3. The complex role of peroxisome proliferator-activated receptor gamma in osteoarthritis

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Abstract. Osteoarthritis (OA), a widespread chronic human health disorder, is the most common form of arthritis and a major cause of disability in developed countries. To date, treatment for OA has been largely palliative, as no treatment exists that can stop the disease from progressing. Although several potential therapeutic approaches have been tested, recent studies suggest that the activation of the transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) is a promising target. PPARγ, a member of the nuclear receptor superfamily, is a ligand-activated transcription factor. Its activation inhibits inflammatory and catabolic responses both in vitro and in vivo, and reduces the development and progression of cartilage lesions in OA animal models. This chapter summarizes recent findings on the effects of PPARγ activation on inflammatory and catabolic responses in chondrocytes and synovial fibroblasts. The role of PPARγ polymorphism in the pathogenesis of OA will also be discussed.

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Introduction

Osteoarthritis (OA) is the most frequent musculoskeletal disorder and the most widespread chronic form of arthritis. It affects approximately 15% of the population and 60% of those over the age of 60, affecting women more frequently than men. OA treatment has become a serious medical concern along with the aging world population. As incidences of the disease are expected to increase over the next few decades, the economic burden related to its medical and social costs is imposing. Improving treatment efficacy and the development of therapeutic strategies to stop the disease progression is becoming increasingly important.

Cartilage damage is a critical event in OA [1]. The articular cartilage is typically regarded as the primary diseased tissue, with increasing deterioration and insufficient tissue repair. During the initiation and progression of OA, chondrocytes, stimulated by the proinflammatory factors, of which interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) appear to be key cytokines, express cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and microsomal prostaglandin E synthase-1 (mPGES-1) as well as matrix metalloproteinases (MMPs), resulting in the degradation of the extracellular matrix components. Moreover, during the disease process, the expression of anabolic factors is substantially downregulated, causing increased cartilage damage.

Synovial inflammation has also been shown to occur in the early stages of OA and can be subclinical [2]. However, synovitis, which is evident at the clinical stage of the disease, can be the cause for a patient to consult a physician. It is the belief that synovitis is induced by the cartilage matrix degradation products that produce wear particles and soluble cartilage-specific neo-antigens, as well as other factors including microcrystals and abnormal mechanical stress. When released into the synovial fluid, these components are phagocytosed by synovial lining macrophages, perpetuating synovial membrane inflammation via the synthesis of mediators. These mediators, in turn, are diffused into the cartilage through the synovial fluid, creating a vicious circle in which cartilage increasingly disintegrates and causes further inflammation. The inflammatory mediators in the synovial membrane are synthesized by several cell types; however, in OA, data suggest the synovial fibroblasts to be the principal producers of catabolic factors.

There is no effective treatment for OA and new therapeutic approaches with the potential to stop or reduce the disease progression are needed. Among such potential targets, peroxisome proliferator-activated receptor gamma (PPARγ) is a promising one for the treatment of OA. This chapter
will describe the role of PPAR\(\gamma\) in the biology of chondrocytes and synovial fibroblasts as well as its complex role in the structural alterations of OA.

**PPAR\(\gamma\)**

PPAR\(\gamma\) receptors belong to the nuclear hormone receptor superfamily, which includes receptors for steroids, thyroid hormone, vitamin D, and retinoic acid. Three PPAR isoforms have been identified: PPAR\(\alpha\), PPAR\(\beta/\delta\) and PPAR\(\gamma\) [3]. PPAR\(\alpha\), primarily present in the liver, heart, and muscle, plays important roles in the catabolism of fatty acid [4]. PPAR\(\beta/\delta\) which is ubiquitously expressed, is implicated in various physiological processes, including lipid homeostasis, epidermal maturation and skin-wound healing, and brain development [5]. PPAR\(\gamma\), the most widely investigated member of the PPARs, exists under at least two isoforms: PPAR\(\gamma\)1 and PPAR\(\gamma\)2. Although derived from the same gene, their production results from the gene alternative promoter and differential mRNA splicing [6, 7]. PPAR\(\gamma\)1 is expressed in several tissues including inflammatory and immune cells, whereas PPAR\(\gamma\)2 is mainly found in adipose tissues. PPAR\(\gamma\) is significantly implicated in glucid and lipid metabolism regulation and has been shown to contribute to diabetes [8], cardiovascular disease [9], carcinogenesis [10], and inflammation [11].

**PPAR\(\gamma\) ligands**

PPAR\(\gamma\) is activated by a number of natural physiological and synthetic agonists. The first natural endogenous agonist of PPAR\(\gamma\) to be identified was the cyclopentanone prostaglandin 15-deoxy-\(\Delta^{12,14}\) prostaglandin J\(_2\) (15d-PGJ\(_2\)) [12], which has since been used extensively in attempts to define the role of PPAR\(\gamma\). Other natural PPAR\(\gamma\) agonists include the essential fatty acids arachidonic acid, docosahexanoic acid, eicosapentanoic acid, and the 15-lipoxygenase (LOX) metabolites 13(S)-hydroxy octadecadienoic acid (13-HODE) and 15(S)-hydroxyeicosatetraenoic (15-HETE) [13]. Nitrolinoleic acid (LNO2), an unsaturated fatty acid derivative induced by nitric oxide (NO)-dependent oxidative inflammatory reactions, has also been reported to activate PPAR\(\gamma\) [14].

Among the many synthetic compounds that bind to and activate PPAR\(\gamma\) are the antidiabetic thiazolidinediones or glitazones, including troglitazone, pioglitazone, ciglitazone and rosiglitazone [8]. Nonsteroidal antiinflammatory drugs such as ibuprofen, indomethacin, fenoprofen, and flufenamic acid have also been reported to bind to and activate PPAR\(\gamma\) [15]. Glitazars, which include muraaglitazar, tesaglitazar, and farglitazar are dual acting PPAR\(\alpha/\gamma\) agonists, currently evaluated in the treatment of type 2 diabetes [16].
Effects of PPARγ activation on inflammatory and catabolic responses

Chondrocytes

Activation of PPARγ has been shown to downregulate several inflammatory responses in chondrocytes (Table 1). Treatment of human OA chondrocytes with 15d-PGJ2 or troglitazone inhibited IL-1β-induced NO and prostaglandin E2 (PGE2) production and iNOS and COX-2 expression [17-19]. PPARγ activation also prevented NO production by the inflammatory cytokines IL-17 and TNF-α, also known for their role in the pathogenesis of OA [18].

In contrast, treatment with the synthetic PPARγ activator rosiglitazone had no effect and the PPARγ antagonist GW9662 did not relieve the inhibitory effect of 15d-PGJ2, suggesting the implication of PPARγ-independent mechanisms.

Table 1. Mechanisms involved in the pathogenesis of OA and effects of PPARγ activation.

<table>
<thead>
<tr>
<th>Mechanism of OA pathogenesis</th>
<th>Effects of PPARγ activation</th>
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<tr>
<td><strong>Chondrocytes</strong></td>
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<tr>
<td>• Enhanced production of MMPs</td>
<td>Reduction in MMP production [17, 18, 26, 27]</td>
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<tr>
<td>• Reduced proteoglycan synthesis</td>
<td>Prevention of proteoglycan degradation [27, 52]</td>
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<tr>
<td>• Increased iNOS expression and NO production</td>
<td>Inhibition of IL-1-induced iNOS expression and NO production [18, 52]</td>
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<tr>
<td>• Increased expression of COX-2 and mPGES-1 and formation of PGE2</td>
<td>Inhibition of COX-2 and mPGES-1 expression and PGE2 formation [19, 21]</td>
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<tr>
<td><strong>Synovial fibroblasts</strong></td>
<td>Suppression of IL-1, IL-6 and TNF-α production [39]</td>
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<tr>
<td>• Increased production of IL-1, IL-6 and TNF-α</td>
<td>Prevention of IL-1-induced MMP production [36]</td>
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<tr>
<td>• Increased production of MMPs</td>
<td>Inhibition of COX-2 and mPGES-1 expression and PGE2 formation [37, 38]</td>
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COX-2, cyclooxygenase-2; IL, interleukin, iNOS, inducible nitric oxide synthase; mPGES-1, microsomal prostaglandin E synthase; MMP, matrix metalloproteinase; OA, osteoarthritis; PGE2, prostaglandin E2; PPARγ, peroxisome proliferator activated receptor gamma; TNF-α, tumour necrosis factor-α.
pathways [20, 21]. Indeed, several studies have reported that 15d-PGJ₂ modulates the expression of a number of genes through PPARγ-independent mechanisms including iNOS in microglial cells and astrocytes [22], the β2-integrin-dependent oxidative burst in human neutrophils [23], and the CD95 ligand in T lymphocytes [24]. Moreover, 15d-PGJ₂ was shown to inhibit the lipopolysaccharide (LPS)-induced expression of iNOS and COX-2 in PPARγ⁻/⁻ embryonic stem cells [25].

Increased production of MMPs plays a critical role in cartilage degradation during OA. Interestingly, PPARγ activators were shown to suppress the production of several MMPs by chondrocytes and to prevent proteoglycan degradation. For instance, our group has shown that 15d-PGJ₂ and troglitazone suppressed IL-1β-induced MMP-13 expression by inhibiting the AP-1 and NF-κB pathways in human OA chondrocytes [18]. Similarly, rosiglitazone blocked IL-1β-induced MMP-1 production in rabbit chondrocytes through DNA binding competition on the composite PPRE/AP1 site in the MMP-1 promoter [26]. In rat chondrocytes, 15d-PGJ₂ and a synthetic activator, GI262570, inhibited IL-1β- and TNF-α-induced MMP-3, MMP-9, and proteoglycan degradation [27]. It was recently demonstrated that 15d-PGJ₂ and the synthetic triterpenoid 2-cyano-3,12-dioxoooleana-1,9-dien-28-oic acid (CDDO) suppress, in a PPARγ-independent manner, MMP-1 and MMP-13 in the human chondrocytic cell line SW1353 [28].

The 15-LOX metabolites 13-HODE and 15-HETE were shown to dose-dependently suppress IL-1β-induced MMP-1 and MMP-13 expression [29]. They also decreased the degradation of type II collagen in human OA cartilage explants treated with IL-1β [29]. Pretreatment with the PPARγ antagonist GW9662 was shown to prevent the suppressive effect of 13-HODE and 15-HETE, suggesting that their effects are mediated by PPARγ [29].

15d-PGJ₂ is generated through dehydration of PGD₂, the biosynthesis of which is catalyzed by two PGD synthases (PGDS): lipocalin PGDS (L-PGDS) and hematopoietic-type PGDS (H-PGDS). L-PGDS is glutathione-independent and H-PGDS glutathione-dependent [30]. L-PGDS (or β-trace) is a member of the lipocalin family, a group of secretory proteins that transport small hydrophobic molecules such as retinoids [31].

In an effort to characterize the PPARγ pathway in OA, the expression of PGDS in cartilage was investigated [32]. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemistry analyses revealed the presence of both H- and L-PGDS in cartilage, with L-PGDS being predominant. In addition, it was demonstrated that the levels of
L-PGDS mRNA and protein were elevated in OA when compared with normal cartilage. The increased expression of L-PGDS in OA cartilage is likely to be mediated by IL-1β, since treatment of chondrocytes with IL-1β enhanced L-PGDS expression in a time-dependent manner. This process requires de novo protein synthesis and involves the mitogen-activated protein kinases (MAPK) c-Jun N-terminal kinase (JNK) and p38, the NF-κB and Notch signalling cascades [32]. L-PGDS, a metabolite of PGD₂, also suppressed IL-1β-induced MMP-1 and MMP-13 production, an effect mediated through the DP1/cAMP/PKA pathway [32]. These data suggest that the increased expression of L-PGDS may represent an attempt to counteract the catabolic effects of IL-1β.

**Synovial fibroblasts**

Early investigations of the role of PPARγ in the biology of synovial fibroblasts demonstrated that its receptor agonists, 15d-PGJ₂ and troglitazone, inhibited the endogenous expression of several inflammatory and catabolic genes including IL-6, IL-8, TNF-α and MMP-3 in human OA synovial fibroblasts [33, 34] (Table 1). PPARγ activators also reduced LPS-induced expression of iNOS, COX-2, IL-1β and TNF-α in rat synovial fibroblasts [35]. In human OA synovial fibroblasts treated with IL-1β, expression of MMP-1, COX-2, and mPGES-1 was inhibited by 15d-PGJ₂ and troglitazone [36-38]. A study in human rheumatoid arthritis synovial fibroblasts [39] demonstrated that 15d-PGJ₂, but not troglitazone, suppressed IL-1β-induced expression of cytosolic phospholipase A₂ (cPLA₂) and COX-2 as well as PGE₂ production. However, pre-treatment with an anti-PPARγ antibody in that study did not reverse the effect of 15d-PGJ₂, suggesting that the effect was mediated via a PPARγ-independent mechanism [39].

**PPARγ-mediated inhibition of gene expression**

A number of mechanisms have been demonstrated to mediate transcriptional suppression by PPARγ, foremost among them its direct binding of key transcription factors and inhibition of their DNA binding and/or transcriptional activity. Indeed, PPARγ has been shown to inhibit transcriptional activity of SP-1, NF-κB, and nuclear factor of activated T cells (NF-AT) via mechanisms involving direct physical protein-protein interactions [40, 41].
PPAR\(\gamma\) can also downregulate transcription by competing with general transcriptional co-activators. In this context, PPAR\(\gamma\) was shown to interact with cAMP-responsive element binding factor (CREB)-binding protein (CBP), p300, steroid receptor co-activator-1 (SRC-1) and thyroid hormone receptor-associated protein (TRAP220) [42, 43]. These co-factors are required for the transcriptional activity of AP-1, NF-\(\kappa\)B, early growth response-1 (Egr-1), signal transducers and activators of transcription (STAT), and NF-AT. Hence, the sequestering of limited amounts of general transcriptional co-activators by activated PPAR\(\gamma\) may be responsible for the suppressive effect of PPAR\(\gamma\).

Post-translational modifications in histone proteins are increasingly recognized as critical in the regulation of gene expression via remodelling of chromatin structure. Acetylation, the most studied modification [44, 45], is controlled by the opposing actions of two classes of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). Generally, acetylation of histones at target promoters by HATs is associated with transcriptional activation, whereas deacetylation by HDACs is associated with transcriptional suppression. For example, the suppression of IL-1\(\beta\)-induced granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-8 expression by the antiinflammatory agents dexamethasone and theophylline appears to be due to the recruitment of HDACs to these gene promoters resulting in histone deacetylation [46, 47]. In human OA synovial fibroblasts, induction of COX-2 by IL-1\(\beta\) is associated with hyperacetylation of histone H3 and H4 at the COX-2 promoter [38]. Treatment with 15d-PGJ\(_2\) inhibits IL-1\(\beta\)-induced COX-2 expression and histone acetylation at the COX-2 promoter. However, reduction in histone H3 acetylation is not correlated with recruitment of HDACs to the COX-2 promoter. Pretreatment of human OA synovial fibroblasts with trichostatin A (TSA), a specific HDAC inhibitor, did not relieve the 15d-PGJ\(_2\) suppressive effect [38]. PPAR\(\gamma\) has also been shown to inhibit transcription by preventing clearance of co-repressor complexes from target gene promoters. Activation of PPAR\(\gamma\) was demonstrated to cause a conformational change leading to the sumoylation of its ligand binding domain by the E3 ligase PIAS1 [48]. Sumoylated PPAR\(\gamma\) binds to the nuclear receptor co-repressor (NCoR)-histone deacetylase-3 (HDAC3) complex, blocking its release from the inflammatory gene promoter [48]. Finally, activators of PPAR\(\gamma\) may also modulate transcription by stimulating specific signalling pathways. Indeed, PPAR\(\gamma\) agonists have been shown to activate several intracellular signalling pathways such as the MAPKs extracellular signal-regulated kinase (ERK), JNK, p38, and the MAPK phosphatase-1 [49].
Reduced expression of PPARγ in OA cartilage

Studies from our group have shown that the predominantly expressed isoform of PPARγ in human cartilage is PPARγ1 and its level of expression is reduced in OA [50]. Similarly, Dumond et al. [51] reported reduced PPARγ expression in cartilage from a rat model of mono-iodoacetate-induced OA. Together, these findings suggest that reduced PPARγ expression in OA cartilage may, at least in part, be involved in increased expression of inflammatory and catabolic genes, promoting articular inflammation and cartilage degradation. Treatment of human OA chondrocytes with IL-1β resulted in a decrease in PPARγ protein expression [50]. PPARγ1 expression was also downregulated by TNF-α, IL-17, and PGE2 [50]. Inhibitors of the MAPK p38, (SB203580) and JNK (SP60012), and of NF-κB signalling, SN-50, MG-132 and caffeic acid phenethyl ester (CAPE), also inhibited IL-1β-induced downregulation of PPARγ1 expression [50]. IL-1β has also been shown to reduce PPARγ protein expression in normal rat chondrocytes [52]. Thus, inhibition of PPARγ expression in chondrocytes by IL-1β may be an important process in the pathophysiology of OA.

PPARγ in experimental models of arthritis

PPARγ has demonstrated its in vivo protective effects in OA animal models. Systemic administration of pioglitazone dose-dependently reduced the size and the depth of cartilage lesions in an experimental guinea pig model of OA (partial medial meniscectomy) [53] in which the histologic severity of cartilage lesions was also reduced. This protective effect appears to be due to reduced expression of MMP-13 and IL-1β, two key mediators in the pathogenesis of OA. Similar reduction in cartilage lesions was found in an anterior cruciate ligament transection canine model of OA [54]. The latter study also showed reduced expression of MMP-1, iNOS, and a disintegrin and metalloproteinase domain with thrombospondin motifs (ADAMTS)-5 as well as a decrease in the activation of the signalling pathways ERK-1/2, p38 and NF-κB [54]. Thus, it seems that PPARγ agonists possess chondroprotective properties by impairing the expression of key genes involved in the pathogenesis of OA and the signalling pathways that mediate their transcriptional activation.

In animal models of rheumatoid arthritis, the protective effects of PPARγ activators have also been demonstrated. The administration of 15d-PGJ2 and
troglitazone inhibited pannus formation and mononuclear cell infiltration in rat adjuvant-induced arthritis [55]. Treatment with rosiglitazone in a mouse model of type II collagen-induced arthritis (CIA) improved the clinical signs and histological features of the disease and reduced plasma levels of IL-1β, TNF-α and IL-6 [56]. A further study demonstrated that pioglitazone and rosiglitazone reduced synovitis and synovial expression of IL-1β and TNF-α in a rat model of CIA [57]. Both thiazolidinediones prevented bone erosion and bone loss [57]. More recently, Sumariwalla et al. [58] used a murine model of CIA to assess a new PPARγ ligand, CLX-090717, and found that it reduced clinical features of arthritis, paw swelling, and the progression of structural damage. Together these data indicate that PPARγ also demonstrates protective effects in other forms of arthritis. This is strengthened by the observation that antigen-induced arthritis is exacerbated in mice heterozygous for PPARγ deficiency [59].

**PPARγ polymorphism in OA**

There are a number of genetic variants in the PPARγ gene, the most prevalent being Pro12Ala and C161T (also known as C1431T). The Pro12Ala polymorphism results from a CCA-to-GCA missense mutation in codon 12 of exon B of the PPARγ gene. Because this mutation is located in the ligand-independent activation domain of the protein, it may cause conformational changes that affect its transcriptional activity [60]. The Pro12Ala polymorphism is associated with improved insulin sensitivity [61], reduced type 2 diabetes risk [62], myocardial infarction [63], and atherosclerosis [64]. The silent C161T (C1431T) polymorphism in the exon 6 is associated with increased plasma levels of leptin [65], decreased risk of coronary artery disease [66], survival of patients with immunoglobulin A nephropathy [67], and longevity [68].

When the effects of Pro12Ala and C161T were assessed on the prevalence and severity of OA in a French Canadian population, data revealed no significant difference in either polymorphism between OA patients and controls [69]. No significant differences were observed after stratification of patients according to age at disease onset and radiographic or functional severity. Moreover, haplotype analysis of both polymorphisms showed no association with OA or its clinical features. Thus, these PPARγ polymorphisms do not appear to contribute to susceptibility to, or severity of, OA in a French Canadian population. However, further studies in other ethnic populations are necessitated to evaluate the role of these and other polymorphisms in the pathogenesis of OA.
Conclusion

Since to date there exists no disease modifying treatment for OA, the investigation of its pathophysiological mechanisms is crucial. Continued research is necessary to develop and improve strategies aimed at reducing or arresting the disease progression. Improved knowledge of the physiological and pathological mechanisms of OA will lead to the development of specific targets in new therapeutic approaches.

The increased inflammatory and catabolic activity in chondrocytes and synovial fibroblasts is believed to contribute to the pathogenesis of OA and related chronic arthritic diseases. Over the last decade, in vitro and in vivo studies have shown that PPARγ agonists inhibit several of these catabolic and inflammatory responses, suggesting their potential benefit in the treatment of arthritis. The thiazolidinedione class of PPARγ agonists is already used in the treatment of type 2 diabetes and is presently being investigated in clinical trials for the treatment of cancer and cardiovascular diseases.

Although the current clinical trials will facilitate future studies assessing PPARγ agonists as anti-arthritis drugs, caution must be exercised. The molecular mechanisms by which PPARγ agonists modulate gene expression in articular cells need to be better characterized and care must be taken to prevent adverse side effects by ensuring that agonists are specific for PPARγ, avoiding PPARγ-independent mechanisms.

References

Role of PPARγ in osteoarthritis