Target Genes of Peroxisome Proliferator-activated Receptor γ in Colorectal Cancer Cells*

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Activation of the nuclear hormone peroxisome proliferator-activated receptor γ (PPARγ) inhibits cell growth and promotes differentiation in a broad spectrum of epithelial derived tumor cell lines. Here we utilized microarray technology to identify PPARγ gene targets in intestinal epithelial cells. For each gene, the induction or repression was seen with two structurally distinct PPARγ agonists, and the change in expression could be blocked by co-treatment with a specific PPARγ antagonist. A majority of the genes could be regulated independently by a retinoid X receptor specific agonist. Genes implicated in lipid transport or storage (adipophilin and liver fatty acid-binding protein) were also activated by agonists of PPAR subtypes α and/or δ. In contrast, PPARγ-selective targets included genes linked to growth regulatory pathways (regenerating gene IA), colon epithelial cell maturation (GOB-4 and keratin 20), and immune modulation (neutrophil-gelatinase-associated lipocalin). Additionally, three different genes of the carci-noembryonic antigen family were induced by PPARγ. Cultured cells treated with PPARγ ligands demonstrated an increase in Ca2⁺-independent, carcinoembryonic antigen-dependent homotypic aggregation, suggesting a potential role for PPARγ in regulating intercellular adhesion. Collectively, these results will help define the mechanisms by which PPARγ regulates intestinal epithelial cell biology.

Peroxisome proliferator-activated receptor (PPAR) isoforms α, δ, and γ constitute a family of transcription factors that are members of the nuclear hormone receptor gene superfami-ly (1). PPAR subtypes form functional heterodimers with members of the retinoid X receptor (RXR) family of nuclear receptors (2).

PPARs play fundamental roles in dietary fat storage and metabolism (3). For example, PPARγ is a central component of the adipocyte differentiation program and regulates genes involved in lipogenesis and peripheral glucose utilization. In contrast, PPARα target genes are involved in fatty acid catabolism and utilization. Potential natural ligands for PPARs include a broad range of polyunsaturated fatty acids (4, 5) and the eicosanoids 15-deoxy-Δ12,14-prostaglandin J₂ (6, 7), leukotriene B₄ (8), (15S)-hydroxyeicosatetraenoic acid (9), and prostacyclin (5, 10). However, each of these molecules has effects independent of their ability to activate PPARs, and their role in PPAR signaling in vivo remains unclear. A number of high affinity synthetic ligands exist for both PPARα and -γ. Hypolipidemic fibrates are activators of PPARγ (11), whereas a class of anti-diabetic drugs known as thiazolidinediones are potent and selective activators of PPARγ (12).

Evidence is emerging that PPARs, and the γ isoform in particular, are important in regulating pathways beyond energy homeostasis (13). For example, activating ligands of PPARγ have been shown to modulate the growth of epithelial cells derived from diverse organs (14–18). This is perhaps most evident in the colon, which was found to express levels of PPARγ mRNA nearly equivalent to that found in adipocytes (19). Within the colon, the highest levels of receptor expression have been observed in post-mitotic, differentiated epithelial cells (20, 21). Consistent with this expression pattern, activation of PPARγ in cultured colon cancer cells induces growth inhibition and an increase in several markers of cellular differentiation (22, 23). Whether or not the anti-neoplastic effects of PPARγ ligands operate in vivo remains controversial. For example, agonists of the receptor reduce pre-malignant intestinal lesions in rats treated with the carcinogen azoxymethane (24) but slightly increase colon polyps in mice that are predisposed to intestinal adenomas because of a mutation in the adenomatosus polyposis coli tumor suppressor gene (25, 26). Finally, 8% of primary colorectal tumors were found to harbor a loss of function mutation in one allele of the PPARγ gene emphasizing a putative role for this receptor as a tumor suppressor in humans (27).

PPARγ may also be important in modulating the intestinal inflammatory response. Ligands for PPARγ have been shown to inhibit the induction of pro-inflammatory cytokines in colonic epithelial cells through an NF-κB-dependent mechanism (28). In mouse models of inflammatory bowel disease, mice treated with PPARγ agonists exhibit decreases in several indices of inflammation. In addition, mice that are heterozygous for the PPARγ gene show an increased sensitivity to chemicals that induce colitis (29).

To understand better how PPARγ regulates different facets of
intestinal epithelial cell biology, we sought to identify PPARγ-regulated genes in a human colorectal cancer cell line using microarray technology. PPARγ-selective targets included genes involved in the regulation of cell growth, epithelial cell maturation, and inflammation. In addition, three different members of the carcinoembryonic antigen (CEA) family were induced by PPARγ. Consistent with this, exposure of colon cancer cells to a PPARγ ligand induced an increase in Ca2+-independent, CEA-dependent homotypic aggregation, suggesting a potential role for PPARγ in regulating intercellular adhesion.

EXPERIMENTAL PROCEDURES

Materials and Cell Culture—The MOSER S (M-S) (30) colon carcinoma line was a gift of M. Brattain (University of Texas Health Sciences, San Antonio, TX), and COS7 cells were purchased from the ATCC. Cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS; HyClone), 1-glutamine (2 mmol/l), penicillin (100 units/ml), and streptomycin (100 μg/ml) in a 5% CO2 atmosphere with constant humidity. For all experiments in which a receptor ligand was added, cells were grown in the above media except regular 10% FBS was replaced with 10% charcoal-stripped FBS (HyClone). All total RNA isolations were done using the TRI-reagent (Molecular Research Center).

Receptor Ligands—All synthetic PPAR and RXR ligands were from GlaxoSmithKline and dissolved in Me2SO. The Me2SO concentration of all experiments was kept constant at 0.1%. The following synthetic ligands were used to introduce the specificity of each induction or repression: rosiglitazone (thiazolidinedione PPARγ agonist (31)), GW7845 (rosiglitazone analogue PPARγ agonist (32)), GW9662 (irreversible PPARγ antagonist (9)), GW7647 (PPARγ agonist (33)), GW1514 (PPARγ agonist (34)), and LG100268 (RXR agonist). Each compound was used at a concentration where it is selective for the indicated isoform.

Western Blot Analysis—M-S cells and COS7 cells transiently transfected with either mPPARγ/pCDNA3.0, hPPARα/pCDNA3.1, or hPPARα/pBKCMV were harvested in ice-cold 1× phosphate-buffered saline (1× PBS), and cell pellets were lysed in RIPA buffer. Centrifuged lysates (50 μg) from each cell line were fractionated on a 4–20% gradient SDS-polyacrylamide gel and electrophoretically transferred to a polyvinylidene difluoride membrane (PerkinElmer Life Sciences). Membranes were blocked for 1 h at room temperature in Tris-buffered saline containing 0.1% Tween 20 (TBST) and 5% powdered milk. Blots were probed with the following primary antibodies overnight at 4 °C: monoclonal anti-PPARγ (Santa Cruz Biotechnology; 1:500), rabbit polyclonal anti-PPARδ (35); 1:500), or monoclonal anti-PPARα (Abcam; 1:500). This was followed by incubation with the appropriate horseradish peroxidase-conjugated secondary antibody (The Jackson Laboratory) at a dilution of 1:50,000 for 1 h. Detection of immunoactive polypeptides was accomplished using an enhanced chemiluminescence system (Amersham Pharmacia Biotech).

Transfections and Luciferase Assays—M-S cells (5.0 × 104/well in 24-well plates) were transfected with a mix containing 0.66 μg/luciferase (a gift of R. Evans, Salk Institute (La Jolla, CA)), GW1514 2 (PPARγ agonist), PPRE3-tk-luciferase (a gift of R. Evans, Salk Institute (La Jolla, CA)), and 1 μg/pCMV (a gift of F. Verdine, Boston College). Filters were pre-hybridized for 4 h at 42 °C in Ultrahyb (Ambion). Hybridization was conducted in the same buffer in the presence of 32P-labeled partial cDNA fragment of the indicated gene. Blots were washed 4 times for 15 min at 50 °C in 2× SSC, 0.1% SDS, and once for 30 min in 1× SSC, 0.1% SDS. Membranes were then exposed to a PhosphorImage screen, and images were analyzed using a Cyclone Storage Phoscope System and Optiquant Software (Hewlett-Packard). Each membrane was analyzed with a [32P]dCTP-labeled probe for 1B15/cyclinG (37) to normalize for differences in RNA loading.

Synthesis of cDNA Fragments—Partial cDNA fragments for adipophilin (25), keratin 20, BFGF, and CEA were generated by PCR with M13 forward and reverse primers using a sequence-validated human IMAGE cDNA clone (Research Genetics) of each gene as a template. Partial cDNA fragments for L-FABP and Gob-4 were generated using reverse transcriptase-PCR and gene-specific primers corresponding to 56–375 base pairs for L-FABP and 238–550 base pairs for Gob-4 (each from the transcriptional start site). The template for these PCR was a random-primer cDNA library of M-S cells treated with either 0.1% Me2SO or 1 μM rosiglitazone. Both of these PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen) and sequenced to confirm gene identity. A 95% confidence interval was obtained for the estimate of “true positives” found in the sample of 14 genes based on Northern blot. This 95% confidence interval can be used to estimate the interval that for 95% of the time will cover true value of the proportion of true positives. This can then be used as an estimate for the proportion of true positives from the original 89 genes the Affymetrix software detected as important.

Northern Hybridization Analysis—Northern blot analysis was performed as described previously (10). Exponentially growing M-S cells were treated with the indicated PPAR or RXR ligand for 24 h or 6 days. Total RNA from each sample (20 μg) was fractionated on a 1.2% agarose-formaldehyde gel and transferred to a Hybond-N nylon membrane (Amersham Pharmacia Biotech). Filters were pre-hybridized for 4 h at 42 °C in Ultrahyb (Ambion). Hybridization was conducted in the same buffer in the presence of 32P-labeled partial cDNA fragment of the indicated gene. Blots were washed 4 times for 15 min at 50 °C in 2× SSC, 0.1% SDS, and once for 30 min in 1× SSC, 0.1% SDS. Membranes were then exposed to a PhosphorImage screen, and images were analyzed using a Cyclone Storage Phoscope System and Optiquant Software (Hewlett-Packard). Each membrane was hybridized with a [32P]dCTP-labeled probe for 1B15/cyclinG (37) to normalize for differences in RNA loading.

Affymetrix GeneChip Expression Measurements—The Affymetrix GeneChip Expression Measurements protocol was used. All samples were treated with 0.1% Me2SO or 1 μM GW7845 for 4 h. The RNA was subsequently treated with DNase I and cleaned up using the RNeasy mini kit (Qiagen). Samples were sent to the DNA Facility at the University of Iowa (Iowa City, IA), where the RNA samples were converted to biotinylated cRNA and probed to the Affymetrix Human GeneChip GeneChip arrays according to manufacturer’s instructions. The blots were imaged using the Cyclone Storage Phoscope System (Hewlett-Packard) and imported into the Pathways Software (Research Genetics) to identify differences in the intensity of cDNA spots between different treatments.

Oligonucleotide Microarray Screening—Total RNA was isolated from M-S cells exposed to 0.1% Me2SO or 1 μM of the PPARγ-ligand GW7845 for 24 h. The RNA was subsequently treated with DNase I and cleaned up using the RNeasy mini kit (Qiagen). Samples were sent to the DNA Facility at the University of Iowa (Iowa City, IA), where the RNA samples were converted to biotinylated cRNA and probed to the Affymetrix Human GeneChip GeneChip arrays according to manufacturer’s instructions. The blots were imaged using the Cyclone Storage Phoscope System (Hewlett-Packard) and imported into the Pathways Software (Research Genetics) to identify differences in the intensity of cDNA spots between different treatments.

Results—Cells were plated at a density of 5.0 × 104/well in 6-well plates and replaced the next day with fresh media and compound. The cells were then harvested in 1× PBS with 1× EDTA. After resuspending the cells in 10 mM EDTA in 1× PBS, the cells were treated with the indicated PPAR or RXR ligand for 24 h or 6 days. Total RNA from each sample (20 μg) was fractionated on a 1.2% agarose-formaldehyde gel and transferred to a Hybond-N nylon membrane (Amersham Pharmacia Biotech). Filters were pre-hybridized for 4 h at 42 °C in Ultrahyb (Ambion). Hybridization was conducted in the same buffer in the presence of 32P-labeled partial cDNA fragment of the indicated gene. Blots were washed 4 times for 15 min at 50 °C in 2× SSC, 0.1% SDS, and once for 30 min in 1× SSC, 0.1% SDS. Membranes were then exposed to a PhosphorImage screen, and images were analyzed using a Cyclone Storage Phoscope System and Optiquant Software (Hewlett-Packard). Each membrane was analyzed with a [32P]dCTP-labeled probe for 1B15/cyclinG (37) to normalize for differences in RNA loading.

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Cell Aggregation Assay—The protocol used was identical to the methods reported by Yan et al. (38). Briefly, exponentially growing M-S cells were treated with 0.1% Me2SO or the indicated ligand for 4 days, with fresh media and compound added after the first 48 h. Cells were then harvested by gentle trypsinization with 0.025% trypsin and 0.01% EDTA. After resuspending the cells in 10 ml EDTA in 1× PBS (at a density of 2 × 106/ml), pelleted cells were resuspended in 5 ml EDTA, 1× PBS. This was followed by disaggregation that was achieved by repeated forceful passages through a Pasteur pipette to generate single cell suspensions. Aliquots were then transferred to a 15 × 100-mm test tube and shaken for the indicated time at room temperature (tubes were placed at a 45° angle). A sample was taken, and the total number of cells and the number of single cells for each sample were counted using a hemocytometer. Each experiment was done in triplicate. For the

2 GW1514 is a closely related analogue of GW501516 that shows comparable human PPARγ potency and selectivity. D. D. Sternbach, unpublished results.
antibody blocking experiments, Fab’ fragments from normal rabbit IgG (Santa Cruz Biotechnology) or a rabbit polyclonal anti-CEA antibody (CEA Ab-2, NeoMarkers) were isolated using papain digestion followed by incubation with protein A-agarose (Santa Cruz Biotechnology) to remove both undigested antibody and Fc fragments. The anti-CEA antibody reacts to CEA and CEA-like proteins including BGP and NCA. Fab’ fragments at a concentration of 1 mg/ml were added to the aggregation experiments when indicated.

RESULTS

Evaluation of Cell Culture System to Monitor PPARγ Target Genes—The M-S colon carcinoma line was evaluated for its suitability in studying gene expression changes in response to PPARγ activation in intestinal epithelial cells. The cell line was found to express protein for all three PPAR subtypes (Fig. 1A). PPARγ transcriptional activity was measured in cells transfected with the PPRE3-tk-luc reporter vector that contains a luciferase cDNA downstream of three tandem repeats of the PPAR-response element (PPRE) from the acyl-CoA oxidase gene (39). PPARγ agonists from two different chemical families (rosiglitazone and GW7845) induced a dose-dependent increase in reporter activity that could be blocked by co-treatment with the PPARγ antagonist GW9662 (Fig. 1B). Either of these two PPARγ agonists also induced a time-dependent decrease in cell number compared with vehicle-treated cells; this decrease in cell growth could be reversed by co-treatment with GW9662 (Fig. 2A). The PPARα-selective compound GW7647 or the PPARβ-selective ligand GW1514 had negligible effects on the growth of the M-S cells (Fig. 2B). Thus, activation of PPARγ activity in these cells induces a specific and significant decrease in cell number. This decrease in cell number was not due to an increase in apoptosis but rather a delay in the G1 phase of the cell cycle (data not shown).

Identification of PPARγ Target Genes Using Microarrays—Two different microarray technologies were used to identify genes induced or repressed in the M-S cells exposed to rosiglitazone (see “Experimental Procedures” for extended details). Membranes containing 5,184 non-control spotted cDNAs (Research Genetics) or oligonucleotide-based arrays that represent 5,600 unique genes (Affymetrix) were used. A short treatment time (24 h) was examined with the intent of identifying direct PPARγ target genes that are responsible for the growth inhibition and differentiation induced by activation of the receptor. An extended treatment time (6 days) was also evaluated to understand the gene expression pattern in cells that have already significantly growth-arrested due to PPARγ activation.

Data from the cDNA filter arrays were found to contain a large number of false positives and were used only to help interpret data obtained from the oligonucleotide-based arrays. By using the difference call decision matrix algorithm, the Affymetrix chip software identified a total of 89 genes (1.6% of transcripts) that were induced or repressed 2.5 times or greater by treatment with PPARγ agonists from two different chemical families (rosiglitazone and GW7845) or -agonist GW9662. Data are represented as fold activation over non-GW9662-treated cells. Cells were harvested, and the dual luciferase assay was performed. Data points represent the mean of three independent experiments. Error bars = S.E.

![Fig. 1. PPARγ is expressed and transcriptionally active in the M-S colon carcinoma cell line.](http://www.jbc.org/)

The nine identified genes fall into several different functional categories. Both adipophilin (40) (also known as adipose differentiation-related protein) and L-FABP (41) are involved in fatty acid transport or storage. Regeneration gene IA (RegIA) encodes a 166-amino acid secreted protein that induces the proliferation of pancreatic β and acinar cells (42). Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kilodalton protein that is thought to blunt inflammatory responses by binding to and sequestering hydrophobic molecules that serve as neutrophil chemoattractants (43). Two of the genes have already significantly growth-arrested due to PPARγ activation.

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been linked to colonic epithelial cell maturation. Gob-4 (also known as hAG-2) is a secreted protein whose expression is strongly associated with mature goblet cells in the intestine (44). Keratin 20 is an intermediate sized filament protein that is strongly expressed in the most differentiated cell types within the mucosal epithelium of the intestine (45, 46). PPARγ also induced three different genes, carcinoembryonic antigen (CEA), NCA, and biliary glycoprotein (BGP), that are all members of the CEA gene family. Proteins in this family have diverse functions in the regulation of cellular adhesion and differentiation (47). Finally, five of the genes (adipophilin, L-FABP, REGIA, GOB-4, and NGAL) were most strongly induced or repressed after 24 h of ligand treatment, whereas keratin 20, CEA, NCA, and BGP were all strongly induced only after 6 days of ligand treatment.

Characterizing the Specificity and Selectivity of Each Target Gene Induction / Repression — The synthetic PPARγ ligands used in our studies may have effects on gene expression independent of their ability to bind to and activate the receptor. Furthermore, since there has been little evidence for differences in the binding affinities of any of the three PPAR subtypes to a particular PPAR response element (48), each of the identified target genes might also be regulated by PPARα and -δ. Such gene targets may potentially be less interesting since ligands for either of these two receptor isoforms have no effects on the growth or differentiation of the M-S cells. To address these issues, the expression of each of the nine putative PPARγ target genes was examined in response to the full panel of synthetic ligands discussed in detail under “Experimental Procedures.”

All nine of the putative target genes could be induced or repressed by PPARγ agonists from two different chemical families (rosiglitazone and GW7845), and the change in expression could be blocked by co-treatment with the PPARγ antagonist GW9662 (Fig. 3-5). This suggests that the ability of PPARγ agonists to regulate the genes identified in this study are likely due to their ability to bind to PPARγ and not via a nonspecific target. Both of the gene targets involved in lipid storage or transport (adipophilin and L-FABP) were also induced by either the PPARα agonist GW7647 and/or the PPARδ agonist GW1514 (Fig. 3). The other seven targets were only activated by ligands for PPARγ (Figs. 4 and 5). In general, the putative function of genes selectively regulated by PPARγ correlated with the biological phenotype induced by PPARγ activation in colon epithelial cells. For example, only PPARγ ligands (and not ligands for PPARα or -δ) were able to regulate genes involved in growth control (e.g. RegIA) or colonocyte maturation (e.g. keratin 20) (Fig. 4).

The PPAR-RXR complex represents a type of RXR-dependent nuclear receptor heterodimer that has been defined as
“permissive” because PPAR does not block the ability of RXR to bind ligand and modulate gene transcription independent of the ligand occupancy status of PPAR (49). We evaluated the ability of each target gene to be regulated independently by the RXR-specific agonist LG100268 or synergistically by both LG100268 and rosiglitazone (Figs. 3–5). Of the nine gene targets, only the three CEA family proteins could not be independently induced by LG100268 (Fig. 5). Finally, there was no evidence that PPARγ and RXR ligands could synergistically regulate any of these nine target genes. Activation of PPARγ Induces an Increase in CEA-dependent Homotypic Aggregation—PPARγ ligands selectively up-regulated three different members of the CEA family of proteins (Fig. 5). The CEA gene family is a member of the immunoglobulin superfamily and consists largely of highly glycosylated cell-surface proteins. These are multifunctional proteins that can act as microbial receptors and regulators of cellular differentiation and adhesion (47). In fact, CEA (50), NCA (51), and BGP (52) have all been implicated in Ca2+-independent homophilic binding to molecules on adjacent cells (versus cadherins that require Ca2+ for homophilic binding). Previous studies have demonstrated that CEA proteins can induce homotypic aggregation of colon cancer cells, suggesting a potential role for these proteins in regulating intercellular adhesion during intestinal development or in the adult intestinal epithelium (50). We determined whether PPARγ ligands could induce cellular aggregation that is dependent on CEA or CEA-like proteins. Cells were forced into single suspension and allowed to re-aggregate over time in a Ca2+-free environment (to exclude cadherin-dependent aggregation). Exposure of M-S cells to rosiglitazone or GW7845 induced a time-dependent increase in the percent of aggregated cells (decrease in the percent of single cells) that could be reversed by co-treatment with the PPARγ antagonist GW9662 (Fig. 6A). This increase in cellular aggregation was dependent upon CEA family proteins since Fab’ fragments of a polyclonal antibody that recognizes CEA, NCA, and BGP could block the ability of PPARγ ligands to induce aggregation (Fig. 6B).

**DISCUSSION**

Although PPARγ was originally identified as a transcription factor essential for adipocyte differentiation, there is now increasing evidence to indicate a role for this receptor in the regulation of other cell types including macrophages, lymphocytes, and epithelial cells. In the case of intestinal epithelial cells, activation of PPARγ in colon cancer cells induces growth arrest and morphological changes consistent with differentiation. Ligands for the receptor also inhibit inflammatory cytokine gene induction in cultured colon cancer cell lines. To begin to address the mechanisms that explain these phenotypes, we sought to identify PPARγ-regulated genes in cultured human colorectal cells.

A majority of what is known about PPARγ and its target genes in other cell types has been gathered through the use of high affinity synthetic PPARγ agonists. However, these compounds are likely to have receptor-independent effects, particularly at high drug concentrations. For example, high micromolar levels of troglitazone or ciglitazone will inhibit inflammatory related genes in macrophages null for PPARγ (53). Genes identified in this study are likely to be true PPARγ targets because their change in expression was seen in response to low concentrations of PPARγ agonists from two different chemical families, and the effect could be blocked by co-treatment with a PPARγ antagonist. Of the nine PPARγ-regulated genes reported in this study, only L-FABP (54) and CEA (22) have been proposed previously as PPAR targets. The three CEA family genes and keratin 20 were only induced after more than 48 h of ligand treatment with maximal induction occurring after 6 days. Because of this, it seems unlikely that PPARγ directly regulates these four genes. On the other hand, adipophilin, L-FABP, RegLA, Gob-4, and NGAL were all induced or repressed as early as 6 h following ligand treatment, and the change in expression could...
be blocked by co-treatment with the protein synthesis inhibitor cycloheximide (data not shown). Thus, these five genes are likely direct PPARγ target genes. Only L-FABP has been reported to contain a functional PPRE in its 5′-regulatory region (54). It is not known how many of the other genes identified in this study also contain functional PREs. This would be particularly interesting to know for the two genes, REGIA and GOB-4, that are repressed by PPARγ activation. No negative cis-PPAR response elements that dictate ligand-dependent repression have been reported. It is possible that PPARγ represses these two genes via a transrepression mechanism that involves competition for limiting amounts of co-activators (55).

Most colon cancer cells express all three PPAR subtypes. Why activation of PPARγ, but not PPARα or PPARδ, is capable of inducing inhibition of colon cancer cell growth and differentiation is not known. Presumably, there are select target genes that only PPARγ is capable of regulating. Experiments reported here support such a notion. Adipophilin and L-FABP, genes with roles in "traditional" PPAR-related functions such as fatty acid transport and storage, are not selectively regulated by PPARγ. In contrast, genes that regulate cell proliferation (REGIA) or are linked to colonocyte maturation (keratin 20) are only induced by PPARγ agonists. The molecular basis for this type of specificity may be due to some combination of unique cis-acting sequences within certain target gene promot-ers and ligand-dependent interactions with isoform-specific co-factors. Castillo et al. (56) reported that the ability of PPARγ to uniquely induce adipocyte differentiation was due to the N-terminal AF-1 domain of the receptor. They further determined that this region binds to a cofactor, PCG-2, that does not interact with other PPAR subtypes. It would be of interest to know if this same domain of PPARγ is necessary for the selective activation of target genes reported here. There may also be unique epithelial cell cofactors that play a role in dictating PPAR subtype specificity.

Two of the more interesting PPARγ target genes identified in this study are REGIA and NGAL. REGIA encodes a 166-amino acid secreted protein that was cloned on the basis of its rapid induction in islets of Langerhans cells that were forced to regenerate following pancreatectomy (57). Reg gene products bind to a cell-surface receptor and have been shown to stimulate the proliferation of both pancreatic β-cells and ductal cells (58). Recently, it was shown that transgenic mice expressing RegIA in islet cells develop multiple tumors including cervical lymphoma, ovarian adenocarcinoma, and hepatocellular carcinoma (59). The non-pancreatic effects of RegIA in this system were presumably due to the high serum levels of RegIA in the transgenic mice. RegIA is overexpressed in a large percentage of colorectal tumors (60) and is also strongly expressed in the regenerating crypt epithelial cells of patients with ulcerative colitis (61). Furthermore, RegIA levels negatively correlate with survival rates following curative surgery of patients with colorectal cancer (62). In tissue culture, RegIA levels negatively correlate with the differentiation status of colon cancer cells (63). Collectively, these data argue that excessive RegIA may act as a factor that inappropriately stimulates intestinal epithelial proliferation and regeneration in pathological settings. The fact that PPARγ negatively regulates RegIA expression may in part explain the ability of the receptor to reduce the malignant potential of colon cancer cells or the level of tissue remodeling during chronic inflammation of the intestine. Because RegIA is a secreted protein that can be detected in the blood (64), serum levels of RegIA might be also a useful clinical indicator of the efficacy of PPARγ ligand therapy in patients with colorectal cancer or inflammatory bowel disease. Finally, RegIA overexpression in pancreatic islet cells induces diabetes (presumably via stimulation of excess islet cell regeneration) (59). Therefore, the observation that PPARγ ligands limit pancreatic β-cell injury and depletion in rats predisposed to insulin resistance (65) may be due to its ability to down-regulate RegIA expression in the pancreas.

NGAL is a member of the lipocalin family (43). Members of this family are characterized by their ability to bind and transport lipophilic molecules such as retinoids. It is possible that NGAL may be involved in pathways that regulate PPARγ ligand availability. Although NGAL was originally isolated as a component of the neutrophilic granule, it is now known to be expressed in epithelial cells from multiple organs (e.g. lung, stomach, and colon) that are exposed to microorganisms (66). Consistent with this, NGAL can bind bacterial formyl peptides and may act as a scavenger receptor to limit the pro-inflammatory effects of these molecules (67). Other pro-inflammatory ligands that NGAL can bind to and potentially sequester are leukotriene B4 and platelet-activating factor. The cyclooxygenase signaling pathway is often induced at sites of inflammation and produces ligands that can act as endogenous activators of PPARγ. The ability of ligand-activated PPARγ to induce anti-inflammatory genes such as NGAL may represent a negative feedback loop to limit the extent of the inflammatory response.

The role of CEA and related genes NCA and BGP in colon epithelial cell biology are complex. In the normal colon, CEA, NCA, and BGP are expressed in the most differentiated epithelial cells exposed to the lumen (68). It has been suggested...
that CEA proteins might regulate rapid adhesive interactions between microvilli. Alternatively, since CEA-surface proteins can act as receptors for bacteria and viruses, these proteins could play an important role in epithelial-microbial interactions. It is possible that PPARγ might regulate either of these processes in the normal colon by up-regulating CEA family proteins. In colorectal tumors (which often have very high levels of CEA) and in the embryonic intestine, CEA is found on adjacent cell membranes (50). In these circumstances, it has been proposed that CEA is a important component of the intercellular adhesive forces that allows the epithelium to develop into a multilayered array. This would suggest that unregulated levels of CEA in the normal adult colon could be pro-oncogenic and in fact overproduction of CEA blocks the differentiation program of rat myoblast cells (69). We have shown that PPARγ ligands induce an increase in CEA-dependent intercellular adhesion. This could point to a role for PPARγ in maintaining proper tissue architecture during embryonic development of the intestine. It might also explain the pro-tumorigenic effects of PPARγ ligands in some model systems. Clearly, the genomic response to activation of PPARγ is complex and will be highly dependent on cellular context. Future studies focused on the regulation and functional significance of the target genes reported here should increase our knowledge of the factors governing PPAR subtype specificity and the biological activity of PPARγ in intestinal epithelial cells.

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