF-κB and AP-1, but also STAT [38]. STATs are a family of latent cytoplasmic proteins activated by extracelluar signaling proteins (mainly cytokines, growth factors, and some peptides) that bind to specific cell-surface receptors [86,87]. 15d-PGJ_2 inhibits interferon γ (IFNγ)-induced STAT phosphorylation [88–90] and DNA binding [88,89] in macrophages and astrocytes through a PPARγ-independent mechanism. Interestingly, the inhibition of IFNγ-activated STAT signaling by 15d-PGJ_2 was mediated by ROS, as the process was ablated by *N*-acetyl-L-cysteine [89]. 15d-PGJ_2 also inhibits IL-6- and IL-10-induced STAT phosphorylation in several cell types [61,91].

4.6. p53

Pande and Ramos [51] suggested that p53 protein may also be a target for direct modification by 15d-PGJ_2 based on the computational study. The tumor suppressive function of p53 protein is mediated by several distinct mechanisms, including cell cycle arrest, apoptosis, and cellular senescence [92]. It has been reported that 15d-PGJ_2 induces apoptosis in neuroblastoma cells [93,94], endothelial cells [57], and chondrocytes [27] through up-regulation of expression [27,57,94] and nuclear accumulation [93,94] of p53. Fitzpatrick and coworkers showed that cyPGs, such as PGA_1 and PGA_2, inactivated p53 by covalently modifying and inhibiting thioredoxin reductase [16,95]. It is well known that p53 has a redox-regulated cysteine (Cys277) in its DNA-binding domain. Therefore, it is plausible that 15d-PGJ_2 can interact with this cysteine residue of p53 protein [96], thereby modulating its transcriptional activity.

4.7. HIF-1

Recently, 15d-PGJ_2 has been reported to induce expression of VEGF in macrophages [97,98], vascular smooth muscle cells (VSMC) [97,99], prostate cancer (PC3) cells [100] and endothelial cells [60]. Analysis of the VEGF promoter region revealed the presence of several potential binding sites for transcription factors including AP-1, AP-2, SP-1, and HIF-1 [101]. As a key transcription factor responsible for hypoxia-induced generation of VEGF [102], HIF-1 is induced in hypoxic cells and bound to the hypoxia response element (HRE). HIF-1 is a heterodimeric transcription factor composed of HIF-1α and HIF-1β subunits. In mammals, three genes have been shown to encode HIF-1α subunits that appear to be regulated in a similar manner (reviewed in [103]).

HIF-1 can also be activated by hypoxia-independent signaling pathways, redox-dependent regulation and protein S-nitrosylation. Recent studies have shown that reducing conditions stabilize HIF-1α, facilitate its DNA binding, and increase its phosphorylation even under normoxic conditions. In contrast, oxidizing conditions dampen the hypoxic response [104]. Nitric oxide (NO) activates HIF-1 activity in normoxia whereas it reduces HIF-1 activity in hypoxia. In normoxia, the DNA binding and transcriptional activities of HIF-1 are increased through S-nitrosylation that impairs HIF-1α ubiquitination and degradation [105]. This may occur by altering the interaction between von Hippel-Lindau tumor suppressor proteins (pVHL) and HIF-1α, for instance, through the S-nitrosylation of pVHL, or changing the proline hydroxylase activity that mediates the activation of HIF-1α. On the other hand, nitric oxide under hypoxic conditions decreases HIF-1 binding and activity through a cGMP-dependent mechanism, suggesting that the effect in hypoxia involves

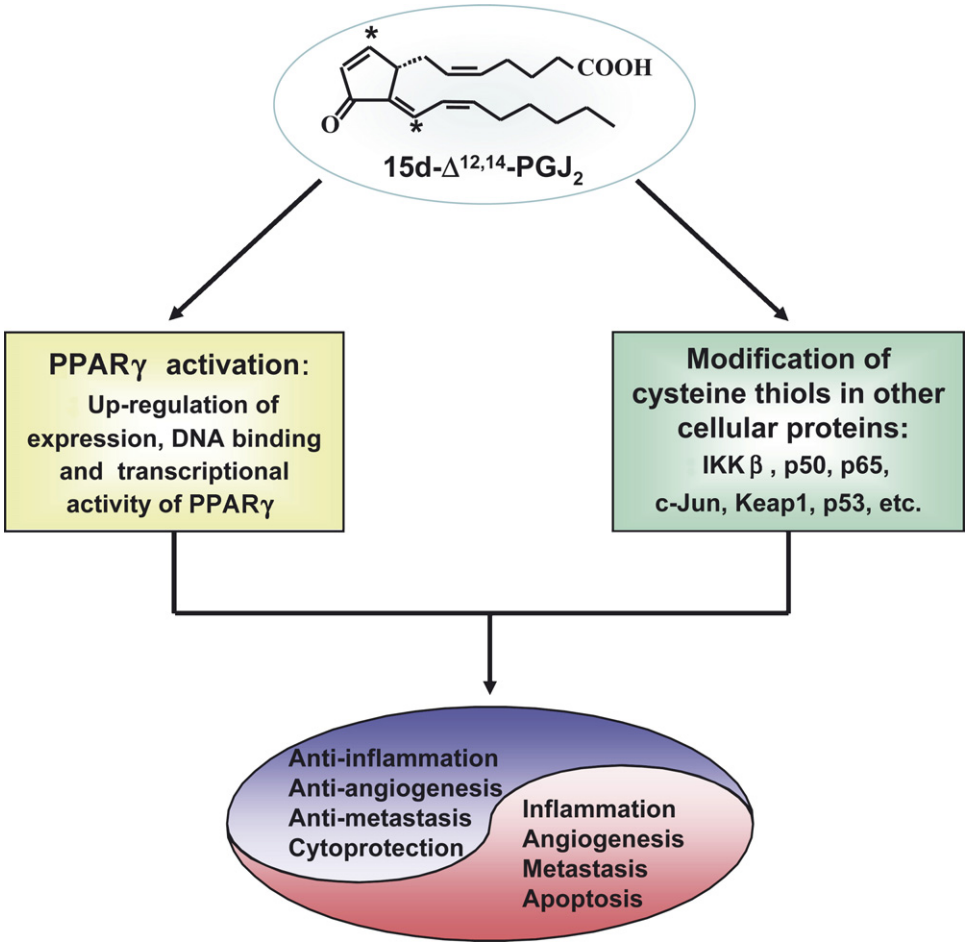

Fig. 4 – Intracellular effects of 15d-PGJ_2. Most of the cellular responses induced by 15d-PGJ_2 are mediated by modulation of redox-transcription factors or their regulators, especially at the critical cysteine thiols that often act as redox-sensors.

activation of guanylate cyclase. However, the reduction in HIF-1 activity attained with NO donors in hypoxia was not affected by a mutation of the cysteine contained within the oxygen-dependent degradation domain in HIF-1α [106].

Yasinska and Sumbayev [107] found that S-nitrosation of Cys800 residue in the HIF-1α transactivation domain by nitric oxide derived from donors and iNOS increases protein transcriptional activity. The increase of HIF-1 transcriptional activity was not observed when Cys800 was replaced with Ala [107]. Cys800 of HIF-1α is known to be critical for HIF-1 protein transactivation by enabling the interaction with CBP [108,109]. Jozkowicz et al. [60] have demonstrated that 15d-PGJ_2 inhibits HIF-1 activity in both normoxia and hypoxia. Although there is little experimental evidence, it is plausible that 15d-PGJ_2 regulates HIF-1α transcriptional activity either by covalent modification of Cys800 or indirectly oxidizing the same sulfhydryl group.

5. Conclusion

15d-PGJ_2, a typical J_2 family cyPG, is an endogenous activator of PPARγ (Fig. 4). Structurally, 15d-PGJ_2 possesses an electrophilic α,β-unsaturated carbonyl moiety in the cyclopentenone ring, which renders this molecule capable of forming Michael reaction adducts with critical cellular nucleophiles. Due to such structural characteristics, 15d-PGJ_2 can act as an electrophile and possibly as a pro-oxidant. Accumulating data demonstrate that 15d-PGJ_2 regulates not only PPARγ, but also other transcription factors such as NF-κB, AP-1, Nrf2, HIF, p53, and STAT. 15d-PGJ_2-mediated covalent or oxidative modification of cysteine thiols present in these redox-sensitive transcription factors or their regulators appears to be critical in many intracellular events exerted by this cyPG. Although there is little experimental evidence, it is plausible that 15d-PGJ_2 modulates CCAAT enhancer-binding protein (C/EBP) and cyclic AMP response element binding protein (CREB) as well. 15d-PGJ_2 has several opposite effects, depending on the concentrations, cell types, intracellular redox status, etc. Further studies will be necessary to define the biochemical functions of this cyPG and to clarify its exact target molecules under physiologic conditions.

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