APC-dependent suppression of colon carcinogenesis by PPARγ

Geoffrey D. Girnun*, Wendy M. Smith†, Stavit Drori*, Pasha Sarraf*, Elisabetta Mueller*, Charis Eng†, Prashant Nambiar‡, Daniel W. Rosenberg‡, Roderick T. Bronson§, Winfried Edelmann¶, Raju Kucherlapati‖, Frank J. Gonzalez, and Bruce M. Spiegelman*††**

*Dana–Farber Cancer Institute and Department of Cell Biology, §Department of Pathology, Harvard Medical School, Boston, MA 02115; †Human Cancer Genetics Program, Ohio State University Comprehensive Cancer Center, Columbus, OH 43210; ‡Center for Molecular Medicine, University of Connecticut Health Center, Farmington, CT 06117; ¶Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461; ‖Harvard Partners Center for Genetics and Genomics, Boston, MA 02115; and **Laboratory of Metabolism, National Cancer Institute, Bethesda, MD 20892

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Activation of PPARγ by synthetic ligands, such as thiazolidinediones, stimulates adipogenesis and improves insulin sensitivity. Although thiazolidinediones represent a major therapy for type 2 diabetes, conflicting studies showing that these agents can increase or decrease colonic tumors in mice have raised concerns about the role of PPARγ in colon cancer. To analyze critically the role of this receptor, we have used mice heterozygous for *Pparγ* with both chemical and genetic models of this malignancy. Heterozygous loss of PPARγ causes an increase in β-catenin levels and a greater incidence of colon cancer when animals are treated with azoxymethane. However, mice with preexisting damage to *Apc*, a regulator of β-catenin, develop tumors in a manner insensitive to the status of PPARγ. These data show that PPARγ can suppress β-catenin levels and colon carcinogenesis but only before damage to the APC/β-catenin pathway. This finding suggests a potentially important use for PPARγ ligands as chemopreventative agents in colon cancer.

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the nuclear hormone receptor superfamily that was initially shown to be a key regulator of fat cell differentiation (1, 2). Subsequent studies showed PPARγ to be the functional receptor for the thiazolideneidione class of antidiabetic drugs such as troglitazone, pioglitazone, and rosiglitazone (3, 47). In recent years, considerable attention has focused on the ability of PPARγ to alter the growth of a variety of cancer-cell types, including those of the colon (reviewed in refs. 4 and 5). Normal colonic mucosa and colonic tumors express abundant PPARγ, and ligands for this receptor can induce changes in gene expression patterns and arrest growth of a variety of colon cancer cell lines. Growth of tumors arising from human colon cancer cells transplanted into nude mice can also be reduced by PPARγ ligands (6, 7). In addition, genetic studies have shown that there are heterozygous loss-of-function mutations in the gene encoding *PPARG* from tumors in ≈10% of human colon cancer patients examined (8). Paradoxically, two studies have suggested that activation of PPARγ actually *increases* tumor growth (9, 10). When $Apc^{+/min}$ mice were treated with PPARγ ligands, there was a small but significant increase in polyp number in the colon. To clarify the genetic role of PPARγ in colon cancer, we have analyzed mice carrying a heterozygous mutation in *Pparγ* in two different models of colon cancer. We report here that PPARγ is a powerful tumor suppressor gene in the colon, and that loss of one allele of PPARγ is sufficient to increase sensitivity to chemical carcinogenesis. However, this tumor-suppressor function depends entirely on the presence of an intact adenomatous polyposis coli (APC) gene. In wild-type mice, PPARγ regulates levels of β-catenin protein. In the presence of a mutant APC, the ability of PPARγ to regulate both β-catenin and colon tumorigenesis is completely lost.

Materials and Methods

Generation of Mutant Mice. Generation of mice with the second exon of the *Pparγ* gene flanked by loxP sites ($Pparγ^{flox/flox}$) has been described (11). Deletion of the neomycin cassette and second exon of *Pparγ in vivo* was achieved by crossing the $Pparγ^{flox/flox}$ mice with an *EIIa*-Cre transgenic line (12). Progeny that lacked the phosphoglycerate kinase (PGK) Neo cassette and exon 2 were identified by an 8-kb *Bam*HI fragment on a Southern blot of tail DNA (11). These mice were bred with a wild-type mouse to generate a colony of mice heterozygous at the *Pparγ* locus ($Pparγ^{+/-}$). Crossbreeding of $Pparγ^{+/-}$ mice was used to generate mixed littermates of wild-type ($Pparγ^{+/+}$) and $Pparγ^{+/-}$ mice for use in these studies. Mice were genotyped by PCR of tail DNA.

Chemical Model of Colon Carcinogenesis. In one group, 12 $Pparγ^{+/+}$ and 15 $Pparγ^{+/-}$ 12- to 14-week-old male mice were injected i.p. with 3.5 mg/kg azoxymethane (Sigma) once a week for 8 weeks. In the second group, 12 $Pparγ^{+/+}$ and 19 $Pparγ^{+/-}$ 12- to 14-week-old male mice were treated with 7.0 mg/kg azoxymethane once a week for 8 weeks. Mice in both groups were examined weekly for rectal bleeding or morbidity until 50 weeks after treatment (3.5 mg/kg dose) or until all mice had developed one of these signs (7.0 mg/kg dose). These signs were used as surrogate markers of endpoint survival, as described (13), at which point, mice were euthanized. Fifteen $Pparγ^{+/+}$ and $Pparγ^{+/-}$ male mice also were treated with saline as a control. After euthanasia of mice in all groups, colons were removed, flushed with PBS, and opened on filter paper. Before fixation, one to three tumors were removed under a dissecting scope and snap frozen for DNA, RNA, and protein analysis. Colons then were fixed in paraformaldehyde for 6 h. Saline-treated mice were euthanized at 50 weeks and 100 weeks of age, and colons were examined. Colons were also removed and processed as above from 16-week-old untreated $Pparγ^{+/+}$ and $Pparγ^{+/-}$ male mice.

Genetic Model of Colon Carcinogenesis. Generation of $Apc^{+/1638N}$ mice has been described (13, 14). $Pparγ^{+/-}$ mice were crossed with $Apc^{+/1638N}$ mice. Thirty-two $Apc^{+/1638N}$:$Pparγ^{+/+}$ and 29 $Apc^{+/1638N}$:$Pparγ^{+/-}$ male mice were observed over 65 weeks. Morbidity and rectal bleeding were used as surrogate markers of survival. After euthanasia, colons were removed, flushed with PBS, opened on filter paper, and fixed in paraformaldehyde or formalin.

Immunoblotting and Immunohistochemistry. Colonic epithelium was isolated by a modified method described by Saam *et al.* (15) by using three mice of each genotype per group. Protein extraction followed by Western blotting of protein lysates from colonic epithelium and tumors was performed as described (16).

Abbreviations: PPARγ, peroxisome proliferator-activated receptor γ; APC, adenomatous polyposis coli.

††To whom correspondence should be addressed at: Dana–Farber Cancer Institute, One Jimmy Fund Way, Boston, MA 02115. E-mail: bruce_spiegelman@dfci.harvard.edu.

Western blotting was performed by using PPARγ (Santa Cruz Biotechnology), β-catenin (Transduction Laboratories, Lexington, KY) or actin (Sigma) antibodies. Densitometry was performed by using IMAGEQUANT (Molecular Dynamics). Segments of colon from *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice were embedded in the same block to ensure uniform treatment of samples, and frozen sections were cut. Immunohistochemistry was performed by using a PPARγ (Santa Cruz Biotechnology) or β-catenin antibody (Santa Cruz Biotechnology). A Cy3 secondary antibody (Jackson Immunologicals, West Grove, PA) was used to determine indirect immunofluorescence by using standard techniques.

Tumor Analysis and Histology. To facilitate counting of polyps, colons were stained with a 0.2% methylene blue solution and examined under a dissecting microscope. For histology, segments of colon were paraffin embedded; 5-μM sections were cut and stained with hemoxylin and eosin.

Mutational Analysis of Murine *Pparγ*. Genomic DNA and RNA were extracted from the frozen tumors as described (17, 18). The genomic DNA then was subjected to mutation analysis by using PCR-based single-strand conformation polymorphism (SSCP) analysis and semiautomated sequencing with primers for all six exons of murine *Pparγ* using standard protocols (19, 20). RNA was subjected to reverse transcription–PCR (RT-PCR) by using specific primers. Two overlapping amplicons were formed and subjected to semiautomated sequence analysis. Oligonucleotide sequences for genotyping and mutational analysis are available upon request.

Results

Reduced Expression of PPARγ in the Colons of *Pparγ*$^{+/-}$ Mice. Mice with homozygous deletions of the *Pparγ* gene are not viable (21, 48); hence, these studies were performed with mice heterozygous for this gene (11). These mice were generated by crossing *Ppar*$^{flox/flox}$ mice with *EIIA*$^{cre/+}$ mice, as described in *Materials and Methods*. Sections from the colons of *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice showed no histological differences (Fig. 1*a*). To examine the PPARγ levels in these mice, protein lysates from the colonic epithelium was isolated and immunoblotted for PPARγ. Reduced PPARγ expression is observed in epithelium isolated from *Pparγ*$^{+/-}$ mice compared with wild-type controls (Fig. 1*b*). Immunohistochemical detection of PPARγ from the colons of 16-week-old mice demonstrated the presence of this protein throughout the length of the crypt (Fig. 1*c*). As expected, reduced PPARγ expression was observed in the heterozygous compared with the wild-type mice.

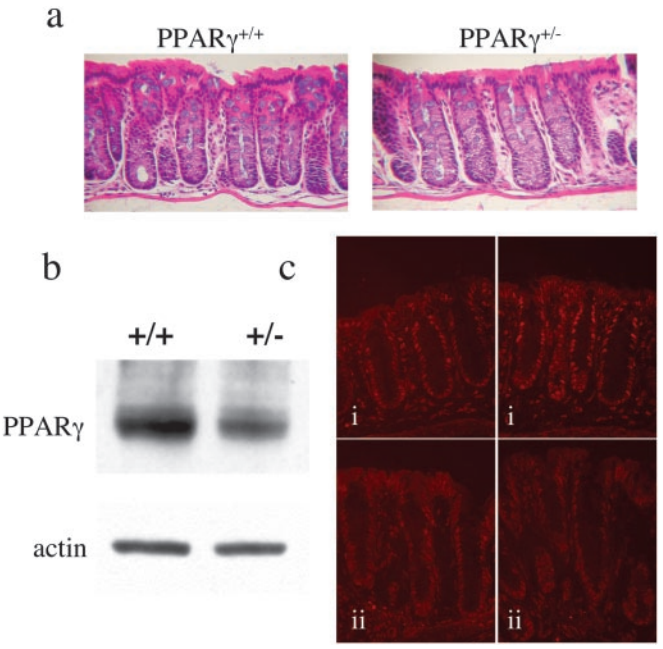
** 
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Fig. 1. Histopathology and PPARγ expression in the colons of untreated *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice. (*a*) Hematoxylin/eosin (H&E) staining of colons from *Pparγ*$^{+/+}$ *Pparγ*$^{+/-}$ mice. Representative H&E staining from one mouse of each genotype. (*b*) Expression of PPARγ from protein lysates isolated from the colonic epithelium of *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice. Protein lysates were obtained from the colonic epithelium of three mice per genotype. A representative immunoblot is shown. (*c*) Immunohistochemistry for PPARγ in the colons of (*i*) *Pparγ*$^{+/+}$ and (*ii*) *Pparγ*$^{+/-}$ mice. Two different sets of immunohistochemistry for PPARγ: one *Pparγ*$^{+/+}$ and one *Pparγ*$^{+/-}$ per set embedded in the same frozen section are shown.

***Pparγ*$^{+/-}$ Mice Are More Sensitive to Azoxymethane-Induced Colon Carcinogenesis.** To investigate the role of PPARγ in colon carcinogenesis, we used a chemical model of carcinogenesis. Azoxymethane is an organotypic carcinogen that induces tumors almost exclusively in the colons of rodents, although it is administered systemically (22). *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice were injected with 3.5 or 7.0 mg/kg azoxymethane or saline as a control, as described in *Materials and Methods*. Mice were euthanized when moribund or when they displayed signs of rectal bleeding. These endpoints were chosen as surrogate markers of survival as described (13) and according to animal care facility guidelines. In the absence of these symptoms, mice in the 3.5 mg/kg group were euthanized 50 weeks after the last injection.

Survival of the *Pparγ*$^{+/-}$ mice (as determined by rectal bleeding or severe morbidity) treated with 3.5 mg/kg azoxymethane was reduced to 53% (7/15) by 50 weeks of posttreatment (Fig. 2*a*). In contrast, survival in *Pparγ*$^{+/+}$ mice was reduced to 92% by the same time point ($P < 0.05$). The presence and number of colonic polyps was determined after euthanasia of all animals. Polyps were found in all mice showing signs of rectal bleeding, as well as in most mice killed due to morbidity.

Mice receiving 7.0 mg/kg azoxymethane showed a survival that was reduced to 50% by 25 weeks after treatment in the *Pparγ*$^{+/-}$ mice (Fig. 2*b*). In contrast, the survival in the *Pparγ*$^{+/+}$ mice was not reduced to 50% until almost 37 weeks. By 37 weeks, all of the *Pparγ*$^{+/-}$ mice had died or required euthanasia. The difference in time to 0% survival between the two groups was 18 weeks, a difference that was highly significant ($P < 0.001$). After euthanasia because of rectal bleeding or severe morbidity, we also examined the proximal and distal colons of these mice for polyp number, size, and pathology. Polyps were found in all mice showing signs of rectal bleeding as well as almost all mice without signs of rectal bleeding. For both groups, there were a significantly greater number of adenomas arising in the colons of *Pparγ*$^{+/-}$ mice compared with those in the *Pparγ*$^{+/+}$ mice treated with azoxymethane (Table 1).

Pathology of Tumors Arising in *Pparγ*$^{+/-}$ Compared with Wild-Type Mice. Histological analysis of the tumors from both groups of mice identified them as either adenomas or adenocarcinomas *in situ* (Fig. 3). However, no discernable histological differences between tumors from *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice were observed. In both genotypes, lobules of some tumor cells grew into the lamina propria, but did not extend down to the level of the muscularis mucosa. The degree of nuclear stratification and dysplasia also were similar. We also examined the colons of saline-control mice of both genotypes and found no polyp formation after 50 weeks and 100 weeks of age (Table 1 and data not shown, respectively). Taken together, these data clearly indicate that PPARγ functions as a tumor suppressor in this

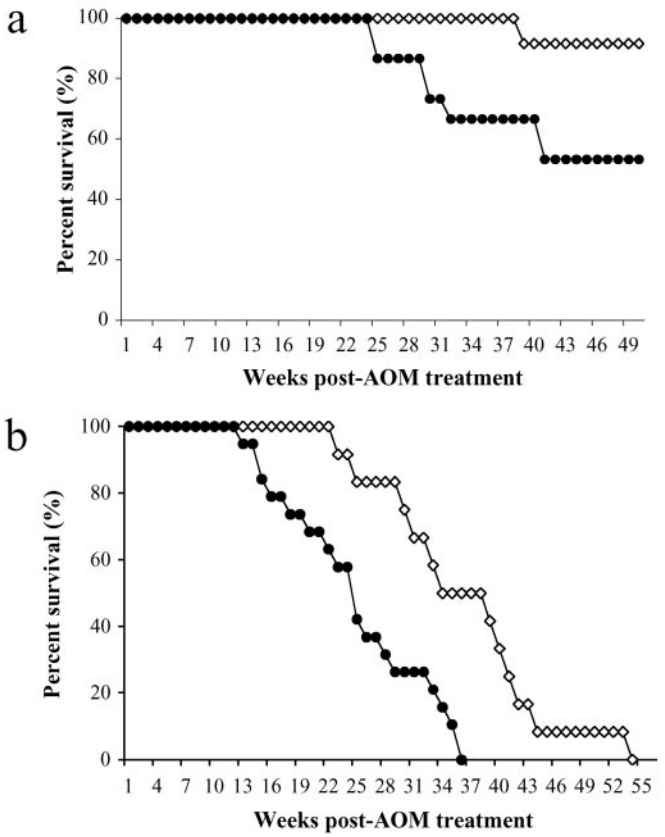

Fig. 2. Survival of *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice after treatment with azoxymethane. (*a*) Survival of mice treated with 3.5 mg/kg azoxymethane. Mice not displaying rectal bleeding or morbidity were euthanized at 50 weeks after the last azoxymethane injections. $P = 0.03$, log-rank test. (*b*) Survival of mice treated with 7.0 mg/kg azoxymethane. $P = 0.001$, log-rank test. ◇, *Pparγ*$^{+/+}$ mice; ●, *Pparγ*$^{+/-}$ mice.

model of colon carcinogenesis, with a strong role in the initiation phase of tumor formation.

Lack of Mutations or Epigenetic Silencing of *Pparγ* After Azoxymethane-Induced Carcinogenesis. The tumor-suppressive function of PPARγ described above was illustrated with a preexisting deletion of one copy of this gene. We then examined the azoxymethane-induced colon tumors for the presence of somatic mutations of the remaining *Pparγ* allele and also analyzed transcript from these tumors to determine whether the remaining allele was silenced by epigenetic means. Genomic DNA and RNA were extracted from 20 and 10 tumors each from *Pparγ*$^{+/-}$ and *Pparγ*$^{+/+}$ mice, respectively. The genomic DNA was subjected to SSCP and direct sequencing of all six exons and flanking intronic regions of *Pparγ*, but no variants were found (data not shown). The RNA was used as a template for RT-PCR as two overlapping amplicons. PCR products were visible for all tumors. These products were subjected to direct sequence analysis; no mutations were detected in any of the tumors (data not shown). Thus, it does not seem that "second hit" somatic mutations or epigenetic silencing of the remaining wild-type *Pparγ* gene are required for colorectal carcinogenesis when promoted by azoxymethane.

Increased β-Catenin Expression Before Carcinogenesis in *Pparγ*$^{+/-}$ Mice. These data showing a decreased latency and increased polyp formation in the *Pparγ*$^{+/-}$ mice strongly suggest that PPARγ functions early in the process of carcinogenesis.

Table 1. Quantification of tumors arising in the colons of *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice following chemical or genetic carcinogenesis

	Genotype	*n*	Tumors/mouse	% tumors (incidence)
Chemical				
Dose (azoxymethane)				
0 mg/kg*	*Pparγ*$^{+/+}$	10	0	0
	Pparγ$^{+/-}$	10	0	0
3.5 mg/kg*	*Pparγ*$^{+/+}$	12	0.42 ± 0.51	42 (5/12)
	Pparγ$^{+/-}$	15	1.47 ± 1.25‡	67 (10/15)
7.0 mg/kg	*Pparγ*$^{+/+}$	10	3.2 ± 2.5	90 (9/10)
	Pparγ$^{+/-}$	17	8.06 ± 5.9§	100 (17/17)
Genetic†				
APC status				
Apc$^{+/+}$	*Pparγ*$^{+/+}$	10	0	0
	Pparγ$^{+/-}$	10	0	0
Apc$^{+/1638N}$¶	*Pparγ*$^{+/+}$	17	1.41 ± 1.37	64.7 (11/17)
	Pparγ$^{+/-}$	20	1.85 ± 1.75	65 (13/20)

Tumors were counted following euthanasia due to rectal bleeding, morbidity, or at 50 (*) weeks post-azoxymethane or saline treatment or 65 (†) weeks of age. ‡, $P < 0.05$, §, $P < 0.01$ compared to *Pparγ*$^{+/+}$ treated with the same dose of azoxymethane. ¶No difference in the number of polyps arising in colons of *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice on the *Apc*$^{+/1638N}$ background, $P = 0.41$.

β-catenin has been shown to play a major role in the early stages of colon carcinogenesis, in part by means of its ability to activate a variety of proto-oncogenes (23, 24). Therefore, we examined the expression of this protein in colonic epithelium isolated from untreated *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice. Much greater β-catenin protein levels (2.7-fold increase) were observed from the colonic epithelium of untreated PPARγ$^{+/-}$ mice compared with *Pparγ*$^{+/+}$ mice (Fig. 4*a*). Immunohistochemistry for β-catenin in the colons of these untreated mice also shows greater staining for β-catenin in the colons of *Pparγ*$^{+/-}$ mice (Fig. 4*b*). In both genotypes, staining for β-catenin appears heaviest at the mucosal portion of the crypts and appears to be primarily associated with the cell membranes. No significant cytoplasmic or nuclear staining was observed in either genotype in the absence of a carcinogen. This finding is consistent with the cellular distribution of β-catenin normally seen in the absence of carcinogenesis (25, 26). We also examined β-catenin expression in tumors arising in the azoxymethane-treated mice. No difference in

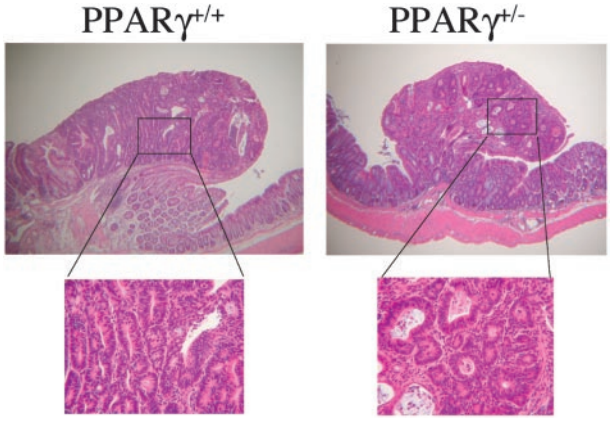

Fig. 3. Pathology of tumors arising in mice after azoxymethane treatment. H&E staining of tumors arising in the colons of *Pparγ*$^{+/+}$ (*Left*) and *Pparγ*$^{+/-}$ (*Right*) mice. (Magnification = ×10.) (*Inset*) Representative H&E sections. (Magnification = ×50.)

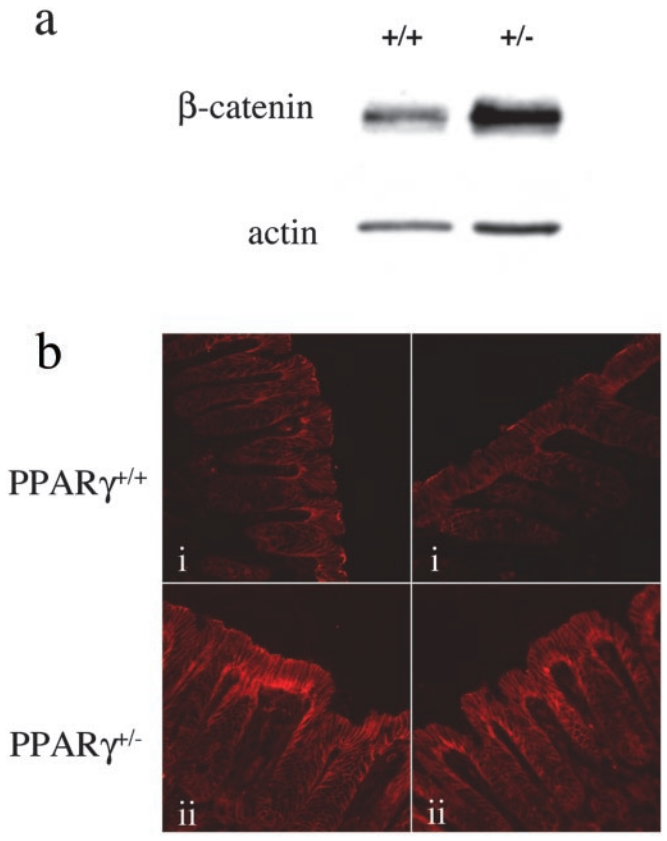

Fig. 4. Expression of β-catenin in the colonic epithelium of untreated mice. (*a*) Expression of β-catenin from protein lysate isolated from the colonic epithelium of *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice before carcinogen treatment. Protein lysates were obtained from the colonic epithelium of three mice per genotype. A representative immunoblot is shown. Actin levels are shown as a loading control. (*b*) Immunohistochemistry for β-catenin in the colons of (*i*) *Pparγ*$^{+/+}$ and (*ii*) *Pparγ*$^{+/-}$ mice before carcinogen treatment. Two different sets of immunohistochemistry for β-catenin, consisting of one *Pparγ*$^{+/+}$ and one *Pparγ*$^{+/-}$ per set embedded in the same frozen section are shown.

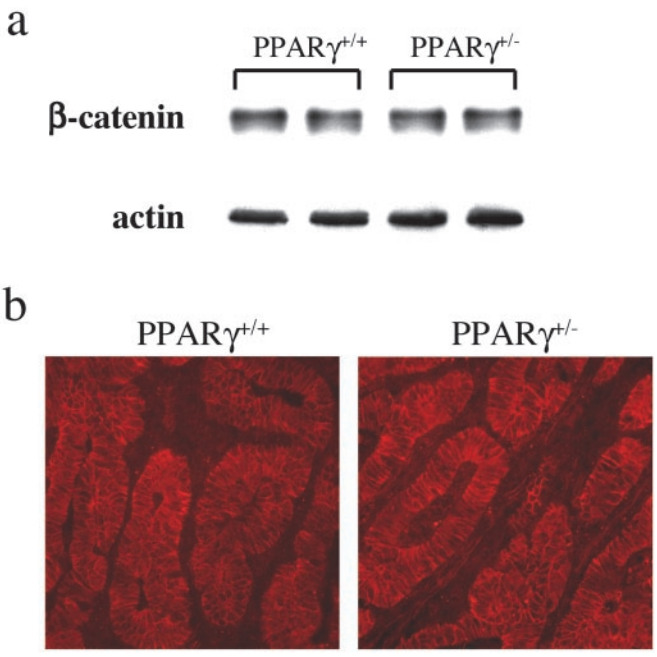

Fig. 5. Expression of β-catenin from tumors arising in azoxymethane-treated mice. (*a*) Expression of β-catenin in protein lysates from tumors arising in *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice after azoxymethane treatment. Representative immunoblots of two different tumors from different mice of each genotype are shown. Actin levels are shown as a loading control. (*b*) Expression of β-catenin in tumors arising in *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ mice by immunohistochemistry.

β-catenin levels were observed in tumors from *Pparγ*$^{+/-}$ compared with *Pparγ*$^{+/+}$ mice (Fig. 5*a*). Additionally, immunohistochemistry of tumors from *Pparγ*$^{+/+}$ and *Pparγ*$^{+/-}$ demonstrates no obvious difference in levels of β-catenin staining (5*b*). Thus, once tumorigenesis has been initiated, β-catenin levels seem insensitive to PPARγ status.

Carcinogenesis and β-Catenin Levels in the Presence of a Mutated *Apc* Allele. If this suppression of β-catenin levels by PPARγ before carcinogenesis represents a major component of its tumor suppressor activity, it might be expected that tumorigenesis initiated by preexisting dysregulation of this pathway would be less sensitive to loss of PPARγ. To test this hypothesis, we used mice with a targeted mutation in the *Apc* gene, a major regulator of β-catenin and a very important tumor suppressor in the colon (27–30). Mice heterozygous for a truncation in the *Apc* gene that results in a protein product that is truncated at amino acid 1,638 (*Apc*$^{+/1638N}$) develop several adenomas in the small intestine and one to two adenomas in the colon after ≈12 months (13, 14). Although these mice ordinarily succumb to small intestinal tumors, we wanted to determine whether neoplasia-related morbidity/mortality could be modulated by PPARγ haploinsufficiency.

We crossed the *Pparγ*$^{+/-}$ mice with *Apc*$^{+/1638}$ mice and isolated protein from the colonic epithelium of the offspring. Immunoblotting of these proteins from the *Apc*$^{+/1638N}$:*Pparγ*$^{+/-}$ and *Apc*$^{+/1638N}$:*Pparγ*$^{+/+}$ mice before polyp formation displayed no difference in β-catenin levels (Fig. 6*a*). We then observed these mice for 65 weeks. As expected, mice began succumbing to tumor burden in their small intestine by about 35 weeks of age (Fig. 6*b*). There was no significant difference in survival between the *Pparγ*$^{+/-}$ and *Pparγ*$^{+/+}$ mice and, most importantly, no difference in the number of tumors formed in the colon (Table 1). Thus, PPARγ has no apparent effect on β-catenin levels or tumorigenesis in the presence of preexisting APC dysfunction.

Discussion

Many recent studies have suggested an anticancer role for PPARγ in a variety of different malignancies, including colon cancer. However, almost all of these data have been of the gain-of-function variety, involving either the addition of PPARγ ligands to transformed cells or the application of PPARγ ligands to animal models of carcinogenesis. The work in colon cancer in particular has led to some controversy. It was demonstrated that PPARγ ligands arrested the growth of several different human colon cancer cell lines and slowed the growth of transplanted tumors in nude mice (6, 7). However, two groups demonstrated that mice carrying a preexisting mutation in the *Apc* gene responded to the application of PPARγ ligands with an increase in polyp formation in colon (9, 10). Because of these conflicting data, we undertook a genetic analysis of PPARγ in colon carcinogenesis. The studies here demonstrate conclusively that PPARγ is a suppressor of colon carcinogenesis and that haploinsufficiency at the *Pparγ* locus can increase sensitivity to chemical carcinogenesis. These data support our previous work that demonstrated a loss of function in one allele of *PPARG* was associated with colon carcinogenesis (8). Furthermore, several other studies have demonstrated that heterozygous mutations or deletions at the *PPARG* locus are associated with a variety of different cancers (31–33). Therefore PPARγ must be included in

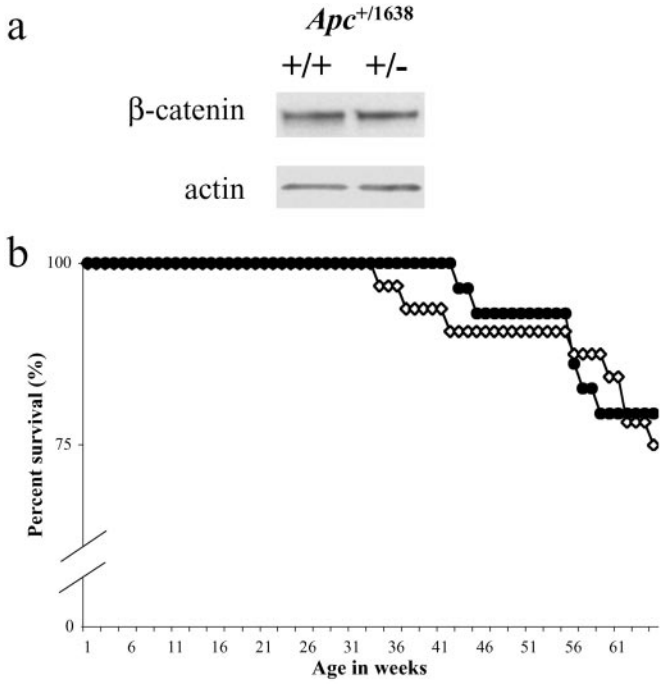

Fig. 6. β-catenin levels in the colonic epithelium and survival of $Apc^{+/1638N}$:$Ppar\gamma^{+/+}$ $Apc^{+/1638N}$:$Ppar\gamma^{+/+}$ mice. (*a*) Expression of β-catenin from protein lysate isolated from the colonic epithelium of $Ppar\gamma^{+/+}$ and $Ppar\gamma^{+/-}$ mice on an $Apc^{+/1638N}$ background. Protein lysates were obtained from the colonic epithelium of three mice per genotype. A representative immunoblot is shown. Actin levels are shown as a loading control. (*b*) Survival of $Ppar\gamma^{+/+}$ and $Ppar\gamma^{+/-}$ mice on an $Apc^{+/1638N}$ background. ◇, $Apc^{+/1638N}$:$Ppar\gamma^{+/+}$ mice; ●, $Apc^{+/1638N}$:$Ppar\gamma^{+/-}$ mice. $P = 0.25$, log-rank test.

a growing list of tumor suppressor genes in which monoallelic inactivation is sufficient to promote carcinogenesis (34, 35).

Although mutations or deletions to one allele of PPARγ may be associated with increased carcinogenesis, it is important to take into consideration posttranslation modification of PPARγ. PPARγ can be inactivated by phosphorylation at Ser-82 of murine PPARγ1 by mitogen-activated protein kinase kinase (MAPKK; refs. 36 and 37). Many cancers have activated Ras, and indeed, this is one of the mechanisms by which azoxymethane is believed to promote carcinogenesis (38–40). Because Ras activation leads to an increase in MAPKK signaling, the PPARγ protein that is expressed from the remaining wild-type allele may well have reduced function (41).

These studies strongly suggest that PPARγ may function as a tumor suppressor during the early steps of tumor formation via regulation of β-catenin. In the absence of any carcinogenic challenge, β-catenin seems to be elevated in the colons of mice heterozygous at the *Pparγ* locus. It is likely that this increased β-catenin primes the epithelium to respond more rapidly to a carcinogenic insult, thereby accelerating tumorigenesis in the $Ppar\gamma^{+/-}$ mice. However, once polyp formation has been accomplished, there is no difference in the β-catenin expression or histological appearance of tumors in the two genotypes.

That PPARγ functions as a tumor suppressor prior to the carcinogenic insult was further demonstrated in the *Apc* mutant mice. In these mice, levels of β-catenin are elevated (42). Thus β-catenin levels seem insensitive to the status of the *Pparγ* gene and furthermore, PPARγ does not seem to play a tumor suppressive role. These data are consistent with a model in which PPARγ is able to maintain lower steady-state levels of β-catenin in the presence of a normal APC pathway. How PPARγ regulates β-catenin levels is not clear, although a recent study (43) demonstrated that application of PPARγ ligands is capable of decreasing β-catenin levels *in vitro* and *in vivo* in adipose cells and tissue. It is also likely that PPARγ functions by controlling the amounts or activities of proteins known to regulate β-catenin levels such as APC, axin1, axin2, GSK3-β, or casein kinase (23, 24, 27, 44).

These data can help to reconcile some of the previous controversy concerning PPARγ and colon cancer, particularly the question of why no decrease in polyp number was observed with the application of PPARγ ligands to $Apc^{+/min}$ mice. Because PPARγ ligands were administered to animals that already had a defect in their APC pathway, alterations in PPARγ activity would not be expected to be efficacious in controlling β-catenin levels or subsequent tumor formation. Why a small increase in tumor formation was observed in these studies is not clear.

Our data have important potential implications for the use of thiazolidinediones in certain patient populations for the prevention or regression of colorectal cancer. This topic is of acute interest because there are currently 2 million patients in the U.S. taking thiazolidinediones, such as rosiglitazone (Avandia) or pioglitazone (Actos) for the treatment of type 2 diabetes. Type 2 diabetics are mainly a middle-aged and sedentary population who are also at increased risk for colon cancer (45, 46). If our murine models have relevance for human colon cancer, thiazolidinediones might be most efficacious in the prophylactic setting of high-risk groups who carry neither germline *APC* mutations nor have multiple existing lesions with somatic *APC* mutations. Of course, it is still possible that PPARγ ligands could have an impact on other aspects of tumor cell growth and/or progression that are not dependent upon the APC pathway and which could not be studied in these murine models. Indeed, our previous work with human colon cancer cell lines demonstrated growth inhibition even in the cell lines with mutant *APC* (6).

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1. Rosen, E. D. & Spiegelman, B. M. (2001) *J. Biol. Chem.* **276,** 37731–37734.
2. Tontonoz, P., Hu, E. & Spiegelman, B. M. (1994) *Cell* **79,** 1147–1159.
3. Lehmann, J. M., Moore, L. B., Smith-Oliver, T. A., Wilkison, W. O., Willson, T. M. & Kliewer, S. A. (1995) *J. Biol. Chem.* **270,** 12953–12956.
4. Gupta, R. A. & Dubois, R. N. (2002) *Am. J. Physiol.* **283,** G266–G269.
5. Sporn, M. B., Suh, N. & Mangelsdorf, D. J. (2001) *Trends Mol. Med.* **7,** 395–400.
6. Sarraf, P., Mueller, E., Jones, D., King, F. J., DeAngelo, D. J., Partridge, J. B., Holden, S. A., Chen, L. B., Singer, S., Fletcher, C., *et al.* (1998) *Nat. Med.* **4,** 1046–1052.
7. Brockman, J. A., Gupta, R. A. & Dubois, R. N. (1998) *Gastroenterology* **115,** 1049–1055.
8. Sarraf, P., Mueller, E., Smith, W. M., Wright, H. M., Kum, J. B., Aaltonen, L. A., de la Chapelle, A., Spiegelman, B. M. & Eng, C. (1999) *Mol. Cell* **3,** 799–804.
9. Saez, E., Tontonoz, P., Nelson, M. C., Alvarez, J. G., Ming, U. T., Baird, S. M., Thomazy, V. A. & Evans, R. M. (1998) *Nat. Med.* **4,** 1058–1061.
10. Lefebvre, A. M., Chen, I., Desreumaux, P., Najib, J., Fruchart, J. C., Geboes, K., Briggs, M., Heyman, R. & Auwerx, J. (1998) *Nat. Med.* **4,** 1053–1057.
11. Akiyama, T. E., Sakai, S., Lambert, G., Nicol, C. J., Matsusue, K., Pimprale, S., Lee, Y. H., Ricote, M., Glass, C. K., Brewer, H. B., Jr., *et al.* (2002) *Mol. Cell. Biol.* **22,** 2607–2619.
12. Lakso, M., Pichel, J. G., Gorman, J. R., Sauer, B., Okamoto, Y., Lee, E., Alt, F. W. & Westphal, H. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5860–5865.
13. Yang, K., Edelmann, W., Fan, K., Lau, K., Kolli, V. R., Fodde, R., Khan, P. M., Kucherlapati, R. & Lipkin, M. (1997) *J. Exp. Zool.* **277,** 245–254.
14. Fodde, R., Edelmann, W., Yang, K., van Leeuwen, C., Carlson, C., Renault, B., Breukel, C., Alt, E., Lipkin, M., Khan, P. M., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8969–8973.

15. Saam, J. R. & Gordon, J. I. (1999) *J. Biol. Chem.* **274,** 38071–38082.
16. Mueller, E., Sarraf, P., Tontonoz, P., Evans, R. M., Martin, K. J., Zhang, M., Fletcher, C., Singer, S. & Spiegelman, B. M. (1998) *Mol. Cell* **1,** 465–470.
17. Dahia, P. L., Aguiar, R. C., Alberta, J., Kum, J. B., Caron, S., Sill, H., Marsh, D. J., Ritz, J., Freedman, A., Stiles, C., *et al*. (1999) *Hum. Mol. Genet.* **8,** 185–193.
18. Mathew, C. G., Smith, B. A., Thorpe, K., Wong, Z., Royle, N. J., Jeffreys, A. J. & Ponder, B. A. (1987) *Nature* **328,** 524–526.
19. Mutter, G. L., Lin, M. C., Fitzgerald, J. T., Kum, J. B., Baak, J. P., Lees, J. A., Weng, L. P. & Eng, C. (2000) *J. Natl. Cancer Inst.* **92,** 924–930.
20. Gimm, O., Dziema, H., Brown, J., de la Puente, A., Hoang-Vu, C., Dralle, H., Plass, C. & Eng, C. (2001) *Int. J. Cancer* **92,** 70–74.
21. Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A. & Evans, R. M. (1999) *Mol. Cell* **4,** 585–595.
22. Druckney, H. (1970) in *Carcinoma of the Colon and Antecedent Epithelium*, ed. Burdette, W. J. (Thomas, Springfield, IL), pp. 267–279.
23. Wong, N. A. & Pignatelli, M. (2002) *Am. J. Pathol.* **160,** 389–401.
24. Bienz, M. & Clevers, H. (2000) *Cell* **103,** 311–320.
25. Inomata, M., Ochiai, A., Akimoto, S., Kitano, S. & Hirohashi, S. (1996) *Cancer Res.* **56,** 2213–2217.
26. Sheng, H., Shao, J., Williams, C. S., Pereira, M. A., Taketo, M. M., Oshima, M., Reynolds, A. B., Washington, M. K., DuBois, R. N. & Beauchamp, R. D. (1998) *Carcinogenesis* **19,** 543–549.
27. van Es, J. H., Giles, R. H. & Clevers, H. C. (2001) *Exp. Cell Res.* **264,** 126–134.
28. Su, L. K., Vogelstein, B. & Kinzler, K. W. (1993) *Science* **262,** 1734–1737.
29. Rubinfeld, B., Souza, B., Albert, I., Muller, O., Chamberlain, S. H., Masiarz, F. R., Munemitsu, S. & Polakis, P. (1993) *Science* **262,** 1731–1734.
30. Powell, S. M., Zilz, N., Beazer-Barclay, Y., Bryan, T. M., Hamilton, S. R., Thibodeau, S. N., Vogelstein, B. & Kinzler, K. W. (1992) *Nature* **359,** 235–237.
31. Smith, W. M., Zhou, X. P., Kurose, K., Gao, X., Latif, F., Kroll, T., Sugano, K., Cannistra, S. A., Clinton, S. K., Maher, E. R., *et al*. (2001) *Hum. Genet.* **109,** 146–151.
32. Mueller, E., Smith, M., Sarraf, P., Kroll, T., Aiyer, A., Kaufman, D. S., Oh, W., Demetri, G., Figg, W. D., Zhou, X. P., *et al*. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10990–10995.
33. Zhou, X. P., Smith, W. M., Gimm, O., Mueller, E., Gao, X., Sarraf, P., Prior, T. W., Plass, C., von Deimling, A., Black, P. M., *et al*. (2000) *J. Med. Genet.* **37,** 410–414.
34. Largaespada, D. A. (2001) *J. Exp. Med.* **193,** F15–F18.
35. Cook, D. L., Gerber, A. N. & Tapscott, S. J. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 15641–15646.
36. Hu, E., Kim, J. B., Sarraf, P. & Spiegelman, B. M. (1996) *Science* **274,** 2100–2103.
37. Adams, M., Reginato, M. J., Shao, D., Lazar, M. A. & Chatterjee, V. K. (1997) *J. Biol. Chem.* **272,** 5128–5132.
38. Singh, J., Kelloff, G. & Reddy, B. S. (1993) *Carcinogenesis* **14,** 699–704.
39. Bissonnette, M., Khare, S., von Lintig, F. C., Wali, R. K., Nguyen, L., Zhang, Y., Hart, J., Skarosi, S., Varki, N., Boss, G. R., *et al*. (2000) *Cancer Res.* **60,** 4602–4609.
40. Bos, J. L. (1989) *Cancer Res.* **49,** 4682–4689.
41. Campbell, S. L., Khosravi-Far, R., Rossman, K. L., Clark, G. J. & Der, C. J. (1998) *Oncogene* **17,** 1395–1413.
42. Smits, R., Kielman, M. F., Breukel, C., Zurcher, C., Neufeld, K., Jagmohan-Changur, S., Hofland, N., van Dijk, J., White, R., Edelmann, W., *et al*. (1999) *Genes Dev.* **13,** 1309–1321.
43. Gerhold, D. L., Liu, F., Jiang, G., Li, Z., Xu, J., Lu, M., Sachs, J. R., Bagchi, A., Fridman, A., Holder, D. J., *et al*. (2002) *Endocrinology* **143,** 2106–2118.
44. Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G. H., Tan, Y., Zhang, Z., Lin, X. & He, X. (2002) *Cell* **108,** 837–847.
45. Hu, F. B., Manson, J. E., Liu, S., Hunter, D., Colditz, G. A., Michels, K. B., Speizer, F. E. & Giovannucci, E. (1999) *J. Natl. Cancer Inst.* **91,** 542–547.
46. La Vecchia, C., Negri, E., Decarli, A. & Franceschi, S. (1997) *Cancer Epidemiol. Biomarkers Prev.* **6,** 1007–1010.
47. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M. & Evans, R. M. (1995) *Cell* **83,** 803–812.
48. Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, T., *et al*. (1999) *Mol. Cell* **4,** 597–609.