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Cellular and Molecular Consequences of Peroxisome Proliferator–Activated Receptor-γ Activation in Ovarian Cancer Cells[1*]

Sara Vignati, Veronica Albertini*, Andrea Rinaldi*, Ivo Kwee*, Cristina Riva†, Rita Oldrini†, Carlo Capella†, Francesco Bertoni*, Giuseppina M. Carbone* and Carlo V. Catapano**

*Laboratory of Experimental Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; †Department of Human Morphology, University of Insubria, Varese, Italy

Abstract

Peroxisome proliferator–activated receptor-γ (PPAR-γ) is a ligand-activated transcription factor. In addition to its canonical role in lipid and glucose metabolism, PPAR-γ controls cell proliferation, death, and differentiation in several tissues. Here we have examined the expression of PPAR-γ in ovarian tumors and the cellular and molecular consequences of its activation in ovarian cancer cells. PPAR-γ was expressed in a large number of epithelial ovarian tumors and cell lines. The PPAR-γ ligand ciglitazone inhibited the growth and clonogenic survival of ovarian cancer cells, inducing cell cycle arrest and cell death. Growth inhibition by ciglitazone was reversed by the PPAR-γ antagonist GW9662, indicating the involvement of PPAR-γ–dependent mechanisms. Microarray-based gene profiling revealed complex changes in the transcriptional program of ovarian cancer cells on treatment with ciglitazone and identified multiple pathways that may contribute to PPAR-γ ligands' antitumor activity. Genes upregulated by ciglitazone were predominantly associated with metabolic, differentiation, and tumor-suppressor pathways, whereas downregulated genes were involved in cell proliferation, cell cycle, cell organization, and steroid biosynthesis. Collectively, our data indicate that PPAR-γ activation by selective agonists is a valid strategy for ovarian cancer therapy and prevention, and should be tested alone and in combination with other anticancer drugs.

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Introduction

Peroxisome proliferator–activated receptors (PPARs) are members of the nuclear hormone receptor superfamily that includes several ligand-activated transcription factors involved in a variety of physiological and pathological processes [1]. The PPAR subfamily consists of three closely related receptors, namely α, β/δ, and γ, which regulate metabolic, developmental, and differentiation pathways [1]. PPARs play important roles in pathological conditions, such as diabetes, atherosclerosis, and chronic inflammation, and are seen as valid therapeutic targets in a variety of human diseases [1]. PPAR-α is involved mainly in fatty acid metabolism and transport [1]. High-affinity ligands of this receptor, such as fenofibrate and bezafibrate, are effective hypolipidemic drugs [1]. PPAR-γ controls adipocyte differentiation, glucose metabolism, and lipid homeostasis, and synthetic PPAR-γ agonists, such as rosiglitazone and pioglitazone, are used as antidiabetic drugs [2]. PPAR-δ, which is the least studied of the three subtypes, is ubiquitously expressed and plays a role in cholesterol and lipid metabolism and in wound healing [3,4].

PPARs have the typical structure of nuclear hormone receptors with DNA-binding, ligand-binding, and transactivation domains [5]. PPARs form heterodimers with 9-*cis* retinoic acid receptor (RXR) and bind to specific peroxisome proliferator–activated receptor response elements (PPREs) in the promoter region of target genes [5]. PPARs bind a diverse group of lipophilic molecules, including long-chain fatty acids, prostaglandins, and leukotrienes [6,7]. Subtle changes in the ligand-binding pocket of each isotype confer distinct ligand specificity [8]. Ligand binding induces conformational remodeling, exposing regions of the receptor needed for interaction with coactivator molecules and for transactivation [8]. PPARs can regulate transcription by additional mechanisms leading to transrepression, instead of transactivation [9]. This aspect adds another level of complexity to the study of PPAR functions in physiological and pathological conditions.

In addition to their role in lipid and glucose metabolism, PPARs play a role in cancer development and represent

Abbreviations: EOC, epithelial ovarian cancer; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide; PPAR, peroxisome proliferator–activated receptor; PPRE, peroxisome proliferator–activated receptor response element; RT-PCR, reverse transcriptase–polymerase chain reaction; RXR, 9-*cis* retinoic acid receptor

Address all correspondence to: Carlo V. Catapano, Laboratory of Experimental Oncology, Oncology Institute of Southern Switzerland, *Via* Vela 6, Bellinzona CH-6500, Switzerland.
E-mail: carlo.catapano@irb.unisi.ch

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promising targets for novel cancer prevention and treatment strategies [10,11]. Numerous studies have suggested that PPAR-γ may act as a tumor suppressor at least in some tissues and cellular contexts [12]. Inactivating mutations, genetic deletions, or chromosomal translocations leading to functional inactivation of PPAR-γ have been detected in cancers of the colon, prostate, and thyroid [12–14]. Natural and synthetic PPAR-γ ligands, such as thiazolidinediones and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, induce the growth arrest and death of transformed cells *in vitro* [11,12,15–19]. PPAR-γ ligands inhibit the growth of human tumor xenografts in nude mice and reduce the frequency of spontaneous and carcinogen-induced preneoplastic and neoplastic lesions in animals [11,12,15,20]. Few studies, however, have reported an increase in the frequency of tumors in mice treated with synthetic PPAR-γ agonists [10,21,22]. PPAR-γ ligands have also been the subject of clinical investigations showing some activity in patients with advanced liposarcoma and prostate cancer [11,12,15,18].

Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer in Western countries, accounting for more deaths than endometrial and cervical cancer deaths combined [23]. EOC derives from the malignant transformation of the ovarian surface epithelium, which is contiguous with the peritoneal mesothelium [24]. Ovarian cancer is frequently diagnosed in advanced stages, with the disease spread in the peritoneum through direct implantation [23]. In these conditions, surgery is rarely curative, and postoperative chemotherapy is required [23]. Current therapies for advanced ovarian cancer are clearly inadequate, and new agents are needed for the treatment of this disease [23,24]. The objective of this study was to determine whether PPAR-γ could be a valid target for ovarian cancer therapy and prevention. We evaluated the expression of this nuclear receptor in ovarian tumors and examined the cellular and molecular consequences of PPAR-γ activation in ovarian cancer cells. Our data suggest that selective PPAR-γ agonists could be very effective agents in ovarian cancer and should be tested alone and in combination with other molecular-targeted agents or cytotoxic drugs.

Materials and Methods

Cell Lines, Reporter Vectors, and Compounds

The ovarian cancer cell lines A2780, OVCAR3, OVCAR5, OVCAR8, OVCAR432, SKOV3, and IGROV1 were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen Life Technologies, San Diego, CA). PREx3-tk-Luc reporter was a gift of Dr. R. Evans (The Salk Institute for Biological Studies, La Jolla, CA), and pRL-SV40 control vector was purchased from Promega Corporation (Madison WI). Ciglitazone and GW9662 were purchased from Alexis Corporation (Lausanne, Switzerland). The compounds were dissolved in DMSO at a concentration of 100 mM and were kept at −20°C. Drug dilutions were prepared in a tissue culture medium for each experiment and used immediately.

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR), Immunoblotting, and Immunohistochemistry

Cells (1×10^5 cells/ml) were plated in six-well plates and treated with ciglitazone. RNA was extracted from control and drug-treated cells using Trizol (Invitrogen Life Technologies). Using the SuperScript One-Step system from Invitrogen Life Technologies (see Table W1 for PCR primers and conditions), RT-PCR was performed. PCR products were separated on agarose gels, stained with ethidium bromide, and visualized with AlphaImager 3400 (Alpha Innotech Corporation, San Leandro, CA). For immunoblotting, cells were lysed in NP-40 lysis buffer (50 mM Tris–HCl, pH 7.4, 250 mM NaCl, 5 mM EDTA, 0.1% NP-40, 10 mM sodium orthovanadate, 2 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 50 mM sodium fluoride) for 30 minutes on ice. Gel electrophoresis and immunoblotting were performed as described [25]. Antibodies against PPAR-γ (H100 and E8), p53 (DO1), c-myc (9E10), and survivin (FL-1452) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against p21 (DCS60), bax, caspase 3 (3G2), and PTEN were purchased from Cell Signaling Technology (Danvers, MA). Antibodies against cyclin D1 (G124-326) and α-tubulin were purchased from Becton Dickinson AG (Basel, Switzerland) and Calbiochem-Merck Biosciences (Darmstadt, Germany), respectively. Peroxidase-conjugated secondary antibodies and the Enhanced Chemiluminescence system were obtained from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). Immunohistochemistry was performed with a mouse monoclonal antibody (E8) for PPAR-γ on 5-μm-thick sections obtained from paraffin blocks of normal ovaries (4), benign tumors, and ovarian tumors (27), including 3 benign tumors (i.e., 1 mucinous cystoadenoma and 2 Brenner tumors), 5 borderline tumors (i.e., 2 mucinous and 3 serous cystoadenomas), 14 surface epithelial carcinomas, 2 malignant mixed müllerian tumors, and 2 granulosa cell tumors. Antigen retrieval was performed by six cycles of 5 minutes each in a microwave in 10 mM citrate buffer (pH 6.0). Tissue sections were incubated overnight with an anti–PPAR-γ antibody (H100) at a 1:200 dilution. The intensity of PPAR-γ staining was scored as: (1+) = *faint*; (2+) = *moderate*; (3+) = *intense*. As negative control, the primary antibody was substituted with nonimmune mouse serum. The specificity of the primary antibody was also tested using tissues with or without known expressions of pertinent antigens.

Luciferase Reporter Gene Assay

Cells were plated in 48-well plates at a density of 5×10^4 cells/ml in RPMI 1640 with 10% charcoal-stripped serum (HyClone, Logan, UT) and transfected with 200 ng of PPREx3-tk-Luc reporter and 20 ng of pRL-SV40 vector using Lipofectamine (Life Technologies, Inc., Gaithersburg, MD). After incubating for 4 hours, the cells were washed and incubated in a medium with or without ciglitazone and GW9662. Cells were harvested 18 hours later to measure Firefly and Renilla luciferase activities using the Dual-Luciferase assay system (Promega Corporation). Data were expressed as percentages of luciferase activity in drug-treated cells, compared to control cells.