**PPARα Activators Inhibit Vascular Endothelial Growth Factor Receptor-2 Expression by Repressing Sp1-Dependent DNA Binding and Transactivation**

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Abstract—Peroxisome proliferator–activated receptors (PPARs) are ligand-activated transcription factors, originally implicated in the regulation of lipid and glucose homeostasis. In addition, natural and synthetic PPAR activators may control inflammatory processes by inhibition of distinct proinflammatory genes. As signaling via the vascular endothelial growth factor receptor-2 (VEGFR2) pathway is critical for angiogenic responses during chronic inflammation, we explored whether known antiinflammatory effects of PPAR ligands are mediated in part through diminished VEGFR2 expression. In this study, PPARα agonists are found to inhibit endothelial VEGFR2 expression, whereas predominant PPARγ ligands remained without discernible effects. Time- and concentration-dependent inhibition is demonstrated both at the level of protein and mRNA VEGFR2 expression. Inhibitory effects of PPARα agonists on transcriptional activity of the VEGFR2 promoter are conveyed by an element located between base pairs −60 and −37 that contains two adjacent consensus Sp1 transcription factor binding sites. Constitutive Sp1-containing complex formation to this sequence is decreased by PPARα treatment, indicating that VEGFR2 gene expression is inhibited by repressing Sp1 site-dependent DNA binding and transactivation. Our coimmunoprecipitation experiments revealed enhanced protein interactions between PPARα and Sp1 on PPARα activation, thus constituting a probable mechanism by which PPARα agonists decrease Sp-dependent binding activity to the VEGFR2 promoter. Hence, molecular mechanisms by which PPARs modulate the rate of gene transcription may include direct interactions between specific transcription factors and PPARs that ultimately result in reduced DNA binding to their respective response elements. (Circ Res. 2004;94:324-332.)

Key Words: endothelial growth factor receptors ■ peroxisome proliferator–activated receptors ■ inflammation ■ transcription factors ■ promoter regions

Vascular endothelial growth factor (VEGF, also referred to as VEGF-A) and its receptors are considered key regulators of blood vessel growth by vasculogenesis and angiogenic sprouting in cancer, wound repair, and ischemic and inflammatory diseases. VEGF is known for its ability to induce vascular permeability, to act as a critical survival factor for endothelial cells, and to promote endothelial proliferation and migration.

Although VEGF receptor-2 (VEGFR2, formerly termed KDR/Flk-1) is detectable only at relatively low levels in the adult vasculature, it may be markedly upregulated by blood vessels during chronic inflammation, tumor growth, and wound repair. Endothelial expression of VEGFR2 closely parallels VEGF expression in angiogenic responses. As a result, suppression of the VEGF/VEGFR2 signaling pathway is intensely explored as therapeutic prospect to interfere with new blood vessel formation.

Increasing evidence suggests that a group of closely related nuclear receptors, called peroxisome proliferators–activated receptors (PPARs), may play a significant role in the control of inflammatory responses with potential therapeutic applications in chronic inflammatory diseases. PPARs comprise a family of three ligand-activated transcription factors characterized by distinct functions, ligand specificities, and tissue distributions. The role of these receptors has been thought originally to be restricted to lipid and lipoprotein metabolism, glucose homeostasis, and cellular differentiation. PPARs are activated by natural ligands, such as eicosanoids and fatty acids. In addition, synthetic antidiabetic thiazolidinediones (TZDs) and lipid-lowering...
fibrates have been shown to act as activators of PPARγ and PPARα, respectively.7 8 As endothelial VEGFR2 expression is increasingly recognized as a key component of the VEGF/VEGFR signaling system during chronic inflammation,9 we hypothesized that PPAR activators may control inflammatory responses in part by targeting VEGFR2 expression. This assumption is also supported by recent experimental evidence, revealing antiangiogenic properties of PPAR activators both in vitro and in vivo.9–11 Thus, PPARs as ligand-activated transcription factors were tested regarding their potential to modulate vascular responses through transcriptional regulation of the VEGFR2 gene.

The present study reveals inhibition of endothelial VEGFR2 expression by different PPARα activators, whereas predominant PPARγ ligands failed to exert such effects. Because PPARα agonists greatly attenuated VEGF-driven capillary-like network formation, antiinflammatory effects of PPARα ligands may be mediated in part via reduced VEGFR2 expression.

Materials and Methods

Reagents
Recombinant human VEGF165 and basic fibroblast growth factor (bFGF) were purchased from R&D Systems. Fenofibrate, Wy14643, ciglitazone, rosiglitazone, troglitazone, and 15-deoxy-A2,14-prostaglandin J2 (15d-PGJ2) were obtained from Biomol; ciprofibrate and fibrates have been shown to act as activators of PPARγ and PPARα, respectively.7

Cell Culture
Human umbilical vein endothelial cells (HUVECs) were purchased from PromoCell (Heidelberg, Germany), cultured until the fifth passage at 37°C and 5% CO2 in Endothelial Cell Growth Medium (PromoCell).

FACS Analysis
HUVECs were incubated with mouse anti-human VEGFR2 mAb (V9134, 1:200 dilution; Sigma) or isotype control mouse anti-human IgG1 (Caltag) for 30 minutes on ice. Cells were then incubated with FITC-conjugated affinity-purified goat F(ab')2 anti-mouse IgG (Dako, Hamburg, Germany) at 1:100 dilution for 30 minutes, and were subsequently analyzed by a BD FACSScan Cytometer (Becton Dickinson). Nonviable cells were identified and excluded by propidium iodide staining.

Western Blot Analysis
Protein extracts were prepared as described previously.12 The membranes were incubated with the indicated primary antibodies (VEGFR1, clone H-225, VEGFR2, clone A-3, Sp1, clone PEP2, all from Santa Cruz, Heidelberg, Germany; PPARα, clone 3B6, Alexis; Tubulin Ab from LabVision), followed by incubation with horseradish peroxidase-conjugated secondary antibodies (anti-mouse or anti-rabbit IgG, Amersham). The blots were developed using the enhanced chemiluminescence detection system (ECL) according to the manufacturer (Amersham).

Comunmunoprecipitation of Sp1/PPARα
For Sp1 immunoprecipitation, 5 × 10⁶ cells were lysed in cold buffer containing 50 mmol/L Tris-HCl (pH 7.6), 150 mmol/L NaCl, and 1% NP-40 (v/v) for 30 minutes on ice. Immunoprecipitations were performed at antibody excess, incubating 200 μg of total lysate with Sp1 antibody (Santa Cruz, clone PEP2) on a rotator at 4°C overnight. Immunocomplexes were then captured with Protein G Sepharose 4 Fast Flow (Amersham) during a 2-hour incubation step.

Cell Proliferation and Cytotoxicity Assay
The effect of PPAR ligands on cell proliferation was measured by quantitating BrdU, utilizing a cell proliferation immunoassay from Roche Diagnostics. Twenty-four hours after seeding, cells were exposed to PPAR activators as indicated. After 6 hours, BrdU was added for 18 hours. The cytotoxic potential of PPAR ligands was determined by the Cytotoxicity Detection Kit (LDH) from Roche. Twenty-four hours after seeding, cells were exposed to different PPAR activators for 24 hours as indicated.

HUVEC Migration Assay
Migration of ECs was assayed by a modified Boyden chamber (BD Falcon 24-well Assay Plates, BD Biosciences) with 8-μm pore size membranes (BD Falcon Individual HTS FluoroBlok Cell Culture Inserts, BD Biosciences). HUVECs, suspended in Endothelial basal medium (EBM) (CellSystems) containing 1% bovine serum albumin (Sigma) and solvent or the respective PPAR agonists, were added to the upper chamber at 1×10⁵ cells/well. Endothelial growth media (EGM) (CellSystems) containing 20% FBS was placed in the lower chamber, and cells were allowed to migrate for 24 hours. After incubation, migrated cells attached to the lower face of the membrane were visualized with Hoechst Stain 33342 (Sigma) and counted in high-power fields.

Two-Dimensional, Short-Term In Vitro Matrigel Assay
The Matrigel assay was performed according to the description by Hernandez and coworkers with minor modifications.13 PPAR concentrations were maintained during the experiment as indicated. After 12 hours, cells were photographed using an inverted phase-contrast photomicroscope. The area covered by cellular extensions and branch points was taken as a measure to reflect formation of spontaneous and VEGF-induced capillary-like structures. Area calculation was performed on photographs of standardized fields from at least five wells per experimental condition, utilizing the public domain Java image-processing program ImageJ (v1.29).

RNA Extraction and Northern Blot Analysis
RNA was prepared as described previously.14 A 331-bp fragment (nt 3803-4134; GenBank accession No. AF035121) of the human VEGFR2 cDNA and a 502-bp coding sequence of the human GAPDH cDNA (nt 286-788; M33197) were used as specific probes. Band intensities were analyzed by densitometry (ImageJ, v1.29).

Transient Transfection and Analysis of Reporter Gene Expression
HUVECs (1.0×10⁶/well, 12-well plates) were transfected with 0.5 μg of appropriate firefly luciferase construct and 0.1 μg pRL-TK vector (Promega) using SuperFect transfection reagent (Qiagen). Human VEGFR2 reporter gene constructs were generously provided by Dr C. Patterson (University of North Carolina, Chapel Hill, NC).15 PPARα expression vector (pSG5-HPPARα) or vector only (each at 0.15 μg) were cotransfected.15 Likewise, Sp1 expression vector (pEV2R/Sp1) or backbone vector only (each at 0.35 μg) were coadministered.17 Twenty-four hours after transfection, control transfectants were left untreated and test transfectants were exposed to PPAR treatment for 24 hours. The activities of luciferases were measured utilizing the Dual-Luciferase Reporter Assay System from Promega.

Preparation of Nuclear Extracts and Gel Mobility Shift Analysis
HUVECs were left untreated or were incubated with PPAR activators for 6 hours. Nuclear proteins were extracted as described previously.12 The DNA binding reactions were performed with or without excess of unlabeled competitor, Sp1 consensus-oligonucleotide (Promega), PPARα, Sp1, Sp3, or RelA/NF-κB antibody (Santa Cruz).
**Statistical Analysis**

Data are expressed as mean±SD/SE from ≥3 independent experiments. Statistical analysis was performed by Student’s t test.

**Results**

**PPARα but not PPARγ Ligands Suppress VEGFR2 Protein Expression by Human Endothelial Cells**

Flow-cytometric analyses showed that incubation with PPARα activators Wy14643 and fenofibrate significantly reduced basilar VEGFR2 protein expression in a concentration-dependent fashion (Figure 1), with significant inhibition noticed at doses of 100 or 200 μmol/L, respectively. Considerable reduction was observed after 16 hours of incubation, whereas a 4-hour treatment only modestly affected VEGFR2 expression (Figure 1E). In contrast to PPARα activators, protein expression was not discernibly suppressed by treatment with PPARγ ligand ciglitazone or 15d-PGJ2 (even at 25 μmol/L, resembling concentrations that compromise membrane integrity, as determined by LDH release assays; Figure 3B). These findings were bolstered by Western blot analyses, revealing inhibition of basal VEGFR2 expression by treatment with PPARα activator Wy14643, but not with PPARγ ligands ciglitazone, troglitazone, 15d-PGJ2, and rosiglitazone (all at 25 μmol/L; Figures 2A and 2C). Interestingly, VEGFR1 expression levels were largely unchanged by treatment with either PPARα or PPARγ ligands.

To explore whether the effects of PPAR agonist were affected by the presence of angiogenic stimuli, according to Xin et al.,10 VEGFR2 expression was also determined in the presence of PMA, VEGF, and bFGF, as well as PMA and growth factors (Figure 2B and 2C). In line with previous data, VEGF was seen to downregulate VEGFR2 expression by cultured ECs.18 This effect was antagonized by simultaneous administration of PMA. Pertinent to the objective, however, the effects of PPAR ligands on VEGFR2 expression were not affected by the presence of angiogenic stimuli. In particular, PPARγ ligands (rosiglitazone, Figure 2C; ciglitazone, not shown) failed to show considerable inhibitory effects under the chosen experimental conditions.

**PPARα Activator Wy14643 Inhibits Basal and VEGF-Induced Formation of Capillary-Like Structures In Vitro**

To evaluate whether PPARα-mediated inhibition of VEGFR2 expression may compromise VEGFR2-dependent endothelial cell functions, we studied the effect of Wy14643 on the ability of HUVECs to form capillary-like structures (Figure 4). Spontaneous and VEGF-induced capillary-like structures, formed by ECs that were either left untreated or were preincubated with Wy14643, was analyzed 12 hours after seeding on Matrigel. Treatment by Wy14643 significantly inhibited both spontaneous and VEGF-induced tube-like formation of HUVECs on Matrigel. These data suggest that...
PPARα activation mediates direct effects on endothelial cell capabilities, which probably involve inhibition of VEGFR2 expression. On the contrary, preincubation with PPARγ ligand ciglitazone revealed no significant suppression on capillary-like structures in the used short-term in vitro Matrigel assay. These findings are in line with our data obtained from EC migration assays (Figure 3C). Whereas the PPARα agonists Wy14643 and fenofibrate inhibited cell migration considerably, PPARγ ligands revealed no significant suppressive effects on the migratory capacity of ECs.

Figure 2. Effects on VEGFR2 and VEGFR1 protein expression by PPARα and PPARγ activators in untreated and stimulated HUVECs. Representative Western blot analyses. A, ECs were left untreated (solvent only; line 1) or were treated with Wy14643 (Wy, 200 μmol/L; lane 2), ciglitazone (Cigl, 25 μmol/L; lane 3), troglitazone (Tro, 25 μmol/L; lane 4), or 15d-PGJ2 (25 μmol/L; lane 5) for 24 hours. Densitometry of bands was quantified using ImageJ (v1.29s); optical densities (ODs) of VEGFR bands were corrected for loading differences based on corresponding tubulin bands; fold difference in ODs of VEGFR bands with respect to untreated controls based on 3 independent experiments: VEGFR2: Wy, 0.02±0.05; Cigl, 0.83±0.21; Tro, 0.89±0.2; 15d-PGJ2, 0.92±0.18; VEGFR1: Wy, 0.79±0.18; Cigl, 1.16±0.17; Tro, 1.29±0.3; 15d-PGJ2, 1.31±0.13. B and C, ECs were treated with solvent only or with the respective PPAR agonist (B, Wy at 200 μmol/L; C, rosiglitazone, Rosi at 25 μmol/L) in the absence or presence of PMA (at 80 nmol/L) or bFGF (at 40 ng/ml); fold difference in ODs of VEGFR bands with respect to untreated controls: VEGFR2: Rosi, 0.92±0.15.

Figure 3. Determination of the cytotoxic potential and the effects on proliferation and migration of PPAR activators on cultured HUVECs. A, Quantification of HUVEC proliferation; average ODs (mean±SD) from 6 wells per experimental condition are displayed; data are expressed as cell proliferation in percentage (%) with regard to solvent controls (=100%; DMSO, 0.3%). B, Quantification of cytotoxicity; average ODs (mean±SD) from quadruplicate determinations per experimental condition were calculated; data are expressed as cytotoxicity in percentage (%). Data displayed are representative of 3 experiments. C, HUVECs were seeded in the top compartment in the absence or presence of PPAR agonists as indicated (Wy14643: Wy, 200 μmol/L; lane 2; fenofibrate, Feno, 200 μmol/L; lane 3; ciglitazone, Cigl, 25 μmol/L; lane 4; rosiglitazone, Rosi, 25 μmol/L; lane 5). Nuclei from cells that migrated to the lower face of the membrane were counted in 5 random high-power fields/filter. Results are the mean±SE of the number of migrated cells per field from 3 independent determinations; Student’s t test. n.s. indicates not significant.
PPARα Activators Suppress Steady-State VEGFR2 mRNA Levels in Human Endothelial Cells

To establish that inhibition of VEGFR2 protein synthesis by PPARα activators corresponds to comparable changes of specific mRNA expression, we analyzed the effects of different PPAR ligands on steady-state VEGFR2 transcript levels in HUVECs (Figure 5). Treatment with PPARα activators considerably suppressed VEGFR2 mRNA accumulation, whereas PPARγ ligand ciglitazone revealed no discernible effects. The inhibitory properties of PPARα activators on VEGFR2 protein expression are thus a result of reduced mRNA steady-state expression, suggesting that the inhibitory properties are mediated at the transcriptional level.

Inhibition of VEGFR2 Promoter Activity in Response to PPARα Activator Wy14643 Is Conveyed by a Proximal Cluster of Sp1 Binding Sites

Analyses of the luciferase (Luc) expressions of 5‘-deletion VEGFR2 promoter–based constructs in controls and Wy14643-treated cells showed significant baseline activity of the −4 kb/+296-bp and −164/+268-bp VEGFR2 Luc constructs that was reduced in response to Wy14643 to about 40% (Figure 6A). Shorter constructs showed less constitutive expression compared with the longer reporter plasmids; however, considerable basal activity was retained, including the ability to significantly suppress reporter gene activity (0.37±0.08; P<0.03). Whereas ciprofibrate was effective in a fashion comparable to that seen by Wy14643, the PPARγ activators ciglitazone and rosiglitazone failed to inhibit VEGFR2 promoter activity (not shown). Because two adjacent Sp1 consensus binding sites are located at position −58 and −44 bp, we next explored the potential impact on transcriptional activation of a mutant −60/+268-bp Luc construct, in which critical two nucleotide-mutations19 were incorporated within the Sp1 sites. Analyses of the mutant −60/+268-bp construct showed loss of both basal and Wy14643-mediated inhibition of reporter gene expression. To further strengthen our assumption that Wy14643-induced inhibition is Sp1-dependent, the effects of PPARα activation on Sp1-mediated VEGFR2 transcription were analyzed (Figure 6B). These experiments revealed that Sp1-driven transcription is indeed subject to inhibition by Wy14643 treatment. In addition, cotransfection of PPARα reduced VEGFR2 promoter activity in a concentration-dependent fashion, an effect that was further enhanced by the presence.
of Wy14643 (Figure 6C). Hence, these data indicate that the repressive effects of fibrates on VEGFR2 occur at the transcriptional level via activation of PPARα.

**Constitutive Sp-Dependent Binding Activity to the −63/−31-bp VEGFR2 Promoter Sequence Is Reduced by PPARα Activator Wy14643**

We next explored whether Sp-dependent binding to the −63/−31-bp VEGFR2 promoter sequence is modulated by PPAR treatment. A DNA probe corresponding to the Sp1 cluster was utilized in EMSAs to investigate effects of PPARα activator Wy14643 on Sp-dependent binding (Figure 7). When incubated with nuclear extracts of untreated HUVECs, constitutive DNA binding activity of distinct complexes was observed (lane 7). In lysates of cells that were treated with Wy14643, a significant decrease in DNA binding activity was detected (lane 8). Treatment with PPARγ ligand ciglitazone did not influence DNA binding activity (lanes 5 and 6). Competition assays further underscored the assumption that nuclear proteins bind to the −63/−31-bp VEGFR2 promoter sequence in a Sp1 site-exclusive manner (lanes 1, 2, and 4). Addition of Sp1 as well as Sp3 antibody led to formation of a more slowly migrating complex that almost disappeared after WY14643 treatment (lanes 9 to 12), whereas addition of PPARα antibody did not exert discernible effects on complex or supershift formation (lanes 13 and 14).

**Sp1/PPARα Protein Interactions Are Subject to Regulation by PPARα Activator Wy14643 in HUVECs**

To determine whether decreased Sp-binding activity to the GC-rich core promoter in response to PPARα activation was mediated by changes in nuclear levels of Sp1 transcription factor, we analyzed nuclear HUVEC proteins for Sp1 expression in response to PPARα activator Wy14643 (Figure 8A). PPARα activation did not induce notable changes in Sp1 or PPARα protein expression, regardless whether HUVECs were incubated a short (1 hour; not shown) or long time (24 hours; Figure 8A). We therefore hypothesized that increases in protein interactions between PPARα and Sp1 may constitute a mechanism by which PPARα activator Wy14643 decreases Sp-dependent binding activity to the −63/−31-bp VEGFR2 promoter. In order to study interactions between PPARα and Sp1 proteins, whole protein extracts of untreated and Wy14643-treated HUVECs were immunoprecipitated with Sp1 antibody and were subsequently subjected to Western blot analyses with antibody directed against PPARα (Figure 8B). Although interactions between PPARα and Sp1 protein were already detectable constitutively, Wy14643 further in-
increased PPARα levels in anti-Sp1 immunoprecipitates. Thus, Sp1/PPARα protein interactions may be enhanced in response to PPARα activation, leading to potential transrepressive effects by which PPARα activators may inhibit VEGFR2 gene transcription.

**Discussion**

Our data indicate that PPARα activators may control inflammatory responses in part by targeting endothelial VEGFR2 expression, representing a key element of the VEGF/VEGFR signaling system during chronic inflammation. Strong evidence revealing a potential role for PPARs in inflammation control stemmed from work on PPARα-deficient mice, which showed a prolonged or exacerbated inflammatory response. Several studies aimed at resolving cellular mechanisms underlying the inflammation control by PPARs unveiled several modes of action that involve different cell types, including macrophages, hepatocytes, smooth muscle cells, and endothelial cells.

At the vascular level, both PPARα and PPARγ activators have been shown previously to repress basal and induced expression of endothelin-1. As another gene activated in inflammatory responses, monocyte-chemoattractant protein-1 expression by endothelial cells was shown to be inhibited by TZDs. In addition, PPARα activation by fibrates and other synthetic agonists has been demonstrated earlier to block the regulated vascular cell adhesion molecule-1 expression by human endothelial cells.

To control for adverse effects due to cytotoxicity, we determined the cytotoxic potential of different PPAR agonists at increasing doses in HUVECs (Figure 3B). These data showed cytotoxicity indices of about 10% with PPARα ligands at concentrations of up to 400 μmol/L. Conversely, the TZD PPARγ agonists revealed cytotoxicities of 15% to 20% by concentrations of 25 μmol/L and about 70% by 50 μmol/L. Consequently, the dose-related cytotoxic profile of PPARα and PPARγ activators is shown to be distinctly different in HUVECs, likely reflecting in part variations in ligand affinity binding. To better allow for comparison of PPARα and PPARγ activators with regard to their biological properties on endothelial gene expression, concentrations of the respective ligands were used that exerted comparable effects on cytotoxicity and cell proliferation (Figures 3A and 3B).
As to important cellular functions, PPARγ activators have been previously implicated in cell cycle withdrawal and in induction of terminal differentiation of several cell types. Inhibitory effects on the growth of different tumor cells were regarded as apparent cellular mechanisms mediating antitumor effects of PPARγ ligands. However, evidence is accumulating that observed antitumor activities in vivo may not be conveyed entirely by effects on the tumor cell itself, but may also reflect antiangiogenic effects on the tumor endothelium, as PPARγ activators suppress proliferation of endothelial cells and induce their apoptosis in vitro. This assumption is bolstered by recent experimental data suggesting that PPARγ ligands can inhibit tumor growth by suppressing angiogenesis in vivo.

We anticipated PPARγ rather than PPARα activators to target VEGFR2 expression, because 15d-PGJ2, which is regarded as a natural PPARγ agonist, was found previously to reduce VEGFR2 mRNA expression by HUVECs grown in growth factor–containing 3-dimensional Collagen Typ I gels. In contrast, we observed in monolayer HUVEC cultures PPARα rather than PPARγ ligands to suppress VEGFR2 expression. The putative discrepancy to the earlier study by Xin et al may presumably be explained by differences in the experimental setup, as HUVECs were seeded in collagen gels as opposed to be grown as monolayers. Whereas 15d-PGJ2 was seen to inhibit tube formation in long-term (48 hours) 3-dimensional collagen assays previously, our experiments on the capacity of PPAR activators to interfere with short-term (12 hours) formation of capillary-like structures after EC seeding on Matrigel revealed no discernible suppression in response to the PPARγ ligand ciglitazone. In the present study, the different findings may be related to the different length of exposure to the respective PPAR ligands. Conceivably, the antiangiogenic effects of PPARγ activators need longer time to take effect, and may therefore be detectable preferentially in long-term in vitro angiogenesis assays. In addition, the apparent antiangiogenic properties of PPARγ activators may be mediated primarily by their capacity to control cell cycling rather than by suppressing endothelial VEGFR2 expression. This assumption is supported by data showing pronounced effects of PPARγ ligands as opposed to PPARα agonists on proliferation and cytotoxicity both in human ECs (Figure 3) and in B lymphocytes and B lymphoma cells.

As a major control point of gene expression, transcriptional activation has been previously identified as a key regulatory mechanism of VEGFR2 expression. Our data indicate that the repressive effects of fibrates on VEGFR2 occur at the transcriptional level via activation of PPARα. A promoter region with two adjacent Sp1 binding sites between base pairs −60 and −37 was identified that conveys Wy14643-mediated transrepression of the VEGFR2 gene (Figure 6A). Our findings support the hypothesis that activation of PPARα inhibits VEGFR2 gene transcription via decreasing Sp1 site-dependent binding to the −63/−31 bp promoter sequence.

At the molecular level, PPARs have been demonstrated previously to repress gene transcription by restricting binding of specific transcription factors to respective response elements, or by competing with coactivators necessary for transcriptional activation. Pertinent to our findings, PPARα activator Wy14643 has been indicated recently to inhibit inducible Sp1/Smad4 complex formation as a mechanism to block transforming growth factor-β-induced β integrin transcription in vascular smooth muscle cells. Because decreased Sp1-dependent binding activity to the GC-rich core promoter in response to PPARα activation was apparently not mediated by changes in nuclear levels of Sp1 transcription factor (Figure 8A), we explored whether direct Sp1-PPARα contacts may constitute a putative mechanism by which PPARα activation may decrease Sp-binding activity to the VEGFR2 promoter. Indeed, our communoprecipitation experiments revealed that PPARα activation may increase direct protein interactions between Sp1 and PPARα (Figure 8B). Thus, transrepression may represent a likely mechanism, by which PPARα activators mediate, at least in part, their inhibitory effects on VEGFR2 gene transcription. Therefore, we here provide first evidence that PPARα activation may exert transrepressive activity via increased interaction with Sp1 transcription factor.

In conclusion, our data identify VEGFR2 expression as an additional target of PPARα activation in endothelial cells. Although all PPAR family members are expressed by vascular endothelium, only PPARα but not PPARγ ligands directly affected VEGFR2 gene expression. As indicated by our studies, molecular mechanisms by which PPARs modulate the rate of gene transcription may include direct interactions between specific transcription factors and PPARs that ultimately result in reduced DNA binding to their respective response elements. Identification of target genes that are repressed in response to PPAR activation as well as further elucidation of the negative control mechanisms will likely help to better define the therapeutic potential and clinical indications of PPAR activators in vascular-dependent diseases.

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