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ACCELERATED PAPER

The nuclear eicosanoid receptor, PPARγ, is aberrantly expressed in colonic cancers

Raymond N.DuBois[1,2,5], Rajnish Gupta[2], Jeffrey Brockman[1], Bandaru S.Reddy[3], Samuel L.Krakow[4] and Mitchell A.Lazar[4,5]

[1]Departments of Medicine and [2]Cell Biology,Vanderbilt University Medical Center, Nashville, TN 37232, [3]American Health Foundation, Valhalla, NY 10595 and [4]Departments of Medicine and Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

[5]To whom correspondence should be addressed. Email: duboisrn@aoi.com

Continuous use of nonsteroidal anti-inflammatory drugs (NSAIDs) lowers the relative risk of colorectal cancer in humans and decreases tumor yield in rodents treated with carcinogens. One well documented target for NSAIDs is prostaglandin endoperoxide synthase (cyclooxygenase) and two isoforms of this enzyme have been identified, cyclooxygenase–1 (COX–1) and cyclooxygenase–2 (COX–2). COX enzymes produce eicosanoid products, some of which have recently been shown to activate transcription mediated by the nuclear hormone receptor peroxisome proliferator activated receptor γ (PPARγ), whose expression is largely restricted to adipose tissue. The present study was undertaken to determine if PPARγ was expressed in colonic tumors. PPARγ messenger RNA (mRNA) and protein levels were assayed in colonic tumors and normal adjacent mucosa, as well as in a variety of human colon cancer cell lines. There was a marked increase in PPARγ RNA levels in four out of four of the colonic tumors compared to paired normal mucosa, where little expression of PPARγ was detected. Western blotting analysis showed that PPARγ protein was expressed in four out of five colonic tumor samples. PPARγ was also expressed in a subset of polyps, and in certain human colon cancer cell lines as well. Additionally, we were able to demonstrate that an eicosanoid, 15 deoxy-Δ12,14 PGJ_2, transactivated transcription of a PPRE-driven promoter in CaCo–2 cells. Thus, we have shown that PPARγ gene and protein expression is elevated in rodent colon tumors, in selected human colon cancer cell lines and that the PPARγ receptor is functional in CaCo–2 cells. Since PPARγ is a ligand-modulated transcription factor, it may provide a novel target for chemopreventive strategies for colorectal cancer.

Introduction

Numerous studies have demonstrated a 40–50% reduction in relative risk for colorectal cancer in individuals taking nonsteroidal anti-inflammatory drugs (NSAIDs*) compared to those not taking these agents (1–6). Individuals with familial adenomatous polyposis (FAP) treated with sulindac have a striking reduction in adenoma size and number (7–11). In azoxymethane (AOM) induced colorectal carcinogenesis, cyclooxygenase (COX) inhibitors exhibit chemoprotective effects as judged by a reduction in the frequency and number of premalignant and malignant lesions (12–14). Reddy *et al.* have also demonstrated a marked reduction of aberrant crypt formation in rats treated with a highly selective cyclooxygenase-2 (COX–2) inhibitor (15).

We have previously demonstrated increased COX–2 expression in human colorectal adenocarcinomas when compared to normal adjacent colonic mucosa (16); these findings have been confirmed by other investigators who have shown elevated levels of COX–2 protein in colorectal tumors by immunoblotting (17) and immunohistochemical staining (18,19). We have also observed markedly elevated levels of COX–2 messenger RNA (mRNA) and protein in intestinal tumors that develop in rodents following carcinogen treatment (20) and in adenomas taken from *Min* mice (21). A recent report by Oshima *et al.* indicates that COX–2 may play an extremely important role in the development of adenomas following loss of APC function (22,23). Adenomas from $APC^{\Delta 716}$ mice were found to have elevated COX–2 levels. Additionally, treatment of $APC^{\Delta 716}$ mice with highly selective COX–2 inhibitors significantly reduced tumor multiplicity. Also, our group has recently demonstrated an 85–90% inhibition of tumor growth in a mouse xenograft model by treatment with a highly selective COX–2 inhibitor (24). Taken together, these results provide strong genetic and pharmacologic evidence for a role of COX–2 in adenoma formation following loss of APC function (25).

The mechanism by which NSAIDs act to reduce the risk of colorectal neoplasia is unknown. We have previously demonstrated increased COX–2 levels in tumors taken from AOM-treated rats by Western and Northern blot analysis (20). We have also shown that forced expression of COX–2 in rat intestinal epithelial cells leads to phenotypic alterations which increase tumorigenic potential. Since these phenotypic alterations can be reversed by treatment with highly selective COX–2 inhibitors, the eicosanoid products formed by the COX–2 enzyme are likely affecting downstream signaling pathways, ultimately regulating gene transcription. One candidate for eicosanoid mediated transcriptional regulation is the PPARγ nuclear receptor that is activated by prostaglandin D_2, prostaglandin J_2, and derivatives thereof (26). At least one PGJ_2 derivative, 15 deoxy-Δ12,14 PGJ_2, is a direct ligand for PPARγ (27,28). In normal tissues, PPARγ expression is predominantly restricted to adipocytes (29,30). Given the aberrant expression of COX–2 in colon tumors, and the potential that this enzyme may serve to produce PPARγ ligands, we undertook these studies to determine if PPARγ was aberrantly expressed in colon tumor cells. Indeed, we found that PPARγ mRNA and protein was expressed not only in intestinal tumors, but in a subset of polyps and colon cancer cell lines. Additionally, we found that 15 deoxy-Δ12,14 PGJ_2 does transactivate PPARγ mediated transcription driven off a

***Abbreviations:** NSAIDs, nonsteroidal anti-inflammatory drugs; FAP, familial adenomatous polyposis; AOM, azoxymethane; COX, cyclooxygenase; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; PPARγ, peroxisome proliferator activated receptor γ; mRNA, messenger RNA.

PPRE-luciferase construct transfected into the CaCo–2 colon cancer cells. This suggests that if COX–2 plays an etiological role in colon carcinogenesis, the mechanism could involve generation of a ligand that activates transcription via PPARγ.

Materials and methods

Carcinogen treatment, tissue procurement and RNA isolation

The experimental design and protocols have been described previously (20,31). Male F344 rats received at weaning were quarantined for 10 days and then randomly distributed by wt into control or experimental groups. Vehicle-treated subgroups and AOM-treated animals were housed (three per cage) in a room maintained under controlled environmental conditions of a 12-h light–dark cycle with 50% humidity at 22°C. Animals received proper care and maintenance according to institutional guidelines. All animals were fed the AIN–76A modified semi-purified diet. All diets were prepared in the laboratory once weekly and were stored at 4°C in airtight containers under nitrogen gas. Starting at 5 weeks of age, animals were allotted to experimental groups. The regimen was continued until termination of the experiment 52 weeks after carcinogen administration. Starting at 7 weeks of age, animals intended for carcinogen treatment were administered AOM s.c. for 2 weeks (15 mg/kg wt once weekly). Colonic tumors and normal tissues were obtained from six different randomly selected AOM-treated rats following the 52 week period. 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. Total RNA was isolated from these samples using previously reported methods (16).

Preparation and labeling of cDNA probes

The rat COX–2 cDNA was isolated and characterized as previously reported (32). The cDNA probes were α-[^{32}P]-dCTP labeled using a commercially available random primer labeling kit (Stratagene, La Jolla, CA) and purified using NICK® column chromatography (Pharmacia LKB, Piscataway, NJ) before use (33).

Northern hybridization analysis

RNA samples were electrophoresed in denaturing agarose gels and transferred to nitrocellulose. Nitrocellulose blots were hybridized using standard conditions followed by 0.1% SSC/0.1% SDS post-hybridization washes at 42°C (16). Blots were exposed for various lengths of time prior to development. Paired carcinoma and normal mucosa were evaluated by Northern hybridization analysis and compared semi-quantitatively using Ofoto and NIH image optical scanning and densitometric software.

Western blotting analysis

The tissues were homogenized at 4°C in RIPA buffer (150 mM NaCl, 1% NP–40, 50 mM Tris, pH 8.0) containing 10 μg/ml aprotinin and 1 mM sodium orthovanadate. Centrifuged homogenates (100 μg) were denatured and fractionated on 7.5% polyacrylamide gels containing SDS then transferred to nitrocellulose membrane using a semidry cell (BioRad). Western analysis was performed as previously described, using a rabbit polyclonal antiserum raised against a peptide derived from the N-terminus of PPARγ that is conserved in both human and mouse PPARγ1 and PPARγ2 (34).

Transfections and luciferase assays

CaCo–2 cells (2.5×10^5) were plated into 24 well plates and allowed to adhere overnight. Cells were transfected with a mix containing 1 μg/ml PPRE3-luciferase (27), 0.5 μg/ml pRL-TK, 1 μg/ml pBluescript as carrier DNA and 20 μg/ml Cellfectin in Opti-MEM (GIBCO-BRL) as per manufacturer's directions for 5 h. The transfection mix was replaced with complete media with or without 15-deoxy-Δ12,14-prostaglandin J_2 (10 μM). After 12 h cells were harvested in 1× luciferase lysis buffer. Relative light units from firefly luciferase activity were determined using a luminomoter (MONO LIGHT 2010) and normalized to the relative light units from runilla luciferase using the Dual Luciferase kit (Promega).

Results

PPARγ gene expression is increased in intestinal tumors

A total of four paired AOM-induced adenocarcinoma and normal mucosa were evaluated for PPARγ expression (Figure 1). Equal loading of each Northern blot was verified by equivalent intensity of 28 S RNA which was visualized by ethidium bromide staining (data not shown). Low to undetectable levels of PPARγ mRNA were observed in each of the normal mucosa samples (N) as can be noted in Figure 1 (upper panel). The degree of elevation of PPARγ mRNA as determined by densitometry ranged from 1.5- to 6-fold in the cancer sample compared to the paired normal mucosa. The tumor sample on the far right lane appears to be under-loaded, so the fold difference in this case is proabably an underestimate (lower panel Figure 1). These results demonstrate that PPARγ RNA levels are increased in most colonic tumors which develop in rodents following carcinogen treatment. We have previously reported that COX–2 is elevated in AOM-induced rat tumors (20) and demonstrate this again in Figure 1 (lower panel).

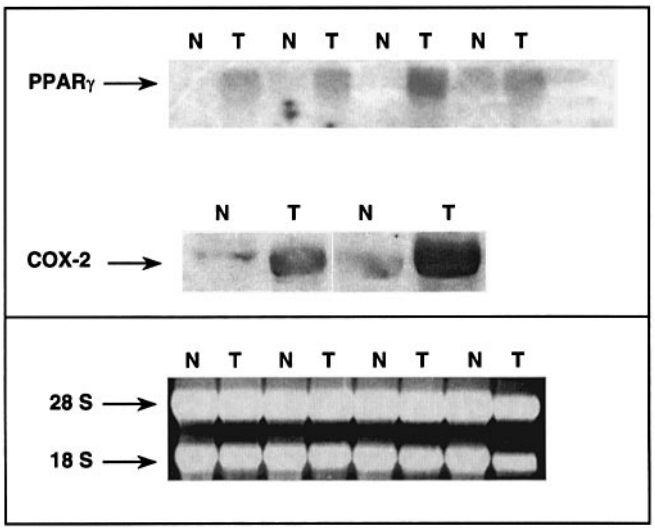

Fig. 1. PPARγ and COX–2 gene expression is increased in intestinal tumor samples from azoxymethane-treated rats. Northern analysis of PPARγ expression in paired normal (N) and tumor (T) from the same rats (upper panel). Each lane contains 30 mg of total RNA. Equal loading was observed by equivalent intensity of 28 S ribosomal bands of an ethidium bromide-stained gel (lower panel).

PPARγ1 protein expression is increased in intestinal tumors

We next proceeded to investigate PPARγ protein levels in colon tumors to determine if elevation of PPARγ RNA levels results in increased translation of PPARγ protein. In one of the colonic tumor samples there was no detectable difference in PPARγ protein levels between the tumor and normal mucosa by Western blotting (Figure 2A). However, in the remaining four samples there was a variable increase in PPARγ protein in the tumor sample compared with the paired normal mucosa. These results clearly demonstrate an increase in PPARγ protein levels in 4 out of 5 of the tumor specimens. There are two isoforms of PPARγ, called PPARγ1 and PPARγ2 (30), that are easily distinguished in the Western assay (34). PPARγ1 is identical to PPARγ2 except that it lacks the first 31 amino acids. PPARγ2 is derived from a different mRNA than PPARγ1, however, the PPARγ1 protein can be produced from the PPARγ2 mRNA by initiation of translation at an internal AUG codon. Since both PPARγ1 and PPARγ2 are expressed in adipocytes, an extract from mouse 3T3-L1 adipocytes is shown in Figure 2A as a control. Interestingly, the colon tumors specifically express PPARγ1 in contrast to the adipocytes which express both PPARγ1 and PPARγ2. The biological significance of this observation is unclear since, to date, no functional differences between the two PPARγ isoforms have been established. Interestingly, other non-adipocyte tissues that express PPARγ also contain predominantly PPARγ1 (35–37).

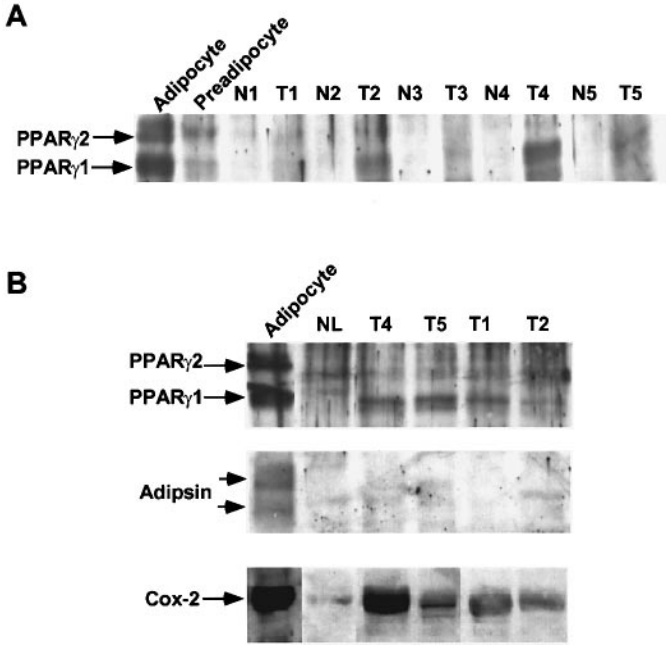

Fig. 2. PPARγ protein expression is increased in adenocarcinoma of the colon. (**A**) Western analysis of PPARγ expression in paired normal and tumor samples. The migration of PPARγ2 and PPARγ1 in adipocyte extract is shown. (**B**) Comparison of protein expression of PPARγ, adipsin, and COX–2 in colon tumors and 3T3-L1 adipocyte extracts.

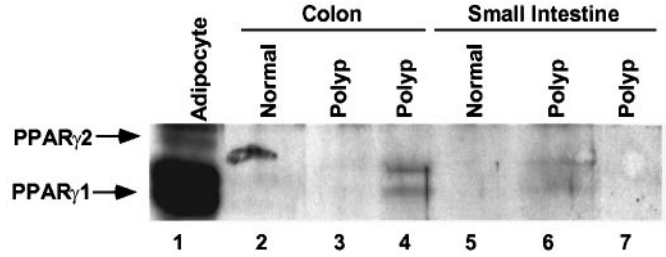

Fig. 3. PPARγ1 is aberrantly expressed in a subset of colonic polyps. Western analysis of PPARγ expression in normal and samples from either colonic or small intestinal polyps. The migration of PPARγ2 and PPARγ1 in adipocyte extract is shown.

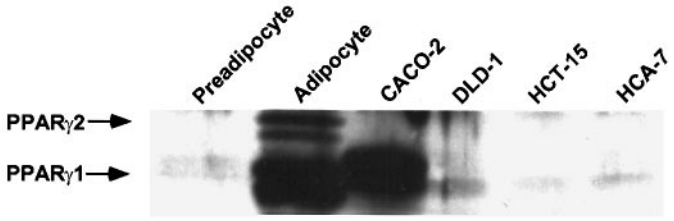

Fig. 4. PPARγ1 is expressed in a subset of human colon cancer cell lines. Western analysis of PPARγ protein expression in the indicated human colon cancer cell lines.

Since PPARγ is expressed at very high levels in adipocytes, it is important to control for fat contamination of the samples. The near absence of PPARγ mRNA or protein expression in the normal samples (Figures 1 and 2A) is reassuring, since peri-intestinal fat contamination might be expected to be more of a concern in the normal tissues. To confirm that the tumor samples were not contaminated by adipose tissue, we performed Western analysis on the four tumors with the highest expression of PPARγ1, as confirmed in Figure 2B. The blot was then reprobed with antibody to adipsin, a fat-specific marker. Figure 2B shows that the extract from adipocytes indeed contained adipsin, as expected, whereas none of the tumor extracts contained detectable levels of adipsin protein, consistent with the lack of adipose contamination. The Western blot was also reprobed with antiserum to COX–2. The results, shown in Figure 2B, show that the tumors which expressed PPARγ1 also expressed COX–2, although there was not a direct correlation between the two suggesting that the expression of each was independent of the other.

PPARγ1 is expressed in a subset of carcinogen-induced intestinal polyps

Much evidence suggests that adenocarcinoma of the colon is a late stage of a multistep process involving numerous changes in gene expression (38). Adenomatous polyps represent an earlier stage, characterized by abnormal proliferation, but lack of metastatic potential. To further characterize the induction of PPARγ during colon carcinogenesis, we examined carcinogen-induced adenomas (polyps) to determine if PPARγ was expressed. Figure 3 shows the results in carcinogen treated rats killed at a time when the major lesions were colonic as well as small intestinal polyps. Again PPARγ protein was not detectable in normal mucosa. By contrast, PPARγ was expressed in a colonic polyp as well as a small intestinal polyp. The closely spaced doublet (also seen in the other PPARγ immunoblots) is due to phosphorylation of PPARγ, with the upper band of the doublet corresponding to the phosphorylated form which has reduced transcriptional activity (39–42). Interestingly, not all polyps, even from the same animal, expressed PPARγ1 (lanes 3 and 7). This could reflect inherent heterogeneity in the tumors, or be the result of sampling polyps at different stages of tumor progression. The latter hypothesis suggests that PPARγ1 expression could be a marker for polyps that are more likely to transform into frank adenocarcinoma.

Expression of PPARγ1 in human colon cancer cell lines. Based upon the rodent data described above, we hypothesized that PPARγ would be expressed in human colon cancer cells as well. To begin to address this, we examined a variety of human colon cancer cell lines for PPARγ expression, including CaCo–2, DLD–1, HCT–15, HCA–7, HCT–116, and SW–480. Interestingly, only a subset demonstrated PPARγ expression, as shown in Figure 4; in those cell lines that did express PPARγ, the only isoform detected was PPARγ1, consistent with what we observed in the rodent model. Of those cell lines in which PPARγ protein was easily detectable, there was a marked variation in expression, with CaCo–2 cells reproducibly expressing by far the highest levels of PPARγ expression, with much lower to undetectable levels in the other cell lines.

PPRE transactivation in CaCo–2 cells. To determine if the PPARγ receptor in the CaCo–2 cells was functional we transfected the cells with a luciferase reporter plasmid containing three copies of a PPAR-response element (kindly provided by R.Evans [27]). Following treatment of the CaCo–2 cells with 15 deoxy-Δ12,14 PGJ2 (10 μm), we observed a >2-fold increase in luciferase activity (Figure 5). These data confirm that the PPARγ in CaCo–2 cells was likely to be functional.

Discussion

Understanding the molecular events involved in the development of colorectal neoplasia has progressed remarkably during

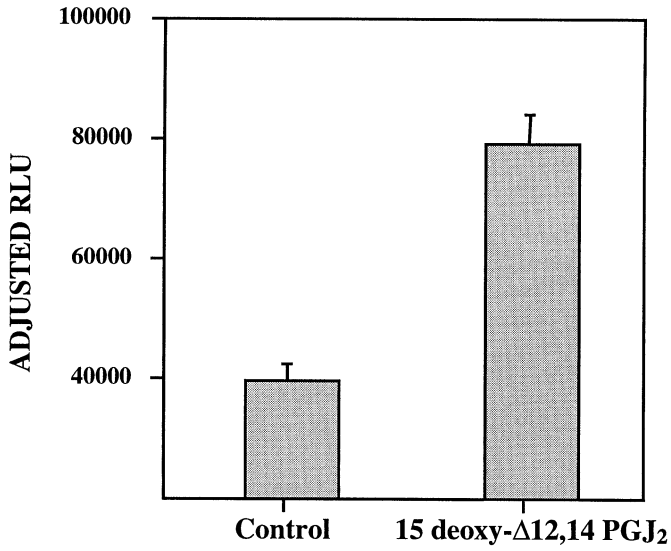

Fig. 5. Transactivation of PPARγ transcription in CaCo–2 cells. CaCo–2 cells were transiently transfected with PPRE3-luciferase (1 μg) and pRL-TK (0.5 μg) by lipid transfection. Cells were treated with or without 15-deoxy-Δ12,14-prostaglandin J_2 for 12 h. Cells were harvested and the Dual-luciferase assay was performed as described in Materials and methods. Representative of two independent experiments: the means of normalized relative light units from three independent transfections are presented. Error bars = SEM.

the past decade. There is a wealth of evidence that dietary factors, especially dietary fat and fiber influence the development of colorectal cancer (43). However, there has been little understanding of how these dietary factors and genetic factors interact (44). Clinical and epidemiological studies have shown a relationship between NSAID use and reduction in relative risk for colorectal cancer (1–6). Although the mechanism whereby NSAIDs mediate these effects are unknown, inhibition of cyclooxygenase enzymes leading to a reduction of eicosanoid production remains a possibility. We and others have shown that mitogen-inducible cyclooxygenase (COX–2) expression is upregulated in human colorectal carcinomas (16–19). Recent reports have shown a link between the tumorogenic effect of APC mutations and arachidonic acid metabolism by observation that deletion of the COX–2 (prostaglandin endoperoxide synthase–2) gene reduces the number of tumors in mice heterozygous for an $APC^{\Delta 716}$ mutation by >6-fold (22). Additional evidence supporting a role for COX–2 comes from studies which show a marked reduction in aberrant crypt formation in rodents treated with highly selective COX–2 inhibitors (15). Recent studies by our group also demonstrate a marked inhibition of tumor growth by treatment with a highly selective COX–2 inhibitor (24). A major question that remains to be answered is which signaling pathways are involved downstream of the COX–2 enzyme? These could provide additional molecular targets for cancer prevention studies.

The results described here demonstrate that PPARγ expression is elevated in AOM-induced rat intestinal tumors, both at the RNA and protein levels. Although PPARγ expression is greatest in adipocytes, expression in other tissues such as liver, muscle, heart, and spleen has also been observed in rodents, rabbits, and humans (35–37). Normal adult rodent intestinal tissues express levels of PPARγ mRNA that are undetectable by standard Northern analysis (29). Thus, the expression of PPARγ in a subset of polyps, cancer cell lines, and adenocarcinomas is aberrant and could play a role in carcinogenesis. Of some interest, we have observed that the highest level of PPARγ expression was found in the CaCo–2 cell line and that the receptor is functional in these cells. We have recently shown that forced expression of COX–2 in CaCo–2 cells leads to a dramatic increase in their metastatic potential (45). It will be important to understand the possible role (if any) of PPARγ in this altered phenotype. It is possible that one of the effects of COX–2 overexpression is to increase local production of a PPARγ ligand, presumably PGJ_2 or a related species. This could provide a key link between dietary fatty acid precursors, eicosanoids, COX–2, and transcriptional regulation which could be involved in colorectal carcinogenesis. Work is underway to determine the biological relevance of aberrant co-expression of PPARγ and COX–2 in colon cancer.

Acknowledgements

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References

1. Giovannucci,E., Egan,K.M., Hunter,D.J., Stampfer,M.J., Colditz,G.A., Willett,W.C. and Speizer,F.E. (1995) Aspirin and the risk of colorectal cancer in women. *N. Eng. J. Med.*, **333**, 609–614.
2. Greenberg,E.R., Baron,J.A., Freeman,D.H.J., Mandel,J.S. and Haile,R. (1993) Reduced risk of large-bowel adenomas among aspirin users. The Polyp Prevention Study Group. *J. Natl Cancer. Inst.*, **85**, 912–916.
3. Thun,M.J., Namboodiri,M.M. and Heath,C.W.J. (1991) Aspirin use and reduced risk of fatal colon cancer. *N. Engl. J. Med.*, **325**, 1593–1596.
4. Thun,M.J., Namboodiri,M.M., Calle,E.E., Flanders,W.D. and Heath,C.W.J. (1993) Aspirin use and risk of fatal cancer. *Cancer Res.*, **53**, 1322–1327.
5. Peleg,I.I., Maibach,H.T., Brown,S.H. and Wilcox,C.M. (1994) Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. *Arch. Int. Med.*, **154**, 394–9.
6. Giovannucci,E., Rimm,E.B., Stampfer,M.J., Colditz,G.A., Ascherio,A. and Willett,W.C. (1994) Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. *Ann. Intern. Med.*, **121**, 241–246.
7. Giardiello,F.M., Hamilton,S.R., Krush,A.J., Piantadosi,S., Hylind,L.M., Celano,P., Booker,S.V., Robinson,C.R. and Offerhaus,G.J. (1993) Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N. Engl. J. Med.*, **328**, 1313–1316.
8. Waddell,W.R. and Loughry,R.W. (1983) Sulindac for polyposis of the colon. *J. Surg. Oncol.*, **24**, 83–87.
9. Waddell,W.R., Gasner,G.F., Cerise,E.J. and Loughry,R.W. (1989) Sulindac for polyposis of the colon. *Am. J. Surg.*, **157**, 175–178.
10. Winde,G., Gumbinger,H.G., Osswald,H., Kemper,F. and Bunte,H. (1993) The NSAID sulindac reverses rectal adenomas in colectomized patients with familial adenomatous polyposis: clinical results of a dose-finding study on rectal sulindac administration. *Int. J. Colorectal. Dis.*, **8**, 13–17.
11. Nugent,K.P., Farmer,K.C., Spigelman,A.D., Williams,C.B. and Phillips, R.K. (1993) Randomized controlled trial of the effect of sulindac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. *Brit. J. Surg.*, **80**, 1618–1619.
12. Reddy,B.S., Nayini,J., Tokumo,K., Rigotty,J., Zang,E. and Kelloff,G. (1990) Chemoprevention of colon carcinogenesis by concurrent administration of piroxicam, a nonsteroidal antiinflammatory drug with D,L-alpha-difluoromethylornithine, an ornithine decarboxylase inhibitor, in diet. *Cancer Res.*, **50**, 2562–2568.
13. Reddy,B.S., Rao,C.V., Rivenson,A. and Kelloff,G. (1993) Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. *Carcinogenesis*, **14**, 1493–1497.
14. Craven,P.A. and DeRubertis,F.R. (1992) Effects of aspirin on 1,2-dimethylhydrazine-induced colonic carcinogenesis. *Carcinogenesis*, **13**, 541–546.
15. Reddy,B.S., Rao,C.V. and Seibert,K. (1996) Evaluation of cyclooxygenase–2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res.*, **56**, 4566–4569.

16. Eberhart,C.E., Coffey,R.J., Radhika,A., Giardiello,F.M., Ferrenbach,S. and DuBois,R.N. (1994) Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology*, **107**, 1183–1188.
17. Kargman,S., O'Neill,G., Vickers,P., Evans,J., Mancini,J. and Jothy,S. (1995) Expression of prostaglandin G/H synthase–1 and –2 protein in human colon cancer. *Cancer Res.*, **55**, 2556–2559.
18. Sano,H., Kawahito,Y., Wilder,R.L., Hashiramoto,A., Mukai,S., Asai,K., Kimura,S., Kato,H., Kondo,M. and Hla,T. (1995) Expression of cyclooxygenase–1 and –2 in human colorectal cancer. *Cancer Res.*, **55**, 3785–3789.
19. Kutchera,W., Jones,D.A., Matsunami,N., Groden,J., McIntyre,T.M., Zimmerman,G.A., White,R.L. and Prescott,S.M. (1996) Prostaglandin H synthase–2 is expressed abnormally in human colon cancer: Evidence for a transcriptional effect. *Proc. Natl Acad. Sci. USA*, **93**, 4816–4820.
20. DuBois,R.N., Radhika,A., Reddy,B.S. and Entingh,A.J. (1996) Increased cyclooxygenase–2 levels in carcinogen-induced rat colonic tumors. *Gastroenterology*, **110**, 1259–1262.
21. Williams,C.W., Luongo,C., Radhika,A., Zhang,T., Lamps,L.W., Nanney,L.B., Beauchamp,R.D. and DuBois,R.N. (1996) Elevated cyclooxygenase–2 levels in *Min* mouse adenomas. *Gastroenterology*, **111**, 1134–1140.
22. Oshima,M., Dinchuk,J.E., Kargman,S.L., Oshima,H., Hancock,B., Kwong,E., Trzaskos,J.M., Evans,J.F. and Taketo,M.M. (1996) Suppression of intestinal polyposis in $APC^{\Delta 716}$ knockout mice by inhibition of prostaglandin endoperoxide synthase–2 (COX–2). *Cell*, **87**, 803–809.
23. Prescott,S.M. and White,R.L. (1996) Self promotion? Intimate connections between APC and prostaglandin H synthase–2. *Cell*, **87**, 783–786.
24. Sheng,H., Shao,J., Kirkland,S.C., Isakson,P., Coffey,R., Morrow,J., Beauchamp,R.D. and DuBois,R.N. (1997) Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase–2. *J. Clin. Invest.*, **99**, 2254–2259.
25. Williams,C.S., Smalley,W. and DuBois,R.N. (1997) Aspirin use and potential mechanisms for colorectal cancer prevention. *J. Clin. Invest.*, **100**, 1–5.
26. Yu,K., Bayona,W., Kallen,C.B., Harding,H.P., Ravera,C.P., McMahon,G., Brown,M. and Lazar,M.A. (1995) Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.*, **270**, 23975–23983.
27. Forman,B.M., Tontonoz,P., Chen,J., Brun,R.P., Spiegelman,B.M. and Evans,R.M. (1995) 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR. *Cell*, **83**, 803–812.
28. Kliewer,S.A., Lenhard,J.M., Willson,T.M., Patel,I., Morris,D.C. and Lehmann,J.M. (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell*, **83**, 813–819.
29. Chawla,A., Schwarz,E.J., Dimaculangan,D.D. and Lazar,M.A. (1994) Peroxisome proliferator-activated receptor (PPAR) gamma: adipose-predominant expression and induction early in adipocyte differentiation. *Endocrinology*, **135**, 798–800.
30. Tontonoz,P., Hu,E., Graves,R.A., Budavari,A.I. and Spiegelman,B.M. (1994) mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes Dev.*, **8**, 1224–1234.
31. Rao,C.V., Tokumo,K., Rigotty,J., Zang,E., Kelloff,G. and Reddy,B.S. (1991) Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, alpha-difluoromethylornithine, 16 alpha-fluoro–5-androsten–17-one, and ellagic acid individually and in combination. *Cancer Res.*, **51**, 4528–4534.
32. DuBois,R.N., Tsujii,M., Bishop,P., Awad,J.A., Makita,K. and Lanahan,A. (1994) Cloning and characterization of a growth factor-inducible cyclooxygenase gene from rat intestinal epithelial cells. *Am. J. Physiol.*, **266**, G822-G827.
33. DuBois,R.N., Awad,J., Morrow,J., Roberts,L.J. and Bishop,P.R. (1994) Regulation of eicosanoid production and mitogenesis in rat intestinal epithelial cells by transforming growth factor-α and phorbol ester. *J. Clin. Invest.*, **93**, 493–498.
34. Xue,J.C., Schwartz,E.J., Chawla,A. and Lazar,M.A. (1996) Distinct stages in adipogenesis revealed by retinoid inhibition of differentiation after induction of PPARgamma. *Mol. Cell. Biol.*, **16**, 1567–1575.
35. Michael,L.F., Lazar,M.A. and Mendelson,C.R. (1997) PPAR gamma1 expression is induced during cyclic AMP-stimulated differentiation of alveolar type II pneumocytes. *Endocrinology*, **138**, 3695–3703.
36. Vidal-Puig,A., Jimenez-Linan,M., Lowell,B.B., Hamann,A., Hu,E., Spiegelman,B., Flier,J.S. and Moller,D.E. (1996) Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J. Clin. Invest.*, **97**, 2553–2561.
37. Vidal-Puig,A.J., Considine,R.V., Jimenez-Linan,M., Werman,A., Pories, W.J., Caro,J.F. and Flier,J.S. (1997) Peroxisome proliferator-activated receptor gene expression in human tissues: effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J. Clin. Invest.*, **99**, 2416–2422.
38. Kinzler,K.W. and Vogelstein,B. (1996) Lessons from hereditary colorectal cancer. *Cell*, **87**, 159–170.
39. Camp,H.S. and Tafuri,S.R. (1997) Regulation of peroxisome proliferator-activated receptor γ activity by mitogen-activated protein kinase. *J. Biol. Chem.*, **272**, 13452–13457.
40. Adams,M., Reginato,M.J., Shao,D., Lazar,M.A. and Chatterjee,V.K. (1997) Transcriptional activation by peroxisome proliferator-activated receptor gamma is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. *J. Biol. Chem.*, **272**, 5128–5132.
41. Hu,E., Kim,J.B., Sarraf,P. and Spiegelman,B.M. (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. *Science*, **274**, 2100–2103.
42. Zhang,B., Berger,J., Zhou,G., Elbrecht,A., Biswas,S., White-Carrington,S., Szalkowski,D. and Moller,D.E. (1996) Insulin- and mitogen-activated protein kinase-mediated phosphorylation and activation of peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.*, **271**, 31771–31774.
43. Smalley,W. and DuBois,R.N. (1997) Colorectal cancer and non steroidal anti-inflammatory drugs. *Adv. Pharmacol.*, **39**, 1–20.
44. Watson,A.J.M. and DuBois,R.N. (1997) Lipid metabolism and APC–Implications for colorectal cancer prevention. *Lancet*, **349**, 444–445.
45. Tsujii,M., Kuwano,S. and DuBois,R.N. (1997) Cyclooxygenase–2 expression in human colon cancer cells increases metastatic potential. *Proc. Natl Acad. Sci. USA*, **94**, 3336–3340.

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