Chemopreventive Effect of Peroxisome Proliferator–Activated Receptor γ on Gastric Carcinogenesis in Mice

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Abstract

Peroxisome proliferator–activated receptor γ (PPARγ) is known to be expressed in several cancers, and the treatment of these cancer cells with PPARγ ligands often induces cell differentiation and apoptosis. Recently, the chemopreventive potential of PPARγ ligands on colon carcinogenesis was reported, although the effect of PPARγ on colon carcinogenesis and the mechanism of the effect remain controversial. In this study, we attempted to elucidate the role of PPARγ in gastric carcinogenesis and explored the possible use of PPARγ ligand as a chemopreventive agent for gastric cancer. *N*-methyl-*N*-nitrosourea (MNU, 240 ppm) was given in drinking water for 10 weeks to induce gastric cancer in PPARγ wild-type (+/+) and heterozygous-deficient (+/−) mice, followed by treatment with PPARγ ligand [troglitazone, 0.15% (w/w) in powder food] or the vehicle alone for 42 weeks. At the end of the experiment, PPARγ (+/−) mice were more susceptible to MNU-induced gastric cancer than wild-type (+/+) mice (89.5%/55.5%), and troglitazone significantly reduced the incidence of gastric cancer in PPARγ (+/+) mice (treatment 55.5%/vehicle 9%) but not in PPARγ (+/−) mice. The present study showed that (a) PPARγ suppresses gastric carcinogenesis, (b) the PPARγ ligand troglitazone is a potential chemopreventive agent for gastric carcinogenesis, and (c) troglitazone's chemopreventative effect is dependent on PPARγ. (Cancer Res 2005; 65(11): 4769-74)

Introduction

Peroxisome proliferator–activated receptor gamma (PPARγ) is a member of a superfamily of nuclear hormone receptors (1). PPARγ heterodimerizes with retinoid X receptor to bind to the PPAR response element, leading to the transcription of downstream genes (2). PPARγ is known to be expressed in various organs, including adipose tissue (3), mammary glands (4), small intestine (5), lung (6), colon (5), and stomach (7), and is also up-regulated in various types of cancer cells. Several specific ligands have been identified, such as the thiazolidinediones (including pioglitazone, rosiglitazone, and troglitazone), 15-deoxy-prostaglandin-J_2, and certain polyunsaturated fatty acids. PPARγ ligands have been reported to induce cell differentiation and apoptosis in several cancers (8–12), suggesting a potential application as anticancer agents. Furthermore, some reports recently suggested that PPARγ ligands can be used as chemopreventive agents for colon, breast, and tongue carcinogenesis (13–16). However, the effect of PPARγ ligands on colon cancer is controversial (17, 18). On the other hand, some recent studies have reported that the biological effect of PPARγ ligand is independent of PPARγ (19–23).

Whereas gastric cancer mortality has markedly declined around the world, it remains the second leading cause of cancer death worldwide (24, 25). Increasing interest has been shown in the chemoprevention of gastric cancer because of the low curable rate and the poor relative survival rate (26–29). Although the anticancer effect of PPARγ ligands has been reported in several gastric cancer cell lines (30–34), no information is available on the role of PPARγ in gastric carcinogenesis or whether PPARγ ligands actually inhibit gastric carcinogenesis.

To address the above questions, *N*-methyl-*N*-nitrosourea (MNU) was used to induce gastric cancer in PPAR wild-type (+/+) and PPARγ heterozygous-deficient (+/−) mice, followed by treatment with a PPARγ ligand, troglitazone, for 1 year. Our results clearly showed that PPARγ plays a protective role in gastric carcinogenesis and that the chemopreventative effect of troglitazone is dependent on PPARγ.

Materials and Methods

Animals. PPARγ knockout mice were generated as described previously (35, 36). Homozygous PPARγ knockout embryos (−/−) died because of placental dysfunction. We therefore used PPARγ heterozygous-deficient mice (+/−) in this study. To minimize the effect of the genetic background on carcinogenesis, one male and one female knockout mouse that had been generated by sister-brother mating for >10 generations were mated and all offspring were genotyped for the *PPARγ* gene. Then, all PPARγ heterozygous-deficient (+/−) offspring were mated again until the fourth generation mice were born. Both the wild-type and the heterozygous-deficient mice used in the present study were littermates. All mice were maintained in plastic cages with hardwood chip bedding in an air-conditioned room with a 12-hour light/12-hour dark cycle and given food (oriental CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) irradiated with 30 Gy of gamma rays and filtered tap water *ad libitum*.

Genetic typing. DNA was extracted from the ear of each mouse. Briefly, the ear was cut and the tissue was immersed into 400 μL of freshly prepared lysis buffer [80% saline sodium citrate, 2.5 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 8.0), 1% SDS, and 80 μg/mL proteinase K (Boehringer Mannheim GmbH, Indianapolis, IN)] and vortexed gently at 37°C overnight. Then 200 μL of phenol and 200 μL of chloroform were added to the lysate, and the mixture was rotated at room temperature for 1 hour, placed on ice for 5 minutes, and centrifuged at 15,000 rpm for 5 minutes. The aqueous phase was then transferred to a new tube. The above process was repeated a second time. Then, 400 μL of chloroform was added to the aqueous phase, and the solution was gently shaken by hand for 1 minute and centrifuged at

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15,000 rpm for 5 minutes. DNA was recovered by the addition of 400 μL isopropanol, decanted, and rinsed with 70% ethanol thrice. The DNA was dissolved in 100 μL of TE buffer. The concentration and purity of the DNA was examined with a spectrophotometer DU 60 (Beckman, Fullerton, CA). The extracted DNA was then subjected to a PCR.

PCR. PCR was done in a TaKaRa PCR Thermal Cycler 480 (TaKaRa Biomedicals, Shiga, Japan) in a total volume of 50 μL containing 100 ng DNA, PCR buffer, 4 μL deoxynucleotide triphosphate (TaKaRa Biomedicals), 0.5 unit γTaq DNA polymerase (TaKaRa Biomedicals), 20 pmol of primer PPARγ sense, 10 pmol of primer PPARγ antisense, and 10 pmol of primer exon for 40 cycles of 30 seconds at 94°C for denaturation, 1 minute at 57°C for annealing, and 1 minute at 72°C for extension. The following primers were used: sense 5′-tctatgaggactgctctgcc-3′, antisense 5′-ggtattcttggagcttcagg-3′, and exon 5′-gccaccaaagaacggagccg-3′. The PCR product derived from the wild-type and knockout loci of *PPARγ* were easily differentiated by agarose gel electrophoresis. The 400-bp band corresponds to the knockout allele, and the 300-bp band corresponds to the wild-type allele (data not shown).

Chemicals. *N*-Methyl-*N*-nitrosourea (MNU; Sigma Chemical, St. Louis, MO) was dissolved in distilled water at a concentration of 240 ppm and freshly prepared thrice per week for administration in drinking water in light-shielded bottles *ad libitum*.

The PPARγ-specific ligand troglitazone was kindly provided by Sankyo Co., Ltd. (Tokyo, Japan). Troglitazone was mixed well in irradiated powder food (CE-2 M, Clea Japan, Inc., Tokyo, Japan) at a concentration of 0.15% (w/w) and freshly prepared thrice per week for administration.

Experimental design. The experimental design was approved by the Institutional Ethics Review Committee for animal experiments at the National Cancer Center. The experiment design is shown in Fig. 1. Sixty-five PPARγ (+/−) and 42 wild-type (+/+) mice, all male, age 4 to 7 weeks, were randomly divided into four groups, respectively. Animals in groups 2, 3, 6, and 7 were given drinking water containing 240 ppm MNU in light-shielded bottles on alternate weeks for a total of 10 weeks exposure, according to the protocol described in a previous report (37). Animals in groups 1, 4, 5, and 8 received only water. Several mice in each MNU-administered group died during the first 10 weeks and were omitted from the final analysis. At experimental week 11, all the drinking water was switched to autoclaved distilled water. The mice in groups 3, 4, 7, and 8 were then given powder food containing 0.15% troglitazone, whereas groups 1, 2, 5, and 6 received only the powder food for 42 weeks, until the end of the experiment. All mice were carefully autopsied at the time of their death, either after having been killed because they had become moribund or at the end of the experiment (week 52).

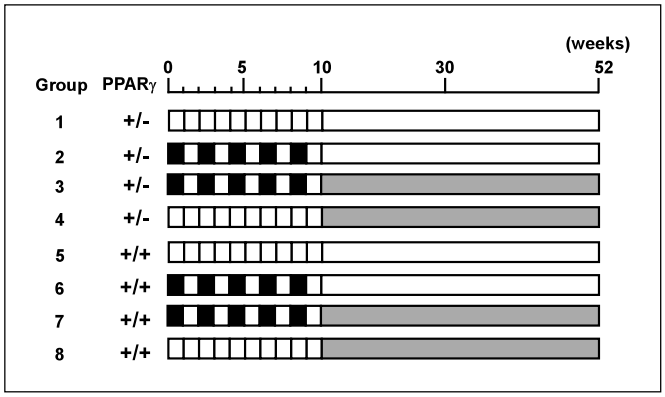

Figure 1. Experimental design. A total of 107 mice were used in the experiment. Groups 1 to 4 consisted of PPARγ-deficient (+/−) mice and groups 5 to 8 of wild-type (+/+) mice. Animals in groups 2, 3, 6, and 7 were given 240 ppm of MNU in drinking water on alternate weeks for a total of 10 weeks of exposure. Animals in groups 1, 4, 5, and 8 received only water. From the 11th week onward, all mice were given autoclaved distilled water, and the mice in groups 3, 4, 7, and 8 were given 0.15% (w/w) troglitazone mixed in powder food, whereas groups 1, 2, 5, and 6 received only the powder food for 42 weeks, until the end of the experiment. *Black,* MNU 240 ppm in drinking water; *white,* vehicle (distilled water and powder food); *thatched,* troglitazone (0.15% mixed in powder food).

Histopathologic analysis. At the end of the experiment, all mice were fasted for 24 hours and killed. Before sacrifice, blood samples were obtained directly from the heart. The stomachs and other organs were carefully examined macroscopically. The excised stomachs were fixed in 4% paraformaldehyde in PBS, cut into 1-mm-wide strips, embedded in paraffin, and cut into 4-μm-thick sections.

The 4-μm-thick sections were stained with H&E and carefully examined. Well-differentiated adenocarcinomas were characterized by excessive glandular proliferation with pronounced structural and cellular atypia invading at least the submucosa, moderately differentiated adenocarcinomas by cellular atypia and atypical glandular structures, and carcinoma *in situ* by glandular proliferation with marked structural and cellular atypia within the gastric mucosa. Other macroscopically abnormal organs were also examined histologically.

Western blot analysis of peroxisome proliferator–activated receptor γ. Tissue samples obtained from mice were dissolved in lysis buffer, and the insoluble tissues were removed by centrifugation. Thirty milligrams of each solubilized lysate were then separated by gel electrophoresis on a polyacrylamide gel containing SDS and transferred to nylon membranes. PPARγ was detected with an anti-PPARγ (H-100) antibody (Santa Cruz Biotechnology, Santa Cruz, CA); visualization was accomplished using an enhanced chemiluminescence system (Amersham Pharmacia Biotech K.K., Buckinghamshire, United Kingdom).

Immunohistochemistry. The expression of PPARγ in the mouse stomach mucosa was examined by immunohistochemistry. The tissue sections were incubated at 4°C overnight with primary antibody for PPARγ, and biotinylated polyclonal anti-rabbit immunoglobulin G/horseradish peroxidase (BD Biosciences, San Jose, CA) was used as the secondary antibody. Then visualization was done. The specificity of the binding was confirmed by omitting the primary antibody, and this staining was used as a negative control.

Statistical analysis. The incidences of gastric cancer were analyzed using Fisher's exact test. Survival curves were drawn using the Kaplan-Meier method and analyzed using the log-rank test. $P < 0.05$ was regarded statistically significant.

Results

Expression of peroxisome proliferator–activated receptor γ in mouse gastric mucosa. The expression of PPARγ in PPARγ wild-type (+/+) and heterozygous-deficient (+/−) mice was examined immunohistochemically using a PPARγ antibody. As shown in Fig. 2*A*, the expression of PPARγ in wild-type (+/+) mice was significantly higher than that in heterozygous PPARγ-deficient (+/−) mice, whereas PPARγ (+/−) mice also exhibited a medial expression of PPARγ, compared with the negative control, because of the heterozygous deficiency of PPARγ in these mice.

To confirm that PPARγ is expressed at discernible levels in the mouse stomach, we also did Western blot analysis. Because PPARγ is well known to be strongly expressed in the colon, the colon was used as a positive control. As shown in Fig. 2*B*, a considerable level of PPAR expression was detected in the stomach of the wild-type mice, although lower than that in the colon, and a weaker level of expression was detected in the heterozygous PPARγ-deficient mice.

Loss of peroxisome proliferator–activated receptor γ promotes gastric carcinogenesis. At the end of the experiment, all surviving mice were killed and their stomachs and other organs were carefully examined. The tumor incidences are summarized in Table 1.

None of the mice in groups 1, 4, 5, and 8 developed cancer. The carcinogen MNU induced gastric carcinoma in 17 of 19 PPARγ (+/−) mice (group 2, 89.5%): one carcinoma *in situ*,

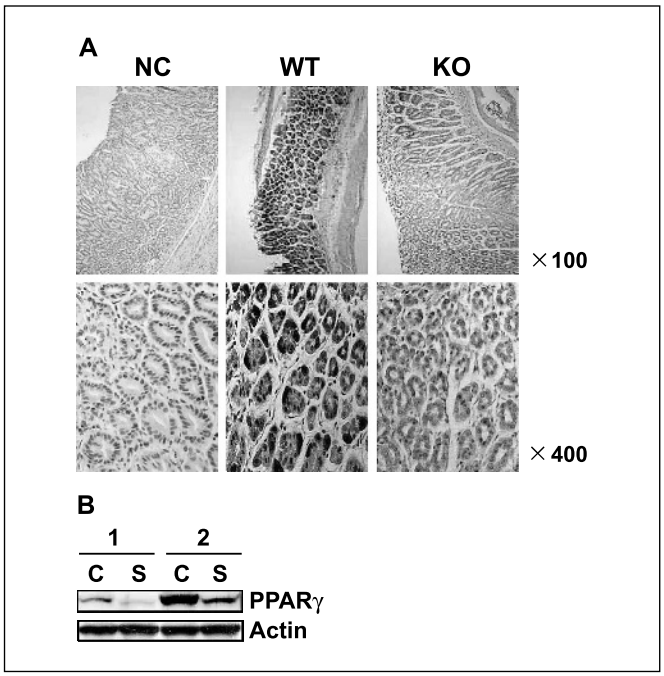

Figure 2. Expression of PPARγ in mouse stomach. *A,* representative section of mouse gastric mucosa. The expression of PPARγ in PPARγ (+/+) and (+/−) mice was examined immunohistochemically with PPARγ antibody. *NC,* negative control; *WT,* wild-type mouse (+/+); *KO,* heterozygous-deficient mice (+/−). *B,* Western blot analysis of PPARγ protein expression in the stomachs and colons of wild-type (+/+) and deficient (+/−) mouse. Thirty milligrams of tissue samples from heterozygous-deficient mice (+/−) (*lane 1*) and wild-type (+/+) (*lane 2*) were separated by SDS-PAGE electrophoresis and visualized using an anti-PPARγ antibody. *C,* colon; *S,* stomach; *Actin,* β-actin.

15 well-differentiated adenocarcinomas, one moderately differentiated adenocarcinoma and even one lymphoma in group 2 (+/−/MNU, total of 19 mice). Gastric carcinoma was only induced in five of the nine wild-type mice that were examined (group 6, 55.5%; $P < 0.05$, compared with group 2; Table 1), all of which were well-differentiated gastric adenocarcinomas. These data suggest that PPARγ deficiency significantly sensitizes mice to MNU-induced gastric carcinogenesis.

Peroxisome proliferator–activated receptor γ activation by troglitazone prevents the development of gastric carcinogenesis. Among the wild-type mice, five of nine mice treated with MNU were found to carry gastric carcinoma (55.5%), but the administration of 0.15% troglitazone for 42 weeks significantly reduced the incidence of gastric carcinogenesis to 1 of 11 mice (9%). However, troglitazone did not inhibit carcinogenesis in the heterozygous PPARγ-deficient mice (MNU 89.5%, MNU + Tro 80%; Table 1). Therefore, the preventative effect of troglitazone was considered dependent on PPARγ.

No appreciable histologic difference was observed in gastric carcinomas between wild-type and heterozygous peroxisome proliferator–activated receptor γ–deficient mice. No significant macroscopic differences in the gastric tumors were observed between the wild-type and PPARγ-deficient mice. The microscopic morphology of the tumors was also carefully examined. Representative images of the macroscopic appearance of stomach adenocarcinoma (Fig. 3*A*) and the microscopic appearance of normal stomach mucosa (Fig. 3*B*), well-differentiated adenocarcinoma (Fig. 3*C*), and moderately differentiated adenocarcinoma (Fig. 3*D*), carcinoma *in situ* (Fig. 3*E*), and lymphoma in stomach (Fig. 3*F*) are shown. The gastric adenocarcinomas were mainly located in the pyloric mucosa and occasionally at the fundopyloric border. No appreciable histologic difference in the gastric carcinomas was observed between the wild-type and heterozygous PPARγ-deficient mice.

Mice died after the 37th week because of carcinogen-induced gastric carcinoma. The survival curves for all eight mice groups are shown in Fig. 4. Before the end of the experiment (52nd week), 10 of 19 mice in group 2, 8 of 15 mice in group 3, 1 of 9 mice in group 6, and 1 of 11 mice in group 7 died. These data suggested that the increased gastric carcinoma burden reduced the survival time of PPARγ (+/−) mice, compared with that of wild-type mice ($P < 0.01$), but there was no difference between the control group and the troglitazone-treated group, either wild-type or PPARγ (+/−) mice. Because of the reported rare but severe hepatotoxicity of troglitazone, we examined the liver of mice treated with troglitazone. Neither degeneration nor necrosis was observed. Focal liver cell hyperplasia was found in one mouse in the group 3, but no dysplasia or cancer was observed. We did not find any abnormality in other organs.

Table 1. Incidence of stomach lesions in eight groups of mice at experiment week 52

Group	PPARγ	MNU	Tro	No. mice	Effective no. mice	Total incident of carcinoma (%)	No. mice with adenocarcinoma (%)			Lymphoma
							Well-differentiated	Moderately differentiated	Carcinoma *in situ*	
1	+/−	−	−	12	12	0	0	0	0	0
2	+/−	+	−	23	19	17 (89.5)*	15 (78.9)	1 (5.3)	1 (5.3)	1 (5)
3	+/−	+	+	19	15	12 (80)	12 (80)	0	0	0
4	+/−	−	+	11	11	0	0	0	0	0
5	+/+	−	−	8	7	0	0	0	0	0
6	+/+	+	−	11	9	5 (55.5)†	5 (55.5)	0	0	0
7	+/+	+	+	13	11	1 (9)	1 (9)	0	0	0
8	+/+	−	+	10	9	0	0	0	0	0

*$P < 0.01$, group 6.
†$P < 0.01$, group 7.

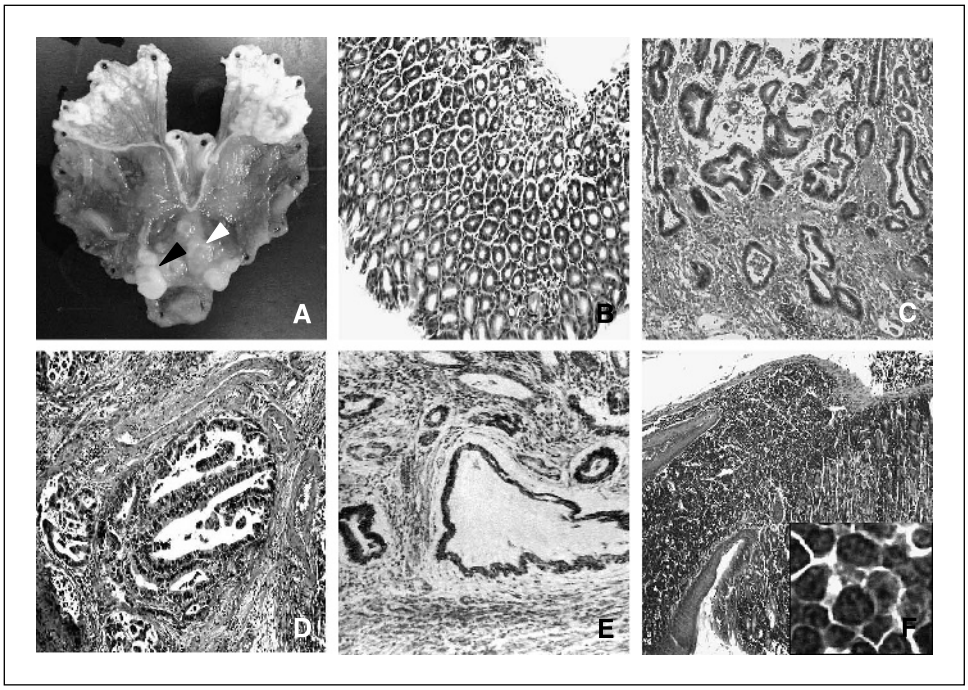

Figure 3. Gross and histologic appearance of representative stomach and its lesions. Stomach with tumor (*A*), the histologic appearance of normal stomach mucosa (*B*), well-differentiated adenocarcinoma (*C*), moderately differentiated adenocarcinoma (*D*), carcinoma *in situ* (*E*), and lymphoma in stomach (*F*). *Inset,* high power of the lymphoma cells.

Peroxisome proliferator–activated receptor γ expression was suppressed in cancerous gastric mucosa. The above observations suggest that PPARγ might exert a tumor suppressor activity in response to endogenous ligand(s). If this is the case, the gastric carcinomas that were induced might have lost their expression of PPARγ because of inhibitory selective pressure from either endogenous or exogenous ligands. To address this question, we examined the expression of PPARγ in cancerous and normal gastric mucosa from the same mouse using immunohistochemistry. The results showed that the expression of PPARγ in the malignant mucosa was much weaker than in the normal mucosa in both wild-type and heterozygous PPARγ-deficient mice, as shown in Fig. 5*A*, and the expression of PPARγ was also lower in the cancerous mucosa of the wild-type mouse treated with troglitazone that developed gastric carcinoma, although a stronger PPARγ expression was observed in the normal mucosa of mice treated with troglitazone. This result was confirmed by Western blot analysis using the protein extracted from either the normal or the cancerous gastric mucosa from wild-type mice (Fig. 5*B*).

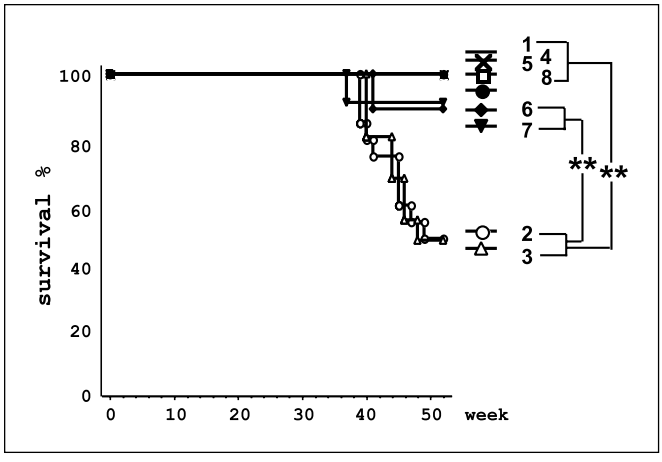

Figure 4. Survival curves of mice. The PPARγ-deficient (+/−) mice exhibited a significantly higher MNU-induced mortality, compared with the wild-type mice. **, $P < 0.01$ (log-rank test); *1-8,* groups 1-8. Mice died after the 37th. Before the end of the experiment (52nd week), 10 of 19 mice in group 2, 8 of 15 mice in group 3, 1 of 9 mice in group 6, and 1 of 11 mice in group 7 died. No death occurred in the groups 1, 4, 5, and 8.

Discussion

Recently, the potential of PPARγ as a target for the prevention and treatment of cancer has been widely explored (38). However, the therapeutic potential of PPARγ ligands has been questioned, based on the results of experiments using animal models for colon cancer, in which the PPARγ ligands increased the development of colon tumors (17). This contradictory result was supplemented by a recent report using transgenic mice that express a constitutive active form of PPARγ in mammary glands showing that PPARγ signaling accelerated tumor development in mammary glands (39). The actual role of PPARγ in cancer has been complicated by recent findings that PPARγ ligands affect cancer cells independent of PPARγ (21, 40–42). To date, no information on the role of PPARγ in gastric carcinogenesis is available.

The results of this study provide clear evidence of the critical importance of PPARγ in gastric carcinogenesis. The loss of one allele of PPARγ significantly enhanced carcinogen-induced gastric carcinogenesis and decreased the survival rate, compared with the wild-type (+/+) littermates. Our results are in good agreement with the report by Nicol et al. that PPARγ haploinsufficiency increased the susceptibility to carcinogen-induced breast carcinogenesis, suggesting that PPARγ acts as a tumor suppressor of skin, ovarian, and breast cancers (43). However, our results are inconsistent with those of one other study that suggested PPARγ acts as a tumor promoter in breast carcinogenesis, instead of a tumor suppressor (39). In that study, the loss of one allele of PPARγ did not influence breast tumorigenesis. This result cannot be easily explained. However, the histologic and pathologic differences between breast

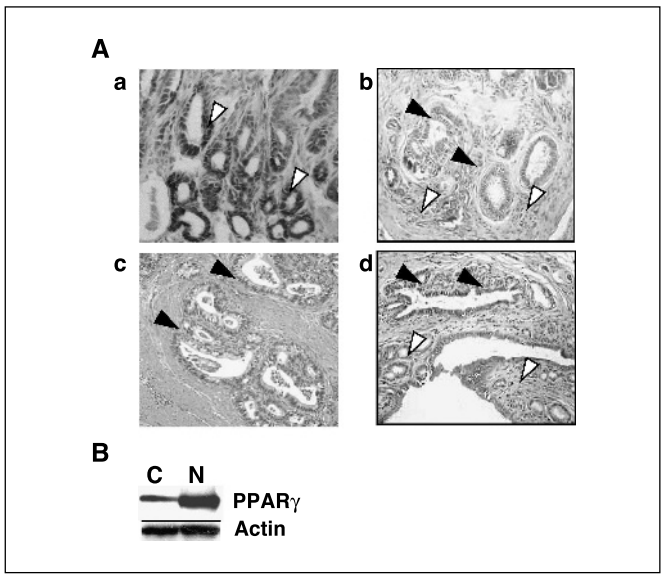

Figure 5. Expression of PPARγ in cancerous gastric mucosa. *A,* the immunohistochemical expression of PPARγ in malignant gastric mucosa was significantly weaker than in normal mucosa. *a, c,* (+/+) mice; *b, d,* (+/−) mice; *open arrow,* normal mucosa; *closed arrow,* malignant mucosa. *B,* Western blot analysis for PPARγ expression in the gastric mucosa of wild-type (+/+) mouse. *N,* normal mucosa; *C,* cancerous mucosa.

and stomach cancer, the method of inducing carcinogenesis (mouse mammary tumor virus/PyV transgenic mice were used to evaluate tumorigenesis in the other study, whereas the carcinogen MNU in drinking water was used to induce carcinogenesis in the present study), and the difference in the genetic backgrounds of the mice used in the two studies [the $(C57BL/6 \times DBA/2)F_1(B6D2_{F1})$ mice were used in that study and the B6 × CBA × ICR mice in the present study] may be responsible for these inconsistencies, although further investigation is certainly needed.

The reduction in PPARγ expression observed among MNU-induced gastric adenocarcinomas from either PPARγ (+/−) or wild-type mice agreed well with the findings of several other reports showing a reduction in PPARγ protein expression in breast and colon cancers, where expression was highest in normal tissue and decreased from benign to malignant states of disease (44–46). Recently, Badawi et al. reported that PPARγ mRNA and protein levels were lower in MNU-induced rat mammary tumors than in normal tissues (47). Our present study showed the suppressed expression of PPARγ in MNU-induced carcinomas, showing a consistent pattern of PPARγ expression. These observations support the hypothesis that PPARγ can exert a tumor suppressing activity.

Furthermore, our results unambiguously showed the chemopreventive potential of a PPARγ ligand, troglitazone, in gastric carcinogenesis. The administration of troglitazone significantly suppressed the formation of MNU-induced gastric carcinoma in wild-type mice. No significant differences in the macroscopic and histologic features of the gastric adenocarcinomas were observed between the wild-type and PPARγ-deficient mice treated with or without troglitazone. Importantly, troglitazone suppressed gastric carcinogenesis without affecting the tumor pathology. In addition, troglitazone's preventive effect was only observed in wild-type mice but not in heterozygous PPARγ-deficient mice, and the reduction in PPARγ expression in the transformed mucosa clearly indicated that this effect was dependent of PPARγ. This result addresses the controversy regarding the dependence of troglitazone on PPARγ, at least in gastric carcinogenesis. However, in the present study, within 52 weeks, troglitazone did not alter the mortality rate. Only one mouse died in the wild-type mice group, either treated with or without troglitazone. It is necessary to extend the experimental period to evaluate troglitazone's effect on the mortality rate. Because troglitazone's preventive effect on gastric cancer seems dependent of PPARγ, as observed in the present study, the preventive effect of other ligands, such as pioglitazone, rosiglitazone and 15-prostaglandin-J_2, on carcinogenesis should be investigated in the future. Recently, the preventive effect of PPARγ against acute gastric mucosal lesions associated with ischemia-reperfusion was reported (48). In that study, PPARγ ligands showed protection against acute gastric mucosal lesions formation induced by ischemia-reperfusion in mice in a dose-dependent manner, and the acute gastric mucosal lesions in PPARγ (+/−) mice was more severe than in wild-type mice. In addition, the inhibition of the up-regulation of tumor necrosis factor-α, intercellular adhesion molecule-1, inducible nitric oxide synthase, apoptosis, and nitrotyrosine formation in the stomach may be responsible for the preventive effect of PPARγ. This may provide us the speculation that the preventive effect of PPARγ against gastric carcinogenesis may be through inhibiting the nuclear factor κB–mediated transcription.

Our present results that PPARγ deficiency sensitizes mice to carcinogen-induced gastric carcinogenesis may provide a way of identifying certain populations susceptible to gastric cancer. In addition, the significant cancer-preventive effect of troglitazone has very important clinical implications. Provided that certain individuals at a higher risk of gastric cancer can be identified, these individuals might actually benefit from the use of PPARγ ligands as chemopreventive agents for gastric cancer.

Taken as a whole, the present study is the first report to show that (a) PPARγ (+/−) mice have an increased susceptibility to MNU-induced carcinogenesis, suggesting that PPARγ may function as a tumor suppressor; (b) the PPARγ ligand troglitazone is a potential chemopreventive agent for gastric cancer; and (c) troglitazone's chemopreventive effect is dependent on PPARγ.

Acknowledgments

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References

1. Green S, Tugwood JD, Issemann I. The molecular mechanism of peroxisome proliferator action: a model for species differences and mechanistic risk assessment. Toxicol Lett 1992;64 Spec:131–9.
2. Bardot O, Aldridge TC, Latruffe N, Green S. PPAR-RXR heterodimer activates a peroxisome proliferator response element upstream of the bifunctional enzyme gene. Biochem Biophys Res Commun 1993; 192:37–45.
3. Keller H, Mahfoudi A, Dreyer C, et al. Peroxisome proliferator-activated receptors and lipid metabolism. Ann N Y Acad Sci 1993;684:157–73.
4. Gimble JM, Pighetti GM, Lerner MR, et al. Expression of peroxisome proliferator activated receptor mRNA in normal and tumorigenic rodent mammary glands. Biochem Biophys Res Commun 1998;253:813–7.

5. Mansen A, Guardiola-Diaz H, Rafter J, Branting C, Gustafsson JA. Expression of the peroxisome proliferator-activated receptor (PPAR) in the mouse colonic mucosa. Biochem Biophys Res Commun 1996;222:844–51.
6. McGowan SE, Jackson SK, Doro MM, Olson PJ. Peroxisome proliferators alter lipid acquisition and elastin gene expression in neonatal rat lung fibroblasts. Am J Physiol 1997;273:1249–57.
7. Huin C, Corriveau L, Bianchi A, et al. Differential expression of peroxisome proliferator-activated receptors (PPARs) in the developing human fetal digestive tract. J Histochem Cytochem 2000;48:603–11.
8. Elstner E, Muller C, Koshizuka K, et al. Ligands for peroxisome proliferator-activated receptorgamma and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. Proc Natl Acad Sci U S A 1998;95:8806–11.
9. Tontonoz P, Singer S, Forman BM, et al. Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor gamma and the retinoid X receptor. Proc Natl Acad Sci U S A 1997;94:237–41.
10. Rumi MA, Sato H, Ishihara S, et al. Peroxisome proliferator-activated receptor gamma ligand-induced growth inhibition of human hepatocellular carcinoma. Br J Cancer 2001;84:1640–7.
11. Heaney AP, Fernando M, Melmed S. PPAR-gamma receptor ligands: novel therapy for pituitary adenomas. J Clin Invest 2003;111:1381–8.
12. Ohta K, Endo T, Haraguchi K, Hershman JM, Onaya T. Ligands for peroxisome proliferator-activated receptor gamma inhibit growth and induce apoptosis of human papillary thyroid carcinoma cells. J Clin Endocrinol Metab 2001;86:2170–7.
13. Badawi AF, Badr MZ. Chemoprevention of breast cancer by targeting cyclooxygenase-2 and peroxisome proliferator-activated receptor-gamma (Review). Int J Oncol 2002;20:1109–22.
14. Brown PH, Lippman SM. Chemoprevention of breast cancer. Breast Cancer Res Treat 2000;62:1–17.
15. Kopelovich L, Fay JR, Glazer RI, Crowell JA. Peroxisome proliferator-activated receptor modulators as potential chemopreventive agents. Mol Cancer Ther 2002;1:357–63.
16. Li J, Lv Y, Dong X, Jin Z. Pioglitazone, a peroxisome proliferators-activated receptor gamma ligand, inhibits dimethylhydrazine (DMH) induced aberrant crypt foci in rats. Beijing Da Xue Xue Bao 2003;35:537–9.
17. Lefebvre AM, Chen I, Desreumaux P, et al. Activation of the peroxisome proliferator-activated receptor gamma promotes the development of colon tumors in C57BL/6J-APCMin/+ mice. Nat Med 1998;4:1053–7.
18. Saez E, Tontonoz P, Nelson MC, et al. Activators of the nuclear receptor PPARγ enhance colon polyp formation. Nat Med 1998;4:1058–61.
19. Wang M, Wise SC, Leff T, Su TZ. Troglitazone, an antidiabetic agent, inhibits cholesterol biosynthesis through a mechanism independent of peroxisome proliferator-activated receptor-gamma. Diabetes 1999;48:254–60.
20. Castrillo A, Mojena M, Hortelano S, Bosca L. Peroxisome proliferator-activated receptor-gamma-independent inhibition of macrophage activation by the non-thiazolidinedione agonist L-796,449. Comparison with the effects of 15-deoxy-δ(12,14)-prostaglandin J(2). J Biol Chem 2001;276:34082–8.
21. Palakurthi SS, Aktas H, Grubissich LM, Mortensen RM, Halperin JA. Anticancer effects of thiazolidinediones are independent of peroxisome proliferator-activated receptor gamma and mediated by inhibition of translation initiation. Cancer Res 2001;61:6213–8.
22. Okano H, Shiraki K, Inoue H, et al. 15-deoxy-δ-12-14-PGJ2 regulates apoptosis induction and nuclear factor-κB activation via a peroxisome proliferator-activated receptor-gamma-independent mechanism in hepatocellular carcinoma. Lab Invest 2003;83:1529–39.
23. Weber SM, Scarim AL, Corbett JA. PPAR{γ} is not required for the inhibitory actions of PGJ2 on cytokine signaling in pancreatic {β}-cells. Am J Physiol Endocrinol Metab 2004;286:E329–36.
24. Roder DM. The epidemiology of gastric cancer. Gastric Cancer 2002;5 Suppl 1:5–11.
25. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. Int J Cancer 2001;94:153–6.
26. Eguchi T, Fujii M, Takayama T. Mortality for gastric cancer in elderly patients. J Surg Oncol 2003;84:132–6.
27. Correa P. Chemoprevention of gastric cancer: has the time come? J Clin Oncol 2003;21:270–1.
28. Kakizoe T. Chemoprevention of cancer: focusing on clinical trials. Jpn J Clin Oncol 2003;33:421–2.
29. Wong JE, Ito Y, Correa P, et al. Therapeutic strategies in gastric cancer. J Clin Oncol 2003;21:267–9.
30. Takahashi N, Okumura T, Motomura W, Fujimoto Y, Kawabata I, Kohgo Y. Activation of PPARγ inhibits cell growth and induces apoptosis in human gastric cancer cells. FEBS Lett 1999;455:135–9.
31. Sato H, Ishihara S, Kawashima K, et al. Expression of peroxisome proliferator-activated receptor (PPAR)-gamma in gastric cancer and inhibitory effects of PPARγ agonists. Br J Cancer 2000;83:1394–400.
32. Takeuchi S, Okumura T, Motomura W, et al. Troglitazone induces G_1 arrest by p27(Kip1)26 induction that is mediated by inhibition of proteasome in human gastric cancer cells. Jpn J Cancer Res 2002;93:774–82.
33. Chen YX, Zhong XY, Qin YF, Bing W, He LZ. 15d-PGJ2 inhibits cell growth and induces apoptosis of MCG-803 human gastric cancer cell line. World J Gastroenterol 2003;9:2149–53.
34. Nagamine M, Okumura T, Tanno S, et al. PPAR gamma ligand-induced apoptosis through a p53-dependent mechanism in human gastric cancer cells. Cancer Sci 2003;94:338–43.
35. Kubota N, Terauchi Y, Miki H, et al. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol Cell 1999;4:597–609.
36. Nakajima A, Wada K, Miki H, et al. Endogenous PPAR gamma mediates anti-inflammatory activity in murine ischemia-reperfusion injury. Gastroenterology 2001;120:460–9.
37. Yamachika T, Nakanishi H, Inada K, et al. *N*-methyl-*N*-nitrosourea concentration-dependent, rather than total intake-dependent, induction of adenocarcinomas in the glandular stomach of BALB/c mice. Jpn J Cancer Res 1998;89:385–91.
38. Sporn MB, Suh N, Mangelsdorf DJ. Prospects for prevention and treatment of cancer with selective PPARγ modulators (SPARMs). Trends Mol Med 2001;7:395–400.
39. Saez E, Rosenfeld J, Livolsi A, et al. PPAR gamma signaling exacerbates mammary gland tumor development. Genes Dev 2004;18:528–40.
40. Clay CE, Namen AM, Atsumi G, et al. Magnitude of peroxisome proliferator-activated receptor-gamma activation is associated with important and seemingly opposite biological responses in breast cancer cells. J Investig Med 2001;49:413–20.
41. Clay CE, Monjazeb A, Thorburn J, Chilton FH, High KP. 15-Deoxy-δ12,14-prostaglandin J2-induced apoptosis does not require PPARγ in breast cancer cells. J Lipid Res 2002;43:1818–28.
42. Place AE, Suh N, Williams CR, et al. The novel synthetic triterpenoid, CDDO-imidazolide, inhibits inflammatory response and tumor growth *in vivo*. Clin Cancer Res 2003;9:2798–806.
43. Christopher JN, Michung Y, Jerrold M, et al. PPAR gamma influences susceptibility to DMBA-induced mammary, ovarian and skin carcinogenesis. Carcinogenesis 2004;25:1747–55.
44. Mueller E, Sarraf P, Tontonoz P, et al. Terminal differentiation of human breast cancer through PPAR gamma. Mol Cell 1998;1:465–70.
45. Wang C, Pattabiraman N, Zhou JN, et al. Cyclin D1 repression of peroxisome proliferator-activated receptor gamma expression and transactivation. Mol Cell Biol 2003;23:6159–73.
46. Bogazzi F, Ultimieri F, Raggi F, et al. Changes in the expression of the peroxisome proliferator-activated receptor gamma gene in the colonic polyps and colonic mucosa of 27 acromegalic patients. J Clin Endocrinol Metab 2003;88:3938–42.
47. Badawi AF, Eldeen MB, Liu Y, Ross EA, Badr MZ. Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor gamma. Cancer Res 2004;64:1181–9.
48. Wada K, Nakajima A, Takahashi H, et al. Protective effect of endogenous PPAR{γ} against acute gastric mucosal lesions associated with ischemia-reperfusion. Am J Physiol Gastrointest Liver Physiol 2004;287:452–8.