# Prostacyclin-mediated activation of peroxisome proliferator-activated receptor δ in colorectal cancer

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**There is evidence from both genetic and pharmacologic studies to suggest that the cyclooxygenase-2 (COX-2) enzyme plays a causal role in the development of colorectal cancer. However, little is known about the identity or role of the eicosanoid receptor pathways activated by COX-derived prostaglandins (PG). We previously have reported that COX-2-derived prostacyclin promotes embryo implantation in the mouse uterus via activation of the nuclear hormone receptor peroxisome proliferator-activated receptor (PPAR) δ. In light of the recent finding that PPARδ is a target of β-catenin transactivation, it is important to determine whether this signaling pathway is operative during the development of colorectal cancer. Analysis of PPARδ mRNA in matched normal and tumor samples revealed that expression of PPARδ, similar to COX-2, is up-regulated in colorectal carcinomas. *In situ* hybridization studies demonstrate that PPARδ is expressed in normal colon and localized to the epithelial cells at the very tips of the mucosal glands. In contrast, expression of PPARδ mRNA in colorectal tumors was more widespread with increased levels in transformed epithelial cells. Analysis of PPARδ and COX-2 mRNA in serial sections suggested they were colocalized to the same region within a tumor. Finally, transient transfection assays established that endogenously synthesized prostacyclin ($PGI_2$) could serve as a ligand for PPARδ. In addition, the stable $PGI_2$ analog, carbaprostacyclin, and a synthetic PPARδ agonist induced transactivation of endogenous PPARδ in human colon carcinoma cells. We conclude from these observations that PPARδ, similar to COX-2, is aberrantly expressed in colorectal tumors and that endogenous PPARδ is transcriptionally responsive to $PGI_2$. However, the functional consequence of PPARδ activation in colon carcinogenesis still needs to be determined.**

Approximately 70–80% of human colorectal carcinomas have increased levels of cyclooxygenase-2 (COX-2) (1, 2), an enzyme that catalyzes the conversion of arachidonic acid (AA) to prostaglandin $H_2$, an unstable endoperoxide intermediate. Prostaglandin (PG) $H_2$ subsequently is converted to one of several structurally related eicosanoids, including $PGD_2$, $PGF_{2\alpha}$, $PGI_2$, and thromboxane $A_2$, by the activity of specific cellular PG synthases. PGs have been shown to play roles in a wide spectrum of biological processes (3). Traditionally, PGs are thought to exert most of their effects through activation of cell surface G protein-coupled receptors. Previous studies using both genetic and pharmacologic approaches have established that COX-2 plays a causal role in the development of colorectal cancer (4, 5). In addition, selective inhibitors of COX-2 inhibit the growth of adenomatous polyps in patients with familial adenomatous polyposis, highlighting the potential clinical utility of these drugs for the prevention and/or treatment of colorectal cancer (6). However, studies on the precise mechanism(s) by which COX-2 promotes tumorigenesis have lacked molecular definition, in large part because of a poor understanding of which eicosanoid receptors are activated by COX-2-derived PGs.

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are members of the nuclear hormone receptor superfamily. Three distinct PPAR isoforms, α, δ, and γ, have been isolated and characterized (7). PPARs bind to sequence-specific DNA response elements as a heterodimer with the retinoic acid receptor (RXR) (8). Although the identity of definitive high-affinity natural ligands for PPARs is lacking, there is evidence that AA metabolites can serve as activating ligands for PPARs. In particular, the $PGD_2$ metabolite, 15-deoxy$^{\Delta 12,14}$ $PGJ_2$ is a potent activator of the PPARγ isoform (9, 10), whereas a stable analog of $PGI_2$, carbaprostacyclin (cPGI), has been shown to activate PPARδ and to a much lesser extent, PPARα (11, 12).

Research on PPARs has revealed that the PPARα and PPARγ isoforms play fundamental roles in such diverse physiological processes as lipid metabolism, immunity, and cellular differentiation (13, 14). For example, PPARγ is considered a master regulator of adipocyte differentiation (15) and also has been shown to play an important role in monocyte/macrophage biology (16–18). There also has been a great deal of interest in PPARs and cancer. Activators of PPARα will induce the formation of hepatocellular carcinomas in rodents (19, 20), whereas ligands for PPARγ have been shown to induce cellular changes consistent with differentiation and reversal of the neoplastic phenotype in liposarcoma (21), breast (22), and colon carcinoma cells (23, 24). In addition, inactivating mutations in PPARγ recently were identified in a subset of colorectal tumors, strongly suggesting that this isoform has a tumor suppressive role during colorectal carcinogenesis (25). Unlike the PPARα and PPARγ receptors, little is known about the physiological role of the PPARδ isoform. Recently, we have reported that this receptor serves an important function in the female reproductive process. COX-2-deficient female mice exhibit multiple reproductive failures, including a defect in embryo implantation (26). The major PG subtype produced at the implantation site is $PGI_2$ and administration of the $PGI_2$ analog, cPGI, to COX-2 −/− mice rescues the implantation defect. Several lines of evidence from our previous study suggested that $PGI_2$ was not signaling through the G protein-coupled prostacyclin (IP) receptor, but rather through activation of PPARδ. For example, the level of PPARδ was significantly increased at the implantation site, whereas little or no IP receptor was expressed at the same location. In addition, a synthetic PPARδ agonist, which cannot activate the IP cell surface receptor, was able to rescue the implantation defect, whereas

Abbreviations: PPAR, peroxisome proliferator-activated receptor; COX, cyclooxygenase; PG, prostaglandin; cPGI, carbaprostacyclin; IP, G protein-coupled prostacyclin receptor; APC, adenomatous polyposis coli; Tcf, T cell factor; AOM, azoxymethane; PPRE, PPAR response element; AA, arachidonic acid.

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cicaprost, a $PGI_2$ analog that can activate the IP receptor, but does not bind to PPARδ, did not rescue the deficiency (11). This study offered evidence that a COX-derived metabolite ($PGI_2$) could have biological effects *in vivo* via activation of a PPAR.

Inactivating mutations in the adenomatous polyposis coli (APC) tumor suppressor gene are thought to be the initiating event for a majority of colorectal cancers (27, 28). A key mechanism by which APC is thought to act is through its ability to bind and target β-catenin for degradation. β-catenin is a protein that interacts with the cytoplasmic domain of E-cadherin and plays an important role in cell/cell contact (29). In colorectal tumors and cell lines with mutant alleles of APC, the levels of β-catenin are significantly elevated and it is no longer colocalized with the cadherin complex, but rather is present throughout the cell (30). Precisely how stabilized levels of β-catenin promote tumorigenesis is still unclear. Stabilized levels of β-catenin act as a transcriptional cofactor via its association with the T cell factor (Tcf) family of DNA-binding proteins (31). In this model, stabilized levels of β-catenin lead to increases in nuclear levels of β-catenin/Tcf complexes. This complex regulates the expression of target genes that could play important roles in neoplastic transformation. For example, there is evidence that c-myc (32), cyclin D1 (33), and the matrix metalloproteinase matrilysin (34) are transcriptional targets of β-catenin/Tcf.

Recently, He *et al.* (35) have identified PPARδ as another β-catenin/Tcf-regulated gene (35). In their study, HT-29 cells induced to express wild-type APC were found to have reduced levels of PPARδ. A Tcf response element was identified in the 5′ regulatory region of the PPARδ gene and further studies showed that β-catenin/Tcf up-regulates expression of PPARδ in colon carcinoma cells. At present, there is no evidence that PPARδ plays any role in colorectal carcinogenesis even though a small number of human colorectal cancer tissues were found to have increased levels of PPARδ (35). The study by He *et al.* (35) provided evidence that PPARδ may have an antiapoptotic function and proposed that high doses (≥100 μM) of nonsteroidal anti-inflammatory drugs induce apoptosis in cultured colorectal carcinoma cells via direct inhibition of PPARδ DNA-binding activity.

In light of our previous work demonstrating that PPARδ is a downstream receptor of COX-2-derived $PGI_2$ during implantation in the uterus and the recent finding that PPARδ expression is regulated by the APC/β-catenin pathway, we hypothesized that COX-2-derived eicosanoids may modulate PPARδ activity in colorectal cancer. To assess the validity of this idea, we first examined tissue localization and expression levels of PPARδ mRNA in colorectal carcinomas from humans, as well as tumors derived from rats treated with the chemical carcinogen, azoxymethane (AOM). In addition, PPARδ transcriptional activity in response to $PGI_2$, cPGI, or other synthetic ligands was assessed by using a transient reporter gene assay in human colorectal carcinoma cells. We found that both endogenously produced $PGI_2$ or exogenous cPGI are effective activators of PPARδ-mediated transcription.

## Materials and Methods

**Cell Culture and Materials.** All cell lines were purchased from the American Type Culture Collection except the HCA-7 rectal adenocarcinoma cell line, which was a kind gift of Susan Kirkland (Imperial Cancer Research Fund, London) (36). Cells were grown in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS (HyClone)/2 mmol/liter L-glutamine/100 units/ml penicillin/100 μg/ml streptomycin in a 5% $CO_2$ atmosphere with constant humidity. AA and cPGI were purchased from Cayman Chemicals (Ann Arbor, MI). All other synthetic PPAR ligands were obtained from Glaxo Wellcome.

**Tissue Procurement and RNA Isolation.** *Human colon cancer tissue.* Colorectal carcinoma specimens were obtained from surgical resections. In each case, accompanying normal mucosa was collected for comparison. All tissues were placed in cryovials, flash-frozen in liquid nitrogen, and stored at −80°C.

*Tissue from AOM-treated rats.* The experimental design and protocols used in the carcinogen treatment of Male F344 rats with AOM have been described (37). Colonic tumors and normal tissues were obtained from six different randomly selected AOM-treated rats (provided by B. Reddy, American Health Foundation, Valhalla, NY). In each case, accompanying normal mucosa from the same animal was collected for comparison. All tissues were placed in cryovials, flash-frozen in liquid nitrogen, and stored at −80°C.

*RNA isolation.* Total RNA from human colon cell lines and rat AOM tissue was isolated by using the TRI reagent (Molecular Research Center, Cincinnati). Total RNA from human colon cancer surgical specimens was isolated by using the TOTALLY RNA Kit (Ambion, Austin, TX).

**Northern Hybridization Analysis.** Northern blot analysis was performed as described (38). Briefly, total RNA (20 μg) was fractionated on a 1.2% agarose-formaldehyde gel and transferred to a Hybond-NX nylon membrane (Amersham Pharmacia). Filters were prehybridized and then hybridized in Ultrahyb (Ambion) buffer containing a $^{32}$P-radiolabeled PPARδ cDNA fragment (kindly provided by M. Breyer, Vanderbilt University, Nashville, TN). Filters were washed four times for 15 min at 50°C in 2× SSC/0.1% SDS, once for 30 min in 1× SSC/0.1% SDS, and then exposed to a PhosphorImager screen and images were analyzed by using a Cyclone Storage Phosphor System and OPTIQUANT software (Hewlett–Packard).

***In Situ* Hybridization.** *In situ* hybridization was performed as described (11). Sense or antisense $^{35}$S-labeled cRNA probes were generated from human PPARδ and COX-2 cDNAs. The probes had specific activities at $2 \times 10^9$ dpm/μg. Sections hybridized with the sense probes did not exhibit any positive autoradiographic signals and served as negative controls.

**Western Blot Analysis.** Exponentially growing cells were harvested in ice-cold 1× PBS, and cell pellets were lysed in radioimmunoprecipitation assay buffer. Centrifuged lysates (50 μg) from each cell line were fractionated on a 10% SDS/PAGE and electrophoretically transferred to a poly(vinylidene difluoride) membrane. Membranes were blocked for 1 h at room temperature in Tris-buffered saline containing 0.1% Tween 20 and 5% powdered milk. For the primary antibody incubation, an affinity-purified rabbit polyclonal antibody for mouse PPARδ was prepared by Research Genetics (Huntsville, AL) and used at a dilution of 1:500 in Tris-buffered saline containing 0.1% Tween 20 + 5% powdered milk (11). This was followed by incubation with a donkey anti-rabbit horseradish peroxidase-conjugated antibody (The Jackson Laboratory) at a dilution of 1:50,000. Detection of immunoreactive polypeptides was accomplished by using an enhanced chemiluminescence system (Amersham Pharmacia).

**Transfections and Luciferase Assays.** *DNA constructs.* Rat COX-2 in the pCB7 expression vector (39) and rat PGI synthase in the pCDNA3 expression vector (40) have been described. PPAR response element 3 (PPRE3)-tk-luc, UAS-tk-luc, PPARδ-pCMX, pGAL4, PPARα-GAL4, PPARγ-GAL4, and PPARδ-GAL4 were kindly provided by R. Evans (Salk Institute, La Jolla, CA).

*Transfections.* U2OS cells ($5.0 \times 10^5$ cells/well using 24-well plates) were transfected by using FUGENE 6 at a lipid/DNA ratio of 3:1. Cells were exposed to a mix containing 150 ng/ml UAS-tk-luc (GAL4 reporter plasmid expressing firefly luciferase)/150 ng/ml PPAR-GAL4/75 ng/ml COX-2, and/or PGI synthase and 1.0 ng/ml pRL-SV40 (control plasmid expressing renilla luciferase) in Opti-MEM (GIBCO/BRL). All transfections were normalized to 450 ng/ml with pCDNA3.1. HCA-7

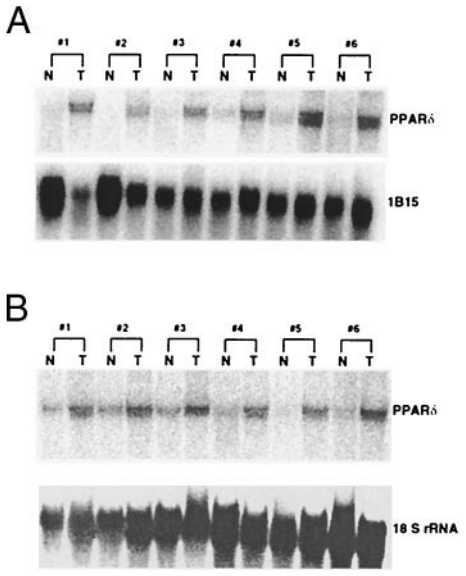


**Fig. 1.** Expression of PPARδ mRNA in matched normal and tumor colon tissue from (*A*) rats treated with the carcinogen AOM and (*B*) human surgical specimens. In each case, total RNA (20 μg) was isolated from six paired samples and analyzed for relative levels of PPARδ mRNA levels by Northern blot hybridization. Each blot was subsequently probed for 1B15 (*A*) or 18S (*B*) to evaluate RNA loading.

cells were transfected with a mix containing 150 ng/ml PPRE3-tk-luc/150 ng/ml pCDNA3.1/1.0 ng/ml pRL-SV40/20 μg/ml Cellfectin in Opti-MEM. In either experiment, the transfection mix was replaced after 5 h with 10% charcoal-stripped FBS containing media supplemented with either 0.1% vehicle (DMSO or ethanol) or the indicated compound.

*Luciferase assay.* After 24 h, cells were harvested in 1× luciferase lysis buffer. Relative light units from firefly luciferase activity were determined by using a luminometer (MGM Instruments, Hamden, CT) and normalized to the relative light units from renilla luciferase by using the Dual Luciferase kit (Promega).

**PG Measurements.** Levels of 6-keto $PGF_{1\alpha}$ were quantified by using a gas chromatography/negative ion chemical ionization mass spectrometric assay as described (38).

## Results

**PPARδ Expression and Localization in Colorectal Carcinomas.** To determine whether PPARδ, similar to COX-2, is aberrantly expressed in colorectal carcinomas, Northern blot analysis was used to examine the relative levels of PPARδ mRNA in paired normal and colon tumor samples from carcinogen-treated rats and human colon cancer specimens. In the rodent tumor samples, the levels of PPARδ mRNA were significantly elevated in tumor tissue when compared with adjacent normal mucosa (Fig. 1*A*). PPARδ also was elevated in all six samples of human colorectal carcinomas evaluated (Fig. 1*B*). The difference in expression between normal and neoplastic tissue are unlikely to be caused by discrepancies in RNA integrity or loading errors because controls were done evaluating levels of 1B15 mRNA (Fig. 1*A*) or 18S ribosomal RNA (Fig. 1*B*) in each lane.

There have been no previous reports describing which cell type(s) within the colon express PPARδ. *In situ* hybridization was used to address this question. Sections from both neoplastic and normal adjacent colonic tissue were probed with antisense PPARδ or COX-2 probes. In the normal colonic mucosa, PPARδ was localized mainly to epithelial cells that reside on the luminal surface of the mucosal glands. In contrast, PPARδ mRNA expression was more widespread in colon carcinomas and localized in dysplastic epithelial cells throughout the section (Fig. 2). As previously reported, COX-2 mRNA was undetectable in the normal mucosa but was expressed in both transformed epithelial cells and stromal cells in colorectal carcinomas. Of interest, analysis of COX-2 and PPARδ mRNA expression in serial tissue sections suggests that both of these genes appear to be expressed in similar regions within a given colorectal tumor.

To use an *in vitro* culture system to study PPARδ activity and function, we examined expression of PPARδ mRNA and protein in a panel of established human colorectal carcinoma cell lines. PPARδ was expressed in all cell lines tested, with some variability in the expression levels between different lines (Fig. 3 *A* and *B*).

**PG-Mediated Activation of PPARδ.** We were interested in exploring the possibility that COX-generated $PGI_2$ could serve as an activating ligand for PPARδ in colorectal carcinoma cells. It is known that cPGI, a stable analog of $PGI_2$, can activate PPARδ (12). Because this is a synthetic ligand that is structurally different from the endogenously produced COX metabolite, $PGI_2$, we questioned whether $PGI_2$ itself can act as a bona fide ligand for PPARδ. However, testing the ability of $PGI_2$ to activate PPARs in a standard gene reporter assay is difficult because of the inherent instability of the compound. For example, it is well established that in neutral or acidic buffers, $PGI_2$ is rapidly hydrolyzed (30–120 s) to 6-keto $PGF_{1\alpha}$. To circumvent this problem, we sought to create experimental conditions in which $PGI_2$ production could be correlated with PPARδ transcriptional activity. For these experiments, the PPAR-GAL4 transactivation assay was used. In this assay, a chimeric receptor is used that contains the ligand-binding domain of a PPAR fused with the DNA-binding domain of the yeast GAL4 transcription factor. Transactivation is detected by cotransfection with a reporter gene containing GAL4 response elements (UAS-tk-luc). U2OS cells that were transiently transfected with PPARδ-GAL4 and UAS-tk-luc were also transfected with expression vectors for COX-2 and PGI synthase (either alone or in combination). Cells then were treated with vehicle, AA (40 μM), or a combination of AA and the selective COX-2 inhibitor, celecoxib (2 μM). Both $PGI_2$ production (as measured by 6-keto $PGF_{1\alpha}$ levels) and PPARδ transactivation were determined for each experimental condition (Fig. 4 *A* and *B*). We found that endogenous $PGI_2$ production correlated well with activation of PPARδ. Importantly, minimal activation was seen with pGAL4, PPARα-GAL4, or PPARγ-GAL4.

**Selectivity for PPARδ Activation.** Finally, transient transfection assays also were performed to determine whether endogenous PPARδ was functionally active in colon carcinoma cells and whether the receptor was responsive to the $PGI_2$ analog, cPGI and other synthetic ligands. The PPAR isoform selectivity of the compounds used in this experiment has been reported as follows: cPGI (PPARα and δ) (12), GW 7647 (PPARα)[*7], and GW 2433 (PPARα and δ) (41). The HCA-7 colon cancer cell line was transfected with the nonspecific PPAR reporter PPRE3-tk-luc. This reporter contains three tandem repeats of the PPRE present in the promoter of the acyl-CoA oxidase gene (8). Addition of PPAR isoform-selective ligands to cells transfected with this reporter allows for the identification of functionally active PPAR isoforms within the cell. Addition of the $PGI_2$ analog, cPGI, to transfected HCA-7 cells results in a dose-dependent increase in luciferase activity (Fig. 5). However, because cPGI has been shown to bind both PPARα and PPARδ, we sought to establish which isoform was activated. To address this issue, PPAR reporter activity also was measured in response to the PPARα-specific ligand GW 7647, and GW 2433, a dual PPARα/δ-selective ligand. GW 7647 did not significantly increase reporter activity compared with vehicle-treated cells at any dose evaluated. However, addition of GW 2433 resulted in a dose-dependent activation of luciferase enzymatic activity. As an additional control, PPAR reporter activity also was

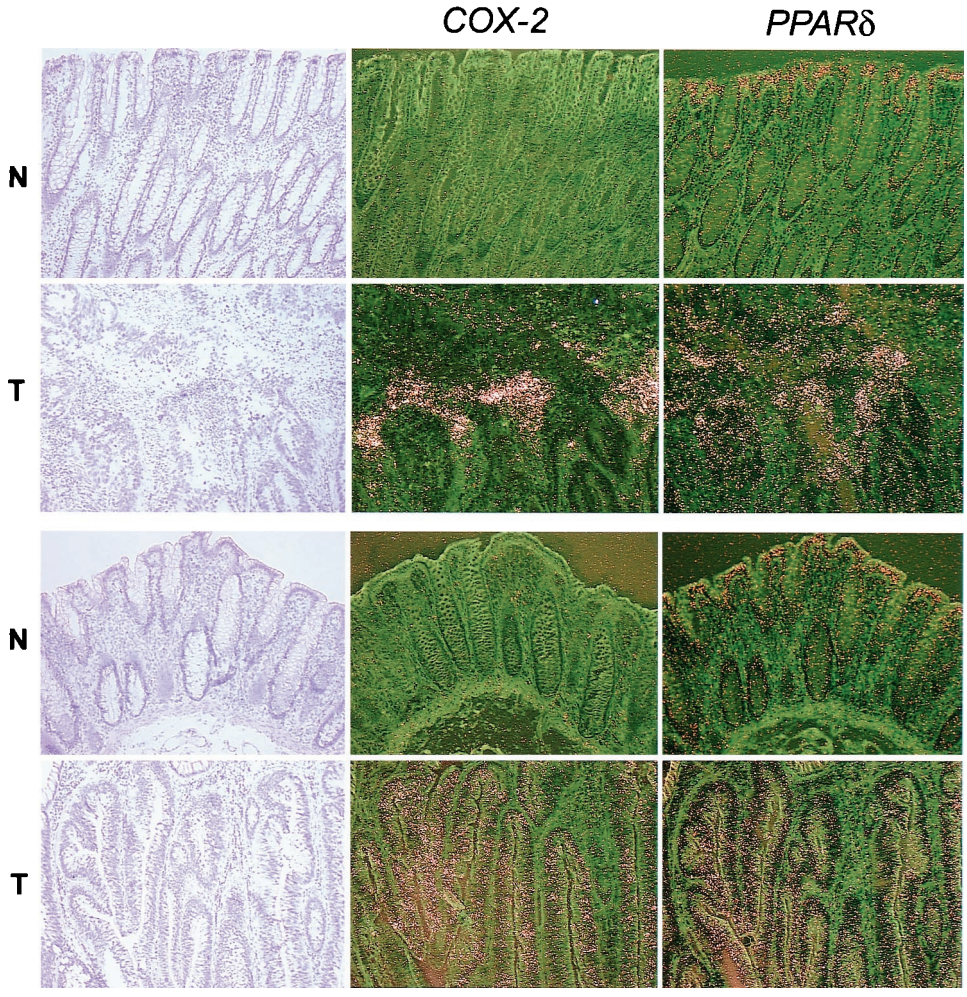


**Fig. 2.** Localization of PPARδ and COX-2 mRNA in normal human colon and colorectal carcinoma. *In situ* hybridization analysis of PPARδ and COX-2 expression in two different matched pairs of normal (N) and cancer tissue (T).

measured in cells cotreated with the selective PPARγ antagonist GW 9662 (42) and either cPGI or GW 2433. Inhibition of endogenous PPARγ activity did not block the induction seen by either of these two compounds. Also, we feel that $PGI_2$-mediated transcriptional activation of PPARδ does not occur via the IP receptor signaling pathway because treatment with a selective IP receptor agonist (cicaprost) did not have an effect (data not shown).

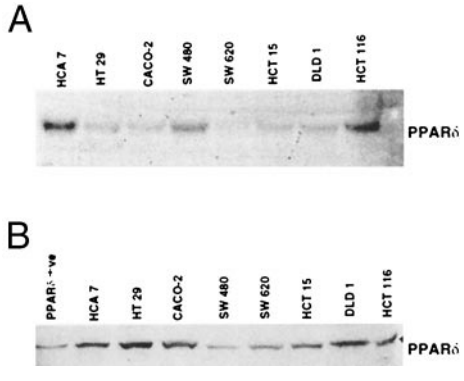


**Fig. 3.** PPARδ (*A*) mRNA and (*B*) protein expression in a panel of colon cancer cell lines. (*A*) Total RNA (20 μg) was isolated from eight different indicated colon carcinoma cell lines and analyzed for PPARδ mRNA expression by Northern blot hybridization. (*B*) Whole-cell protein lysates from the indicated cell lines (50 μg) were analyzed for PPARδ protein expression by using immunoblot analysis.

## Discussion

Oshima *et al.* (43) have assessed the development of intestinal polyps in $Apc^{\Delta 716}$ mice in a wild-type and homozygous null COX-2 genetic background. The number and size of polyps were reduced dramatically in the COX-2 null mice compared with COX-2 wild-type mice. In addition, treatment of the $Apc^{\Delta 716}$ COX-2 wild-type mice with a selective COX-2 inhibitor (MF tricyclic) also caused a reduction in polyp burden (43). This experiment implies that COX-2 plays an important causal role in promoting the development of tumors arising in an APC mutant genetic background. Evaluation of other cancer models also demonstrates that treatment with selective COX-2 inhibitors reduces tumor growth dramatically (44–46). Although the levels of several prostaglandins have been shown to be elevated in colorectal tumors, the mechanism(s) by which COX-2 promotes tumor development is still largely unknown. There is evidence that COX-2 overexpression in colonic epithelial cells can increase metastatic potential (47), promote resistance to inducers of apoptosis (39), and induce expression of angiogenic growth factors (48). However, the relevant downstream pathways affected by COX-2-derived eicosanoids that could potentially play a role in inducing these biological phenotypes are not known.

The hypothesis that COX-2-generated $PGI_2$ could exert biologic effects via activation of PPARδ arises from two recently published observations: (*i*) COX-2-derived $PGI_2$ promotes embryo implantation in mice via activation of PPARδ (11); and (*ii*) β-catenin/Tcf positively regulates the expression of PPARδ in human colon cancer cells (35). As a first step in exploring this

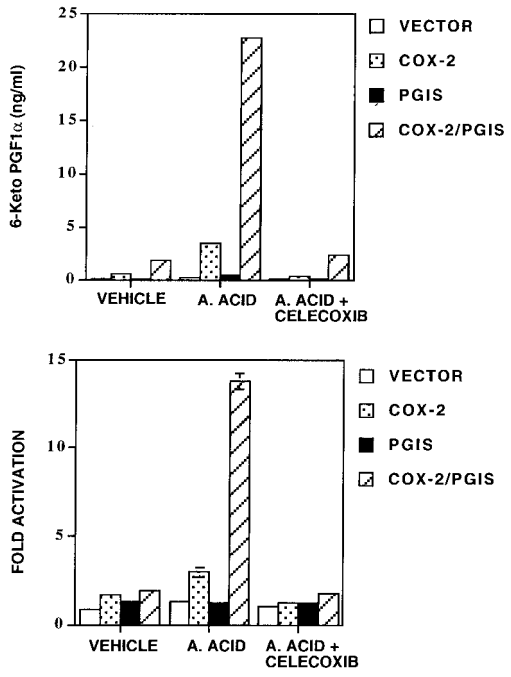


**Fig. 4.** Endogenous production of $PGI_2$ correlates with PPARδ transactivation. U2OS cells were transiently transfected with UAS-tk-luciferase, pRL-TK, PPARδ-GAL4, and combinations of expression vectors for COX-2 and PGI synthase (PGIS). Cells were then treated with vehicle (0.1% ethanol), AA (40 μM), or AA + the selective COX-2 inhibitor celecoxib (2 μM) for 24–36 h. (*A*) Media were harvested and used to measure 6-keto $PGF_{1\alpha}$ levels (represented as the mean from two independent transfections ± SEM). (*B*) Cells were harvested and the dual luciferase assay was performed as described in *Materials and Methods*. Data are presented as -fold activation and represent the mean from three independent transfections. Error bars equal SEM. No significant activation was seen in cells transfected with pGAL4, PPARα-GAL4, or PPARγ-GAL4.

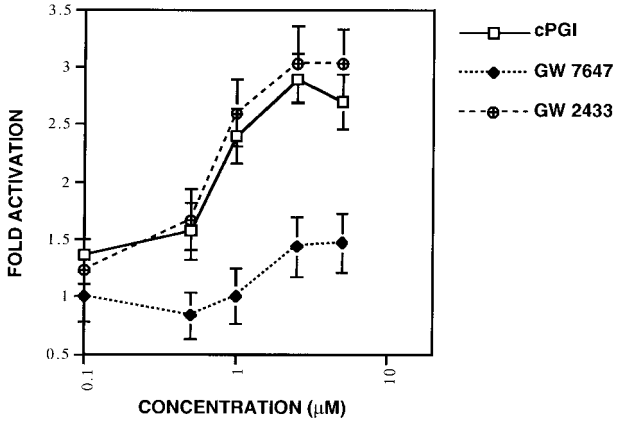


**Fig. 5.** Transactivation of endogenous PPARδ in human colon cancer cells. (*A*) HCA-7 cells were transiently transfected with PPRE3-tk-luciferase and pRL-TK plasmids followed by treatment with increasing doses of the following ligands (published PPAR isoform selectivity in parenthesis): cPGI (PPAR $\alpha/\delta$), GW 2433 (PPAR $\alpha/\delta$), or GW 7647 (PPAR $\alpha$) for 24 h. Cells were harvested and the dual luciferase assay was performed as described in *Materials and Methods*. Data are presented as -fold activation relative to vehicle treated (0.1% DMSO) and represent the mean from three independent transfections. Error bars equal SEM.

idea, the experiments described in this report were designed to determine the expression, localization, and transcriptional responsiveness of PPARδ in colorectal cancer.

Analysis of PPARδ mRNA levels in normal and tumor tissue from rats treated with the carcinogen, AOM, suggests that PPARδ is significantly up-regulated in tumors in this model system. This result was not surprising in light of our previous report demonstrating elevated nuclear β-catenin levels in AOM-induced rat colon carcinomas (49). Importantly, the up-regulation of PPARδ in tumor tissue compared with adjacent normal mucosa also was seen in six different human colon carcinoma specimens, confirming a previous report (35). These results are strikingly parallel to the expression pattern of COX-2 in colorectal carcinomas. We previously found that tumors from rats treated with AOM have elevated levels of COX-2 (37) and many different groups have published that COX-2 levels are increased in a majority of human colorectal cancers (1, 2, 50, 51). In addition, introduction of wild-type APC into the HT-29 colon carcinoma line leads to a reduction in both COX-2 (52) and PPARδ (35). Thus, COX-2 and PPARδ expression appears to be coordinately up-regulated during colorectal carcinogenesis.

*In situ* hybridization analysis of the normal colonic mucosa suggests that PPARδ is predominantly expressed in colonic epithelial cells. Furthermore, its expression appears to be concentrated in the most differentiated cells located at the luminal surface of the mucosal glands. In contrast, PPARδ was highly expressed in epithelial cells located throughout the dysplastic glands found in the neoplastic tissue. These results also confirm the data obtained by Northern blot analysis, that is, PPARδ is aberrantly expressed in colorectal carcinomas. It is interesting to note that PPARδ is highly expressed in the most differentiated cells in normal colonic mucosa and is also widely expressed in dedifferentiated carcinoma cells. The biological significance of this apparent paradox still needs to be determined, but it suggests that PPARδ expression alone is not proneoplastic. Finally, the observation that COX-2 and PPARδ mRNA are colocalized to similar regions within a tumor strengthens our hypothesis that signaling between these pathways could be operative *in vivo*.

By using the PPAR-GAL4 transactivation assay in combination with cells transfected with COX-2 and/or PGI synthase, we were able to establish a correlation between PPARδ transactivation in a COX-2 and PGI synthase-dependent manner. This is evidence that an endogenously synthesized eicosanoid can serve as an activator of PPARδ. Because 6-keto $PGF_{1\alpha}$ does not activate any of the PPARs (data not shown), it is more than likely that the active ligand being generated in this experiment is $PGI_2$. However, the possibility of this pathway producing an unknown eicosanoid product that can act as a ligand for PPARδ cannot be completely ruled out. Importantly, this same experiment performed with either pGAL4, PPARα-GAL4, or PPARγ-GAL4 resulted in minimal reporter activity. This suggests that the COX/prostacyclin synthase pathway is both specifically and selectively upstream of the PPARδ isoform. Interestingly, when an identical experiment was performed with COX-1 instead of COX-2, there was no PPARδ transactivation and minimal prostacyclin produced (data not shown). Protein lysates from transfected cells showed strong immunoreactivity for COX-1 by immunoblot analysis. There was a 21.4-fold increase in $PGE_2$ levels in the medium taken from COX-1-transfected cells versus vector-transfected cells that was absent after treatment with indomethacin. Thus, at least in our model system, COX-2, but not COX-1, appears capable of coupling to PGI synthase to produce high levels of $PGI_2$ and transactivation of PPARδ.

Finally, we were able to establish that PPARδ is expressed and transcriptionally responsive in human colorectal carcinoma cells. In addition, we determined that cPGI activates endogenous PPARδ in these cells. Importantly, based on the results of this study, it appears as if cPGI is a relevant and appropriate synthetic ligand that can mimic the ability of $PGI_2$ to activate PPARδ. It is likely that the ability of either cPGI or GW 2433 to activate PPAR reporter activity is specifically caused by endogenous PPARδ activation, because no such activation was seen with GW

7647, a pure PPAR$\alpha$ selective ligand. In addition, cotreatment with the PPAR$\gamma$ antagonist, GW 9662, did not block GW2433 or cPGI-induced transactivation, suggesting that these compounds are not responsible for activation via PPAR$\gamma$.

At this time, we have no direct evidence that PPAR$\delta$ plays a role in promoting or inhibiting colon cancer formation. It has previously been suggested that high doses of the nonsteroidal anti-inflammatory drug, sulindac, promotes apoptosis of carcinoma cells through inhibition of the DNA-binding activity of PPAR$\delta$ (35). However, we were unable to detect any effect of selective COX-2 inhibitors on PPAR transcriptional activity by using pharmacologically relevant doses (1–10 $\mu$M). In addition, there was no effect of the PPAR$\delta$ activators GW2433 or cPGI on the proliferation of colon carcinoma cells in culture.

In summary, we show that expression of PPAR$\delta$, similar to COX-2, increases dramatically during colorectal carcinogenesis and demonstrate that PPAR$\delta$ is functionally active in human colorectal carcinoma cell lines. Because both COX-2 and PPAR$\delta$ are up-regulated in colon tumors, colocalized to similar regions within a given colorectal tumor, and because a COX-generated ligand, $PGI_2$, activates PPAR$\delta$, we speculate that COX-2 may, in part, modulate cellular processes through activation of this receptor. However, the functional consequence of COX-2 activation of PPAR$\delta$ in colorectal tumors still needs to be determined. If PPAR$\delta$ is found to play a causal or protective role in the development of colorectal cancer, then modulators of this pathway may have therapeutic potential in humans.

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