PPARγ Ligand Inhibits Osteopontin Gene Expression Through Interference With Binding of Nuclear Factors to A/T-Rich Sequence in THP-1 Cells

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Abstract—Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear receptor superfamily that acts as a key player in adipocyte differentiation, glucose metabolism, and macrophage differentiation. Osteopontin (OPN), a component of extracellular matrix, is elevated during neointimal formation in the vessel wall and is synthesized by macrophages in atherosclerotic plaques. In the present study, we investigated the molecular mechanisms regulating OPN gene expression by PPARγ in THP-1 cells, a cell line derived from human monocytic leukemia cells. Northern and Western blot analyses showed that exposure of THP-1 cells to PMA (phorbol 12-myristate 13-acetate) increases OPN mRNA and protein levels in a time-dependent manner. PMA-induced OPN expression was significantly decreased by troglitazone (Tro) and other PPARγ ligands. Transient transfection assays of the human OPN promoter/luciferase construct showed that PPARγ represses OPN promoter activity, and the PPARγ-responsive region within the OPN promoter lies between −1100 and −970 relative to the transcription start site. Site-specific mutation analysis and electrophoretic mobility shift assays indicated that a homeobox-like A/T-rich sequence between −990 and −981, which functions as a binding site for PMA-induced nuclear factors other than PPARγ, mediates the repression of OPN expression by Tro. Furthermore, concatenated A/T-rich sequences conferred the PPARγ responsiveness on the heterologous promoter. Taken together, these data suggest that PPARγ ligand inhibits OPN gene expression through the interference with the binding of nuclear factors to A/T-rich sequence in THP-1 cells. (Circ Res. 2002;90:348-355.)

Key Words: peroxisome proliferator-activated receptor γ  osteopontin  macrophage  transcription factors

Accumulation of macrophages in the vessel wall is a hallmark of atherosclerosis. Macrophages are present in every phase of atherogenesis.1 In the process of progressing atherosclerosis, macrophages and lymphocytes are activated and release hydrolytic enzymes, cytokines, chemokines, and growth factors, which induce further damage of the vessel wall. Production of extracellular matrix from activated macrophages in the vessel wall accelerates the progression of atheroma.2

Osteopontin (OPN) is a phosphorylated glycoprotein, which is originally isolated from bone,3 and has been implicated in a variety of vascular pathology including proliferation and migration of endothelial cells, macrophages, and vascular smooth muscle cells.4–6 It has recently been reported that phosphorylated OPN is an inhibitor of vascular smooth muscle cell calcification.7 These findings suggest that OPN may play an important role in mediating many aspects of the atherosclerotic process.

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily and acts as a ligand-inducible transcription factor.8 Troglitazone (Tro) is an insulin-sensitizing agent that acts as a PPARγ ligand.9 Prostaglandin derivative 15-deoxy-Δ12,14-prostaglandin J1 (15-d-PGJ1) is well known as a natural ligand for PPARγ.10 Some of the nonsteroidal antiinflammatory drugs such as indomethacin (IDM), fenoprofen, ibuprofen, and flufenamic acid are capable of activating PPARγ.11 Ligand-activated PPARγ forms a heterodimer complex with the 9-cis retinoic acid receptor, RXR. This complex binds to the PPAR-responsive element, PPRE, within the promoters of PPARγ-target genes.12,13 PPARγ has been known to be essential for adipocyte differentiation and the control of cellular lipid uptake.14 Recent studies have demonstrated that PPARγ is expressed at high levels in the foam cells in atherosclerotic lesions and can promote differentiation of monocytes to macrophages.15 Furthermore, it has been reported that PPARγ agonists suppress the elaboration of inflammatory cytokines from macrophages.16,17 These lines of evidence suggest that Tro or PPARγ ligands regulate the development and progression of atherosclerosis. Recently, we have reported that the PPARγ agonist reduces OPN gene expression.18 However, the mo-

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lecular mechanism of PPARγ-mediated OPN gene expression remains to be determined.

In the present study, we examined the regulation of OPN gene expression by PPARγ ligand in THP-1 cells and defined the molecular mechanisms behind the observation. We determined the cis-regulatory sequence within the OPN gene promoter, which is targeted by PPARγ. Our results represent one of the molecular mechanisms by which PPARγ regulates the extracellular matrix gene implicated in atherosclerotic progression and vascular calcification.

Materials and Methods

Cell Cultures

THP-1, C2/2 (rabbit aorta-derived smooth muscle cells), and COS-7 cells (African monkey kidney cells) were obtained from American Type Culture Collection (ATCC; Manassas, Va). Suspension cell cultures of THP-1 cells were grown to a density of 1×10^6 cells/mL in RPMI 1640 medium supplemented with 10% (vol/vol) fetal bovine serum (FBS). C2/2 cells derived from rabbit-cultured vascular smooth muscle cells were previously described.19,20 THP-1 and COS-7 cells were grown at 37°C in a 5% CO₂ atmosphere in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS and 1% penicillin/streptomycin.

Northern Blot Analysis

Total RNA was isolated using the ISOGEN reagent (Nippongene) in accordance with the manufacturer’s instruction. Northern blot analysis was performed as previously described.20 A 533-bp fragment of human OPN cDNA sequence (obtained from ATCC) was used as a probe and labeled with [γ-³²P]dCTP (Amersham Corp) by using a random primer DNA labeling kit (Boehringer Mannheim).

RT-PCR Assays

Total RNA from THP-1 cells was reverse-transcribed by random 9-mers with the use of reverse transcriptase and amplified with Taq DNA polymerase (Takara).

Western Blot Analysis

THP-1 cells were incubated with or without phorbol 12-myristate 13-acetate (PMA) and Tro at a density of 1×10⁶ cells/mL and harvested in cell lysis buffer at 12 and 48 hours after transfection, respectively. Western blot analysis was performed as previously described.22 A 533-bp fragment of human OPN cDNA sequence (obtained from ATCC) was used as a probe and labeled with [γ-³²P]dCTP (Amersham Corp) by using a random primer DNA labeling kit (Boehringer Mannheim).

Construction of the Luciferase Gene

For generation of luciferase reporter genes, the following forward and reverse primer were used in a PCR reaction with -32 P]dCTP (Amersham Corp) by using a random primer DNA labeling kit (Boehringer Mannheim).

Transfection and Luciferase Assays

Transfection of THP-1 cells has been performed by the electroporation method as previously reported.21 Briefly, suspension cultures of 5×10⁶ cells were transfected with 3 μg of reporter gene along with either 3 μg PPARγ/pCDNA3 or pCDNA3 as described.22 Expression Plasmids

Mouse PPARγ cDNA was kindly provided by Dr T. Kadowaki (University of Tokyo, Japan). For generation of the coding region of mouse PPARγ, cDNA was amplified by PCR using the upstream primer with a KpnI site (underlined), 5'-GGGGGTACC-ATGGGTTAACCTCGGGGAGGATG-3', and the reverse primer with a XdeI site (underlined), 5'-CCCTCTAGACTAAAGCTCGCCTGATGAT-3'. The PCR product was gel-purified, digested, and subcloned into the KpnI/XhoI site of pcDNA3 (Invitrogen).

Electrophoretic Mobility Shift Assays (EMSAs)

Nuclear extracts from THP-1 cells were prepared as previously described.22 The sequences for the sense strand of double-stranded oligonucleotides used as probes or competitors in EMSAs were as follows, with a consensus motif underlined and modifications of wild-type sequence in bold: OPN: (−995/−975), 5'-TCGAAAATTTCTCTATTTGATG-3', mut-1, 5'-TCGGGAAATTCTCTTATGTG-3', mut-2, 5'-TGCAAGTTTCTCTATGTG-3', mut-3, 5'-TGCAAAATCCTCTATTGATG-3', mut-4, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-5, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-6, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-7, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-8, 5'-TGCAAAATCCTCTCTTGTGATG-3'; PPRE, 5'-GACCCAGGACAAAGGCTCGCT-3', Ets-1, 5'-TGCAATAGCGAAGGATGCTACAC-3', homebox protein, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-9, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-10, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-11, 5'-TGCAAAATCCTCTCTTGTGATG-3', mut-12, 5'-TGCAAAATCCTCTCTTGTGATG-3'; and Sp1, 5'-ATTCGAGTGGGGCCGGGGAGGACG-3'. Binding reactions were performed as previously described.

Recombinant Adenovirus Expression Constructs

The recombinant adenovirus vectors were generated as previously described.24 AxCA/LucZ and AxCA/PPARγ were prepared by inserting the β-galactosidase or PPARγ cDNAs, respectively, into the Ad E1−deleted region under the control of the CAG promoter.
Results

Effect of PMA on OPN mRNA Levels
To determine whether OPN mRNA levels are regulated in THP-1 cells, we performed Northern blot analysis and measured OPN mRNA levels after induction of differentiation from monocytes to macrophages with PMA. Whereas the OPN mRNA expression was virtually undetectable in untreated cells, PMA stimulation significantly increased OPN mRNA levels in a time-dependent manner (Figure 1).

Effect of Troglitazone on PMA-Induced OPN Expression
To investigate the effect of PPARγ on OPN expression, THP-1 cells were incubated with either PMA alone or in combination with Tro, a PPARγ ligand. Northern blot analysis showed that PMA-induced OPN mRNA expression was markedly attenuated in the presence of Tro (Figure 2A). It should be noted that the decrease in OPN mRNA levels was not due to the nonspecific toxic effect of Tro, because Tro had no inhibitory effects on scavenger receptor-A (SR-A) and PPARγ mRNA levels (Figure 2B). As shown in Figure 2C, an inhibitory effect of Tro on OPN mRNA expression was dose-dependent: 1 μmol/L of Tro has a marked effect on OPN expression, and 10 μmol/L of Tro completely inhibited OPN expression. The concentration of Tro used in this study seems to be relevant to the clinical situation because the effective concentration of Tro in the human plasma is ~1.3 μmol/L.²³ Western blot analysis confirmed that PMA induces and Tro inhibits the expression of OPN protein. OPN protein levels were detectable after 24 hours of exposure to PMA, and OPN protein was not detected in the presence of Tro (Figure 2D).

PPARγ Ligands Inhibit OPN Expression
To determine whether the inhibitory effect of Tro on OPN mRNA expression is due to the action of Tro as a PPARγ ligand, we next examined the effects of other PPARγ ligands on OPN expression. We incubated THP-1 cells with either PMA alone or in combination with Tro, IDM, 15-d-PGJ2, or aspirin (Asp) for 24 hours and harvested them for Northern blot analysis. IDM and 15-d-PGJ2 but not Asp have been described to be ligands of PPARγ.¹¹ As shown in Figure 3, either IDM or 15-d-PGJ2 completely abolished the induction of OPN mRNA expression by PMA. Consistent with our expectation, Asp had no effect on OPN mRNA expression. These results allowed us to speculate that the inhibitory effect of Tro on OPN expression is mediated through the activation of PPARγ.

Effect of PPARγ on OPN Promoter Activity
To investigate whether PPARγ regulates OPN expression at the transcriptional level, we performed transient transfection
assays. The luciferase reporter construct containing the OPN promoter region spanning from −1500 to +87 in front of the luciferase reporter gene, designated as OPN-1500Luc, was transiently transfected into THP-1 cells, and transfected cells were then treated with PMA either in the presence or absence of Tro (Figure 4). PMA stimulated luciferase activity of this construct, and more importantly, its activity was significantly repressed by Tro in the presence of PMA. The repression of OPN promoter activity by Tro seems to be dependent on PMA stimulation because Tro had no effect on the OPN promoter in the absence of PMA. We next determined whether PPARγ overexpression represses OPN promoter activity. Cotransfection of the PPARγ expression plasmid reduced OPN promoter activity either in the absence or presence of PMA. These data are consistent with the hypothesis that Tro inhibits OPN expression at the transcriptional level through the activation of PPARγ.

To delineate the sequence responsible for the repression of the OPN promoter by PPARγ, progressive deletion of 5′-flanking region was performed. As shown in Figure 5A, PMA-induced activity was observed in −1000Luc and −970Luc, in which the 5′ ends correspond to −1000 and −970, respectively. Luciferase activity of the construct −1000Luc was reduced by Tro to the same extent as shown in −1500Luc. In contrast, the decrease in the promoter activity by Tro was not observed in −970Luc (Figure 5A).

We reasoned that if Tro inhibits the luciferase activity of −1500Luc and −1000Luc, but not −970Luc, through PPARγ activation, then overexpression of PPARγ could exert the same effects on the OPN promoter as those seen by Tro stimulation. As shown in Figure 5B, overexpression of PPARγ decreased the luciferase activity of −1500Luc and −1000Luc but not −970Luc in THP-1 cells.

To further assess the role of PPARγ in the repression of OPN promoter activity, we used the C2/2 cells, smooth muscle cells derived from rabbit aorta, as the possible PPARγ-deficient cells. Figure 5C reveals that in the absence of PPARγ expression vector Tro has only minimally increased luciferase activity of 3xPPRE/Luc, which contains three copies of rat acly-CoA oxidase PPRE in front of the TK basal promoter. The ability of 3xPPRE/Luc to respond to Tro was verified by showing that Tro enhanced the PPARγ-induced activity of 3xPPRE/Luc activity. These data suggest that C2/2 cells express little, if any, functional PPARγ. As shown in Figure 5D, PPARγ overexpression strongly reduced the promoter activity of −1500Luc and −1000Luc but not −970Luc in C2/2 cells. These results indicate that PPARγ reduces OPN promoter activity in C2/2 and THP-1 cells and suggest that the sequence between −1000 and −970 contains a regulatory element responsible for PPARγ-induced repression of OPN promoter activity regardless of the cell types.

### Identification of Nuclear Factor–Binding Sites Between −1000 and −970

To determine whether the sequence between −1000 and −970 of the OPN promoter interacts with the nuclear proteins, we performed EMSAs by using double-stranded oligonucleotides containing the sequence from −995 to −975 as a probe. Results showed that incubation of nuclear extracts prepared from PMA-stimulated THP-1 cells gave rise to two prominent shifted complexes, C1 and NS. To determine the sequence specificity of these complexes, we
PPARγ antibody had no effect on complex C1 formation. The integrity of anti–PPARγ antibody was confirmed by incubating the nuclear extract prepared from AxCA/PPARγ-infected cells with the probe containing PPRE, a PPAR-responsive element. Expectedly, the PPRE probe gave rise to a DNA:protein complex, and anti–PPARγ antibody supershifted this complex (Figures 7A and 7B). These results suggest that PPARγ does not bind to the sequence OPN (−995/−975).

To better characterize the nuclear proteins participating in the complex formation, we tested whether some of the sequences known as targets of DNA binding proteins can compete for the binding to the probe. Searching for the DNA binding proteins whose recognition sequence resembles the probe sequence reveals that the sequence 5′-AATTTCCTTA-3′ is similar to the DNA binding sequence for homeobox proteins, whose consensus binding sequence is 5′-CAATTTAA-3′. As shown in Figure 7D, the addition of the unlabeled oligonucleotide containing the consensus sequence for the homeobox protein but not for CARG, Sp1, and Ets-1 has measurable effects on complex formation. These data suggest that the A/T-rich sequence between −990 and −981, which may be recognized by homeobox proteins or related proteins but not by PPARγ, is important for the inhibitory effects of PPARγ on OPN gene expression.

Effect of Mutation of A/T-Rich Sequence on the OPN Promoter Activity

The role of the A/T-rich sequence in the inhibitory effects of PPARγ on OPN gene expression prompted us to explore the possibility that mutation of this sequence may abolish the effects of Tro on OPN promoter activity. We made constructs in which the mutation was introduced into this 10-bp sequence in the context of −1000Luc (Figure 8A). The constructs −1000 mut2Luc and −1000 mut3Luc, both containing a mutation within the 10 base pairs, failed to respond to the overexpression of PPARγ. The construct −1000 mut1Luc, which contains a mutation outside the 10 base pairs, exhibited the same reduction in luciferase activity in response to PPARγ overexpression.

To further verify that PPARγ represses OPN promoter activity through the A/T-rich sequence, we made a construct, 4xA/T-TK/Luc, in which 4 copies of fragments containing 5′-AATTTCCTTA-3′ were inserted into HSV-1 TK-luciferase promoter vector TK/Luc. As shown in Figure 8B, the promoter activity of the 4xA/T-TK/Luc was reduced by the PPARγ expression vector. From these results, we conclude that the 10-bp sequence 5′-AATTTCCTTA-3′ mediates the inhibitory effects of PPARγ on OPN promoter activity. It is also of interest to note that luciferase activity of 4xA/T-TK/Luc is lower than that of TK/Luc, suggesting that the A/T-rich sequence when multimerized may act as a negative element.

Discussion

Increasing evidence indicates an important role of PPARγ activation in modulating the development and progression of atherosclerosis. Several reports have demonstrated that PPARγ ligands inhibit growth factor–mediated growth and
migration of vascular smooth muscle cells.\textsuperscript{26,27} Furthermore, PPAR\textsubscript{γ} ligands exert their role as negative regulators of macrophage activation and inflammatory cytokine production.\textsuperscript{16,17} We have recently found that the expression of the OPN gene is induced upon stimulation by PMA and that PPAR\textsubscript{γ} ligands reduce PMA-induced OPN gene expression.\textsuperscript{18} In the present study, we explored the molecular mechanisms behind this observation. We demonstrated that OPN gene expression can be regulated at the transcriptional level in monocytic leukemia cells, THP-1 cells. In addition, we demonstrated that the A/T-rich sequence on the OPN promoter lying between −990 and −981 serves as an essential motif for the repression of the transcription by PPAR\textsubscript{γ}. Results of the EMSA suggest that PPAR\textsubscript{γ} inhibits the binding of the nuclear factor to the OPN promoter.

Our findings that PPAR\textsubscript{γ} ligands inhibit the expression of the OPN gene will carry considerable clinical significance from the viewpoint of the effectiveness of PPAR\textsubscript{γ} agonists as antiatherosclerotic drugs. Such an effect of PPAR\textsubscript{γ} ligands on OPN expression suggests the beneficial role of PPAR\textsubscript{γ} activators because OPN has recently been implicated in neointimal formation, atherosclerogenic progression, and vascular calcification.\textsuperscript{5–7} This assumption is supported by previous reports indicating that PPAR\textsubscript{γ} activators prevented the induction of inflammatory cytokine production and matrix metalloproteinase production from stimulated monocytes/macrophages.\textsuperscript{28} Furthermore, PPAR\textsubscript{γ} activators have been suggested to inhibit the initiation of atherosclerosis by inhibiting the expression of VCAM-1 in endothelial cells.\textsuperscript{29}

**Figure 7.** EMSA. A, Nuclear extracts (10 μg) prepared from either unstimulated, PMA-treated, PMA+Tro-treated, or Tro-treated THP-1 cells were incubated with \textsuperscript{32}P-labeled −995/−975 probe. Position of the sequence-specific DNA:protein complex (C1) is indicated. Same experiments were performed using the Sp1 consensus sequence as a probe. B, COS-7 cells were infected with AxCA/LacZ or AxCA/PPAR\textsubscript{γ}. Nuclear extracts prepared from COS-7 cells were incubated with −995/−975 probe or PPRE probe. Sequence for PPRE probe is shown in Materials and Methods. DNA:protein complex containing PPAR\textsubscript{γ} is indicated. NS indicates nonspecific complex. C, Supershift assays using nuclear extracts prepared from PMA-treated THP-1 cells were performed using indicated antibodies. Supershifted complex (SS) is indicated by arrow. D, Competition assays. Nuclear extracts (10 μg) prepared from either PMA-stimulated THP-1 cells were incubated with \textsuperscript{32}P-labeled −995/−975 probe, in the absence or presence of a 100-fold molar excess of indicated unlabeled competitors. Sequence of the unlabeled competitor mut-8, which contains mutation within the sequence −990/−981 is indicated by bold letters. Consensus binding sequences for homeobox binding proteins (Homeo), CArG, Sp1, and Ets-1 are shown in Materials and Methods. Position of the sequence-specific DNA:protein complex (C1) is indicated.

**Figure 8.** Effect of mutation of the sequence 5′-AATTTCTTA-3′ on OPN promoter activity. A, Effect of the mutation of A/T-rich sequence, 5′-AATTTCTTA-3′, on OPN promoter activity. Wild-type (−1000Luc) or mutated constructs (−1000 mut1Luc, −1000 mut2Luc, and −1000 mut3Luc) were cotransfected with either pcDNA3 or PPAR\textsubscript{γ}/pcDNA3. Mutated bases are shown in Figure 6A. Luciferase activities are expressed relative to that of pGL3, which is set at 1.0. Values are mean±SEM for 3 separate experiments. B, Luciferase construct TK/Luc or 4xA/T-TK/Luc, which contains 4 copies of the A/T-motif in front of the HSV-1 TK promoter, was transfected with the indicated expression plasmid. Luciferase activities are expressed relative to that of TK/Luc, which is set at 1.0. Values are mean±SEM for 3 separate experiments.
All these observations favor the hypothesis that PPARγ ligands act as antiatherosclerotic medicines.

It is worth stressing that under our experimental conditions, we found that Tro inhibits the apolipoprotein E (apoE) mRNA expression, which is highly induced in the absence of Tro. We also noted that induced expression of SR-A and lipoprotein lipase (LPL) during differentiation from monocytes to macrophages was augmented by Tro (data not shown). Considering the fact that apoE is essential for LDL receptors to recognize LDL particles, the reduction of apoE by Tro seems to be unfavorable to atherosclerotic lesions. Similarly, an increase in LPL and SR-A expression by Tro may aggravate the lesions. Taken together with the previous report by Tontonoz et al., we showed that PPARγ activators promote monocytic differentiation, we need to be aware that activation of PPARγ may have unfavorable effects on preexisting vascular lesions. Recent studies, however, showed that PPARγ activators do not enhance the ox-LDL uptake of macrophages, although CD36 gene expression from macrophages is increased by PPARγ activators. More recent work demonstrated that Tro inhibits the fatty streak lesion formation in apoE-knockout mice. These results suggest that despite the pleiotropic effects of Tro on the gene expression in the vascular wall, it acts as an antiatherosclerotic rather than proatherosclerotic medicine.

The specificity of an inhibition of OPN gene expression by Tro should be stressed because Tro contains a vitamin E moiety that endows it with some antioxidant properties. Our findings show that both 15d-PGJ2 and IDM, which act as PPARγ ligands, inhibited OPN expression to a similar extent as Tro. The inability of Asp, which is not considered to be a PPARγ activator, to inhibit OPN expression supports our findings that inhibition of OPN expression is not due to the nonspecific effects of antiinflammatory agents.

Although PPARγ is known to change gene transcription through its responsive element, PPRE, several reports have indicated that PPARs regulate gene expression independent of PPRE. Tolon et al. have reported that PPARγ inhibits prolactin gene activation by inhibiting the function of GHP-1, a transcription factor implicated in pituitary-specific gene expression. Ricote et al. postulated that PPARγ ligands inhibit the expression of the inducible form of NO synthase, gelatinase B, and SR-A expression by interfering with the function of AP-1, STAT-1, and NF-kB. In the present study, we defined the A/T-rich sequence located at −990 as a regulatory element of PPARγ. We partially characterized the factors, which recognize the A/T-rich sequence by competitive EMSA, and showed that homeobox proteins or related factors may constitute the complex formation with this element. Identification of DNA-binding protein on A/T-rich sequence will merit further studies.

In conclusion, the present study showed that OPN production was significantly reduced in response to the ligand-activated PPARγ. Although our studies do not address the question whether PPARγ activators serve as antiatherosclerotic medicine in vivo, our findings may provide new insight into the molecular mechanisms by which activators of PPARγ modify vascular lesions because OPN expression is intimately associated with the development of atherosclerosis.

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References


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