PPAR-\(\gamma\) Activation Inhibits Angiogenesis by Blocking ELR+CXC Chemokine Production in Non-small Cell Lung Cancer\(^1\)

Venkateshwar G. Keshamouni, Douglas A. Arenberg, Raju C. Reddy, Michael J. Newstead, Shalini Anthwal and Theodore J. Standiford

Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109, USA

Abstract

Activation of peroxisome proliferator–activated receptor-\(\gamma\) (PPAR-\(\gamma\)) results in inhibition of tumor growth in various types of cancers, but the mechanism(s) by which PPAR-\(\gamma\) induces growth arrest has not been completely defined. In a recent study, we demonstrated that treatment of A549 (human non small cell lung cancer cell line) tumor-bearing SCID mice with PPAR-\(\gamma\) ligands troglitazone (Tro) and pioglitazone significantly inhibits primary tumor growth. In this study, immunohistochemical analysis of Tro-treated and Pio-treated tumors with factor VIII antibody revealed a significant reduction in blood vessel density compared to tumors in control animals, suggesting inhibition of angiogenesis. Further analysis showed that treatment of A549 cells \textit{in vitro} with Tro or transient transfection of A549 cells with constitutively active PPAR-\(\gamma\) (VP16–PPAR-\(\gamma\)) construct blocked the production of the angiogenic ELR +CXC chemokines IL-8 (CXCL8), ENA-78 (CXCL5), and Gro-\(\alpha\) (CXCL1). Similarly, an inhibitor of NF-\(\kappa\)B activation (PDTC) also blocked CXCL8, CXCL5, and CXCL1 production, consistent with their NF-\(\kappa\)B–dependent regulation. Conditioned media from A549 cells induce human microvascular endothelial cell (HMVEC) chemotaxis. However, conditioned media from Tro-treated A549 cells induced significantly less HMVEC chemotaxis compared to untreated A549 cells. Furthermore, PPAR-\(\gamma\) activation inhibited NF-\(\kappa\)B transcriptional activity, as assessed by TransAM reporter gene assay. Collectively, our data suggest that PPAR-\(\gamma\) ligands can inhibit tumor-associated angiogenesis by blocking the production of ELR+CXC chemokines, which is mediated through antagonizing NF-\(\kappa\)B activation. These antiangiogenic effects likely contribute to the inhibition of primary tumor growth by PPAR-\(\gamma\) ligands.

Introduction

Peroxisome proliferator–activated receptors (PPARs) are a family of ligand-activated transcription factors consisting of three isotypes: PPAR-\(\alpha\), PPAR-\(\delta\), and PPAR-\(\gamma\). PPARs serve as intracellular sensors to fatty acids and fatty acid derivatives, which in turn act as endogenous ligands to PPARs, leading to transcriptional regulation of pathways involved in lipid and glucose metabolism. PPARs heterodimerize with retinoid X receptors (RXRs), forming a complex that regulates target gene expression. Ligand binding results in a conformational change in the receptor that permits the dissociation of corepressors and concomitant association of coactivators. The heterodimer–coactivator complex binds to specific response elements (PPREs) in the promoter regions of target genes to regulate transcription. PPAR-\(\gamma\) is highly expressed in adipose tissues and is a master regulator of adipocyte differentiation \([1,2]\). In humans, PPAR-\(\gamma\) is expressed in multiple other tissues, including breast, colon, prostate, lung, placenta, and pituitary tissues \([3–6]\). PPAR-\(\gamma\) is a molecular target for thiazolidinediones (TZDs), a class of insulin-sensitizing agents such as troglitazone (Tro), rosiglitazone, and pioglitazone (Pio). PPAR-\(\gamma\) activation is antiproliferative by virtue of its differentiation-promoting effects. For example, ligands activating PPAR-\(\gamma\) were effective in arresting the growth of dedifferentiated tumor cells in multiple tumor types \([3,5,7–9]\), making PPAR-\(\gamma\) a potential therapeutic target for cancer. In a recent study, we have shown that PPAR-\(\gamma\) ligands Tro and Pio inhibit the growth of NSCLC cells \textit{in vitro} and \textit{in vivo} \([8]\). We have also shown that PPAR-\(\gamma\) ligands promote differentiation of tumor cells and block spontaneous metastasis in a xenograft model \([8]\). However, the mechanisms by which these ligands inhibit tumor growth and metastasis have not been completely defined.

Abbreviations: NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; PPAR-\(\gamma\), peroxisome proliferator–activated receptor-gamma; HMVEC, human microvascular endothelial cells; CM, conditioned medium; ENA, epithelial neutrophil-activating peptide; GRO, growth-related oncogene; IL, interleukin; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; HPF, high-power field; Tro, troglitazone; Pio, pioglitazone.

Address all correspondence to: Theodore J. Standiford, MD, Division of Pulmonary and Critical Care Medicine, University of Michigan Medical Center, 6301 MSRB III, 1150 West Medical Center Drive, Ann Arbor, MI 48109. E-mail: standif@med.umich.edu

\(^1\)This research is supported, in part, by an American Lung Association grant (RG-134-N to T.J.S.), a Flight Attendant Medical Research Institute grant (N005884 to V.G.K.), and National Institutes of Health grants (P050 HL60289 and HL57243 to T.J.S.).

Received 7 September 2004; Revised 28 September 2004; Accepted 2 October 2004.

Copyright © 2005 Neoplasia Press, Inc. All rights reserved 1522-8002/05/$25.00 DOI 10.1593/neo.04601
Angiogenesis is an essential component of tumor progression and metastasis. A variety of factors have been found to modulate angiogenesis [10]. Net tumor-induced angiogenesis is believed to be due to an imbalance in the expression of angiogenic factors relative to angiostatic factors [11]. Among the factors that can regulate angiogenesis is a unique family of 8- to 10-kDa molecules known as CXC chemokines. These molecules are initially characterized as a family of neutrophil chemotactic factors during inflammation. However, their angiogenic activity is distinct from their ability to induce inflammation. The CXC chemokine family consists of a number of structurally related peptides that either promote or inhibit angiogenesis. All of the angiogenic members of CXC chemokine family such as IL-8 (CXCL8), ENA78 (CXCL5), and Gro-α (CXCL1) contain a three–amino-acid motif of glutamic acid–leucine–arginine (ELR motif) residues, which precedes the first cysteine residue in their CXC motif present in their amino terminus [12]. The angiogenic activity of ELR+CXC chemokines is mediated through the CXC motif ligand receptor 2 (CXCR2) [13]. CXCR2 has been shown to bind all ELR+CXC chemokines with high affinity [14–17]. Previous studies have established the role of these ELR+CXC chemokines in human NSCLC [10]. The transcription factor nuclear factor–kappa B (NF-κB) regulates the expression of several genes involved in inflammation, including that of CXC chemokines [18–22]. Promoter regions of CXC chemokine genes contain NF-κB–binding sites. The expression of CXCL8, CXCL5, and CXCL1 is upregulated in response to different stimuli in an NF-κB–dependent fashion [18–21,23]. PPAR-γ, in addition to its differentiation-promoting and anti proliferative effects, has been described as a negative regulator of macrophage function [24]. It suppresses the production of inflammatory cytokines/chemokines, in part by antagonizing activities of transcription factors AP-1, STAT, and NF-κB [25]. In the present study, we investigated the role of PPAR-γ in regulating ELR+CXC chemokine expression from tumor cells, the effect of such regulation on endothelial cell function, and its implications for tumor-associated angiogenesis and primary tumor growth in NSCLC.

Materials and Methods

Cell Culture

The A549 human lung adenocarcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 medium with glutamine, supplemented with 10% FBS and antibiotics penicillin and streptomycin. Human microvascular endothelial cells (HMVEC-1) kindly provided by Dr. Edwin Ades and Mr. Francisco J. Candal (Center for Disease Control, National Center for Infectious Diseases, Atlanta, GA) and Dr. Thomas Lawley (Emory University, Atlanta, GA), were maintained in MDCB131 medium (GibcoBRL, Grand Island, NY) to which fetal calf serum (FCS) (10%), 100 U/ml penicillin, 100 mg/ml streptomycin, 1% l-glutamine, 10 ng/ml endothelial growth factor (EGF), and 1 mg/ml hydrocortisone had been added. Media were changed in all cell lines every 48 to 72 hours. All cell lines were incubated at 37°C with 5% CO2.

Tumor Xenografts in SCID Mice

Six-week–old CB17-SCID mice were subcutaneously injected with A549 cells on either side of the dorsal flank. Six mice per group were treated once daily by oral gavage for 8 weeks with Tro (200 mg/kg) or Pio (25 mg/kg) or placebo, formulated by suspending drugs in an aqueous solution of 2% carboxymethylcellulose and 0.2% Tween 20 and then sonicating for 5 minutes. After 8 weeks, animals were sacrificed and tumors were dissected from the mice, fixed in formalin, and embedded in paraffin for thin sectioning.

Quantification of Vessel Density in Tumors

Quantitation of vessel density was performed using the method described previously [26,27]. In brief, tissue sections from paraffin-embedded tumors of control-treated, Tro-treated (200 mg/kg), and Pio-treated (25 mg/kg) A549 xenografts were dewaxed and rehydrated. Slides were stained for endothelial cells using antibody to factor VIII–related antigen conjugated to horseradish peroxidase (EP-OS staining reagent; DAKO Corporation, Carpinteria, CA) for 1 hour at room temperature. 3,3′-Diaminobenzidine (Vector Laboratories, Burlingame, CA) was used for localization of factor VIII–related antigen. After optimal color development, sections were immersed in water and coverslipped. Tissue sections were scanned at low magnification (×40) to identify vascular hot spots. Areas of greatest vessel density were then examined under higher magnification (×200) and counted. Any distinct area of positive staining for factor VIII–related antigen was counted as a single vessel. Two investigators, blinded to the identity of the specimen, independently scored sections from each tumor. The average of three high-power fields (HPFs) per section was taken, and the results for each treatment group were expressed as the mean ± SE, representing a minimum of 12 tumors per group.

ELR+CXC Chemokine ELISA

ELR+CXC chemokines [IL-8 (CXCL8), ENA-78 (CXCL5), and GRO-α (CXCL1)] were quantitated by a double-ligand method described previously [28]. In brief, flat-bottom 96-well microtiter plates (Immuno Plate F96; Nunc, Rochester, NY) were coated with the appropriate purified Abs overnight at 4°C and were then washed three times with PBS with 0.05% Tween 20 (washing buffer). Nonspecific binding sites were blocked with PBS and 2% BSA. After washing three times, samples (neat and 1:10 dilution) and standards were added as 50 μl aliquots and incubated at 37°C. Plates were washed three times and incubated with the respective biotinylated Ab at 37°C. Plates were then washed three times and incubated with poly peroxidase–avidin substrate (Endogen, Woburn, MA) at 37°C. Subsequently, plates were incubated with the peroxidase substrate (DAKO Corporation) at room temperature to the desired extinction. The reaction was terminated with 0.5 M H2SO4. Plates were read
at 490 nm in an ELISA reader. Standards were prepared as 1/2 log dilutions of purified recombinant Ab, from 100 to 0.001 ng/ml per well.

**Endothelial Cell Chemotaxis Assay**

Chemotaxis membrane filters (5.0 μm pore size) (Osmonics, Livermore, CA) were prepared by bathing in 2.8% glacial acetic acid for 24 hours, followed by two 1-hour rinses in deionized, distilled water. The filters were then transferred to a 0.01% gelatin bath to soak for 24 hours. Filters were dried under a laminar flow hood, and stored at room temperature for up to 1 month. Endothelial cell chemotaxis assays were performed in 12-well blind well chemotaxis chambers (Neuro Probe, Gaithersburg, MD). Briefly, human endothelial cells (HMEC-1) were suspended at a concentration of 10⁶ cells/ml in serum-free media with 0.1% BSA. Endothelial cells were placed into each of the bottom wells (165 μl). Membranes were placed over the wells, the gasket applied, and the chambers sealed. Chambers were inverted and incubated at 37°C with 5% CO₂ for 1 hour to allow for endothelial cell adherence to the membrane. Chambers were then reinverted and the test media were placed in the upper well (116 μl). Chambers were reincubated for 2 hours. Membranes were then scraped to remove any unmigrated endothelial cells from the lower chamber, fixed in methanol, and stained with a modified Wright-Giemsa stain, and cells that had migrated through the membrane were counted in 5 HPF (×200). Results were expressed as the mean number of endothelial cells that had migrated per HPF ± SE. Each sample was assessed in triplicate. Experiments were performed at least three times. Each experiment was accompanied by a positive control (VEGF 100 ng/ml) and a negative control (nonconditioned media) to account for variable chemotaxis from one assay to the next.

**Transient Transfection**

The constitutively active VP16–PPAR-γ fusion cDNA was obtained from Dr. Mitchel Lazar (University of Pennsylvania, Philadelphia, PA) and was subcloned into mammalian expression vector pcDNA under the control of CMV promoter. A549 cells were seeded in six-well plates and allowed to reach 60% to 70% confluency. The cells were transfected in the absence of serum with 2 μg/well of either VP16–PPAR-γ construct or the empty vector. After 4 hours of transfection, cells were washed and grown in the presence of 20% serum. After 24 hours, cells were treated with TNF-α in the absence of serum. Conditioned media were collected after 24 hours of TNF-α treatment for measuring chemokine levels.

**Cell Proliferation**

Cell proliferation was measured using Promega’s (Madison, WI) aqueous one-solution cell proliferation assay according to the manufacturer’s protocol. It is based on the cellular conversion of a tetrazolium salt into a soluble formazan product as a measure of proliferation. In brief, 2000 cells were plated in each well of a 96-well plate in RPMI 1640 medium with 10% serum. After 24 hours, cells were treated with various concentrations of PPAR-γ agonists. After 48 hours of incubation with agonists, the aqueous one-solution reagent was added and incubated for 2 to 3 hours. The intensity of the color was measured at 490 nm using a 96-well plate reader.

**NF-κB Activation Assay**

NF-κB activation was assayed using Active Motif’s (Carlsbad, CA) ELISA-based transactivation TransAM kit following the manufacturer’s protocol. The NF-κB TransAM kit contains a 96-well plate with immobilized oligonucleotides encoding an NF-κB consensus site (5’-GGGACTTTCC-3’). The active form of NF-κB contained in the cell extract specifically binds to this oligonucleotide. The primary antibody used to detect NF-κB recognizes an epitope on p65 that is accessible only when NF-κB is activated and bound to its target DNA. A horseradish peroxidase (HRP)–conjugated secondary antibody provides a sensitive colorimetric readout that is quantified by a spectrophotometer at 450 nm with a reference wavelength of 655 nm.

**Statistical Analysis**

For most of the analyses, unpaired t test was employed to determine P values, unless otherwise specified.

**Results**

**PPAR-γ Activation Inhibits Angiogenesis in A549 Xenografts**

We have previously shown that PPAR-γ ligands inhibit the growth of NSCLC cells in vitro and in vivo in part by promoting tumor cell differentiation [8]. A549 tumor-bearing SCID mice, with six mice in each group, were treated with either Tro (200 mg/kg) or Pio (25 mg/kg) or placebo once daily by oral gavage for 8 weeks. A significant reduction (65%) in primary tumor growth was observed at the end of 8 weeks [8]. Immunohistochemical analysis of primary tumors with factor VIII antibody, an endothelial cell marker, showed decreases in blood vessel density in Tro-treated and Pio-treated tumors at 8 weeks, compared to the placebo-treated control (Figure 1A). Quantitative analysis of blood vessel density by manual counting of factor VIII–stained vessels in the tumor sections revealed approximately 60% decrease in vessel density in Tro-treated and Pio-treated groups compared to the control (Figure 1B).

**PPAR-γ Activation Blocks Expression of Angiogenic Chemokines in Tumor Cells**

Tumor cells are one of the major sources for angiogenic modulators during tumor progression, and CXC chemokines expressed by tumor cells are believed to be attributed to tumor-associated angiogenesis. Specifically, ELR+CXC chemokines, including CXCL8, CXCL5, and CXCL1, mediate both in vivo and in vitro angiogenic activities by directly stimulating proliferation and migration of endothelial cells [29–33]. A549 cells secrete significant amounts of CXCL8, CXCL5, and CXCL1 constitutively, and increase
the expression of chemokines on stimulation with TNF-α (Figure 2A). The secreted levels of each chemokine were assessed by the respective chemokine-specific ELISA in the conditioned media collected from A549 cells. The conditioned media were collected from A549 cells after 24 hours of various treatments as indicated, in serum-free media. Treatment of A549 cells with the PPAR-γ agonist Tro blocked constitutive and TNF-α induced expression of CXCL8, CXCL5, and CXCL1 in a dose-dependent manner (Figure 2, A–C). Of note, treatment of A549 cells with Tro for 24 hours at the concentrations used did not effect proliferation or apoptosis significantly, as assessed by MTT assay and ssDNA ELISA, respectively [8]. Fusion of the potent viral transcription factor VP16 with PPAR-γ renders it constitutively active in a ligand-independent fashion [34]. Transient transfection of VP16–PPAR-γ into A549 cells also blocked CXCL8, CXCL5, and CXCL1 protein expression (Figure 2, D–F). This suggests that inhibition of CXC chemokine expression by Tro is dependent on PPAR-γ activation.

Inhibition of Angiogenic Chemokines Reduces Endothelial Cell Chemotactic Activity by Tumor Cells

One of the key endothelial cell functions that contribute to the progression of angiogenesis is endothelial cell migration. ELR+CXC chemokines induce endothelial cell migration through chemotactic signaling. To assess the effect of blocking ELR+CXC chemokine secretion by tumor cells on angiogenesis, we assayed the conditioned media from Tro-treated A549 cells for its ability to induce endothelial cell chemotaxis. Conditioned media from unstimulated A549 cells induced appreciable endothelial cell chemotaxis (Figure 3), which was significantly reduced in the conditioned media from Tro-treated A549 cells, consistent with the observed chemokine expression under those conditions. TNF-α–stimulated conditioned media induced significantly more endothelial cell chemotactic activity compared to the conditioned media from unstimulated cells. Importantly, Tro treatment also abolished the TNF-α–induced endothelial cell chemotaxis in the A549 conditioned media.

As an important control, we found that Tro treatment did not have any effect on the growth of HMVEC cells during the (2-hour) course of the chemotaxis assay. However, long-term treatment of up to 3 days with Tro inhibited HMVEC cell growth (Figure 4). This suggests that CXCL8, CXCL5, and CXCL1 secreted by A549 cells are important regulators of endothelial cell chemotaxis, and that inhibiting the expression of these chemokines in tumor cells will attenuate endothelial cell chemotaxis. In addition, Tro can also inhibit endothelial cell growth directly on long-term exposure.

PPAR-γ Activation Antagonizes NF-κB Activation

It is known from previous studies that expression of ELR+CXC chemokines and angiogenesis can be regulated by NF-κB activity [20]. It has also been shown that PPAR-γ agonists can antagonize NF-κB activation [24,25]. In the present study, we sought to elucidate whether PPAR-γ...
agonists can antagonize NF-κB activation, and, if so, whether the expression of ELR+CXC chemokines is NF-κB–dependent in NSCLC cells. Consistent with previous studies, we observed that pharmacologic inhibition of NF-κB activation by pyrrolidinecarbodithioic acid (PDTC) [35] blocked the expression of all the three ELR+CXC chemokines (Figure 5). Furthermore, the PPAR-γ agonist Tro inhibited both constitutive as well as TNF-α–induced NF-κB activation in A549 cells in a dose-dependent manner, as determined by ELISA-based transcriptional reporter assay. This suggests that modulation of NF-κB activity is involved in PPAR-γ ligand-induced inhibition of ELR+CXC chemokine expression.

Discussion
Lung cancer is the leading cause of cancer-related death for both men and women in the United States [36]. In most cases, lung cancer patients are diagnosed with advanced inoperable disease and left with the only option of systemic chemotherapy. Unfortunately, conventional therapies may have reached a ceiling of clinical impact as evidenced by the 5-year survival rates of 14% and 5% for NSCLC and SCLC, respectively. More innovative strategies and newer therapeutic agents are urgently needed for the control of this disease. Recent advances in cell and developmental biology led to the identification of novel antitumor approaches with potential therapeutic targets [37,38]. Because PPAR-γ is identified as a master regulator of adipocyte differentiation, it is being evaluated as a therapeutic target for promoting differentiation in dedifferentiated tumor cells. Ligands activating PPAR-γ have been shown to induce differentiation and inhibit tumor growth in multiple tumor types [3,5,7–9]. We and others have shown that PPAR-γ ligands can also inhibit the growth of NSCLC cells both in vitro and in vivo [39,8]. However, the precise mechanisms with which these ligands inhibit tumor growth are still not clear. In this study, we present an additional mechanism that may, in part, contribute to tumor growth inhibition achieved by PPAR-γ ligands.

Angiogenesis is a rate-limiting step in tumor development. Avascular tumors are limited in size by the diffusion distance of oxygen, nutrients, and cellular waste through the interstitium (approximately 100–200 mm). Although tumors often

---

Figure 2. Effect of PPAR-γ activation on ELR+CXC chemokine expression. Chemokine expression was quantified in A549 conditioned media by using chemokine-specific ELISA as described in Materials and Methods section after 24 hours of various treatments in the absence of serum. (A–C) CXCL5, CXCL8, and CXCL1 expression in conditioned media of unstimulated and TNF-α–stimulated (10 ng/ml) A549 cells in the presence and absence of PPAR-γ ligand Tro (10 and 20 μM). (D–F) CXCL5, CXCL8, and CXCL1 expression in conditioned media of unstimulated and TNF-α–stimulated (10 ng/ml) A549 cells in the presence and absence of transient VP16–PPAR-γ expression. Each column represents the mean ± SE from three independent experiments in (A)–(C) and two independent experiments in (D)–(F).

Keshamouni et al.
co-opt the existing vasculature, an angiogenic switch (i.e., the production of factors that induce angiogenic sprouting of the vasculature) is required for propagation, invasion, and subsequent metastasis of the tumor. A variety of factors, including ELR+CXC chemokines, are implicated in the growth and maintenance of new vasculature serving the tumor. In this study, we demonstrate the role of PPAR-γ in the regulation of ELR+CXC chemokine expression in NSCLC cells.

Figure 3. Endothelial cell chemotactic activity of A549 conditioned media after treatment with PPAR-γ ligand. Conditioned media from A549 cells were collected after various treatments in the absence of serum as indicated after 24 hours and assayed for its ability to induce endothelial cell chemotactic activity as described in Materials and Methods section. The number of cells that migrated through the membrane was counted in 5 HPF (>200). Results were expressed as the mean number of endothelial cells that had migrated per HPF ± SE from three independent experiments. Concentrations used are 10 and 20 μM Tro, and 10 ng/ml TNF-α. Serum-free RPMI 1640 medium alone was used as a negative control (Ctrl).

Figure 4. Effect of PPAR-γ ligand on endothelial cell proliferation. Cell proliferation was assayed by using CellTiter 96 Aqueous One Solution assay kit after treating serum-stimulated HMVEC for 3 days with different concentrations of Tro. Percent proliferation was calculated relative to the rate of proliferation in untreated cells, using the mean from three independent experiments.

kines in NSCLC, it is clear that the ability of PPAR-γ ligands to inhibit the expression of these chemokines by tumor cells will impact on the angiogenic potential of tumors. As predicted, conditioned media from Tro-treated A549 cells showed significant reduction in the ability to induce endothelial cell chemotaxis in a dose-dependent manner. Furthermore, PPAR-γ ligands also exerted direct growth-inhibitory effects on endothelial cells—consistent with previous reports [41] providing an additional mechanism whereby PPAR-γ can regulate angiogenic responses.

The transcription factor NF-κB plays an important role in regulating inflammatory and immune responses in mammalian systems. CXCL8, CXCL5, and CXCL1 chemokines have NF-κB binding sites in their promoter elements [42,43]. In conditions such as diabetic retinopathy and rheumatoid arthritis, TNF-α was shown to induce angiogenic ELR+CXC chemokines in a NF-κB-dependent manner [23]. Here we show that pharmacologic inhibition of NF-κB blocks the expression of CXCL8, CXCL5, and CXCL1 in A549 cells. Furthermore, PPAR-γ ligands inhibit constitutive as well as TNF-α–induced transcriptional activation of NF-κB in A549 cells. This suggests that modulation of NF-κB activity is involved in PPAR-γ ligand-induced inhibition of ELR+CXC chemokine expression. It has been proposed that PPAR-γ activation leads to consumption of transcriptional coactivators required for NF-κB activation by competitive recruitment, thus resulting in inhibition of NF-κB activation [24]. However, it has recently been shown that PPAR-γ forms a complex with the p65 subunit of NF-κB. As a result, the newly formed complex is exported from the nucleus, leading to the attenuation of NF-κB–mediated gene expression [44]. The ability of PPAR-γ ligands to antagonize TNF-α–induced NF-κB activation may have additional implications, including the possibility of sensitizing tumor cells to TNF-α–mediated apoptosis. Given the important role ELR+CXC chemokines play in the modulation of angiogenesis in NSCLC and the inhibition of ELR+CXC chemokine expression by PPAR-γ ligands in tumor cells, resulting in decreased ability of the tumor cells to stimulate angiogenesis, it is likely that the observed decrease in tumor vessel density by Tro or Pio
treatment is due to PPAR-γ-mediated inhibition of expression of angiogenic chemokines. The overall inhibition of tumor growth by Tro may be due to a combination of different PPAR-γ-dependent and PPAR-γ-independent effects.

In summary, our data suggest that PPAR-γ activation can inhibit tumor-associated angiogenesis by blocking the expression of ELR+CXC chemokines, which is mediated through NF-κB activation. These antiangiogenic effects likely contribute to the inhibition of primary tumor growth achieved by PPAR-γ ligands.

Acknowledgements

We thank Mitchell Lazar (University of Pennsylvania) for providing VP16–PPAR-γ cDNA constructs. V. G. Keshamouni is a Parker B. Francis Fellow in Pulmonary Research.

References


Figure 5. Effect of PPAR-γ ligand on NF-κB activation and effect of NF-κB inhibition on ELR+CXC chemokine expression. (A) NF-κB activity was assayed in unstimulated and TNF-α–stimulated (10 ng/ml) A549 cells in the presence and absence of PPAR-γ ligand Tro (10 and 20 μM) or NF-κB inhibitor PDTC (50 and 100 μM), using an ELISA-based TransAM kit. (B–D) CXCL5, CXCL8, and CXCL1 expression in conditioned media of TNF-α–stimulated (10 ng/ml) A549 cells in the presence and absence of NF-κB inhibitor PDTC (50 and 100 μM). Each column represents the mean ± SE from two independent experiments.


