0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 5
Printed in U.S.A.

Ligands for Peroxisome Proliferator-Activated Receptor γ Inhibit Growth and Induce Apoptosis of Human Papillary Thyroid Carcinoma Cells

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ABSTRACT

Ligands for peroxisome proliferator-activated receptor γ (PPARγ) induce apoptosis and exert antiproliferative effects on several carcinoma cell lines. The present study investigates the expression of PPARγ and the possibility that agonists for PPARγ also inhibit the growth of human thyroid carcinoma cells. We examined this hypothesis using six cell lines, designated BHP thyroid carcinoma cells, which originated from patients with papillary thyroid carcinoma. RT-PCR analysis revealed that the thyroid carcinoma cell lines BHP2–7, 7–13, 10–3, and 18–21 express PPARγ. More PPARγ was expressed in carcinoma than in adjacent normal thyroid tissue in three of six samples of human papillary carcinoma of the thyroid.

PPARγ-positive thyroid carcinoma cells were treated with agonists of PPARγ, troglitazone, BRL 49653, and 15-deoxy-Δ12,14-prostaglandin J2. Troglitazone (10 μmol/L), BRL 49653 (10 μmol/L), and 15-deoxy-Δ12,14-prostaglandin J2 (1 μg/mL) decreased [^{3}H]thymidine incorporation and reduced cell number, respectively, in BHP carcinoma cell lines that expressed PPARγ. Under low serum conditions, ligands for PPARγ induced condensation of the nucleus and fragmentation of chromatin into nucleosome ladders. These findings indicate that the death of thyroid carcinoma cells is a form of apoptosis.

To investigate the molecular mechanism of the apoptosis, we assessed expression of the apoptosis-regulatory genes *bcl-2*, *bax*, and c-*myc*. Troglitazone significantly increased the expression of c-*myc* messenger RNA but had no effect on the expression of *bcl-2* and *bax* in thyroid carcinoma cells. These results suggest that, at least in part, the induction of apoptosis in human papillary thyroid carcinoma cells may be due to an increase of c-*myc*.

Troglitazone (500 mg/kg·day) significantly inhibited tumor growth and prevented distant metastasis of BHP18–21 tumors in nude mice *in vivo*.

Taken together, these results suggest that PPARγ agonist inhibit cell growth of some types of human thyroid cancer. (*J Clin Endocrinol Metab* **86:** 2170–2177, 2001)

HUMAN PAPILLARY CARCINOMA has a relatively good prognosis (1, 2). The primary disease associated with differentiated papillary thyroid carcinoma is usually removed by surgery and by ^{131}I radiation, which are potentially curative (3–5). On the other hand, during the course of tumor progression, the morphological and functional characteristics of differentiated thyroid carcinoma may disappear. In dedifferentiated thyroid carcinoma, the expression of thyroid-specific genes, namely thyroglobulin, thyroid peroxidase, and TSH receptor is diminished (6, 7). Clinically, several papillary thyroid carcinomas lose the ability to accumulate radioiodine. This phenomenon is associated with a worse prognosis and prevents effective treatment. The shortage of curative therapies for undifferentiated thyroid carcinoma has resulted in a demand to rapidly develop novel therapies.

The peroxisome proliferator-activated receptor γ (PPARγ) belongs to the nuclear hormone receptor superfamily, and it plays an important role in the differentiation of adipocytes (8, 9). Ligands for PPARγ induces apoptosis and exert antiproliferative effects on several carcinoma cell lines (10–13). In the present study, we examined the expression of PPARγ and the effects of PPARγ ligands on the growth of human papillary thyroid carcinoma cells. We also investigated the ability of PPARγ agonist to inhibit the growth of thyroid carcinoma *in vivo*.

Materials and Methods

Cells and samples

All thyroid papillary carcinoma cell lines (BHP2–7, 7–13, 10–3, 14–9, 18–21, and 19–24) were maintained in RPMI 1640 medium supplemented with 10% FBS in a 5% CO_2-95% air atmosphere at 37 C, as described previously (14, 15). Neoplastic human thyroid tissues [papillary carcinoma (n = 6), follicular adenoma (n = 2)] and adjacent normal thyroid tissue (n = 4) were surgically resected with the informed consent of the patients.

Chemicals

Troglitazone was provided by Sankyo Co., Ltd. (Tokyo, Japan). Pioglitazone was provided by Takeda Pharmaceutical Co. (Osaka, Japan). All other reagents were purchased from Sigma (St. Louis, MO).

RT-PCR

Total RNAs were prepared using acid-guanidium thiocyanate phenol chloroform (16). RNA (10 μg) was reverse transcribed with Rous-associated virus reverse transcriptase (Takara Shuzo Co., Kyoto, Japan) at 42 C for 60 min in a 40-μL mixture in the presence of random primer. One microliter of a reverse-transcribed mixture was amplified by PCR. The

Received December 27, 2000. Revision received October 25, 2000. Rerevision received January 23, 2001. Accepted February 5, 2001.

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primers for PPARγ were 5′-TCTGGCCCACCAACTTTGGG-3′ (nucleotides 113–132, upstream) and 5′-CTTCACAAGCATGAACTCCA-3′ (nucleotides 453–472, downstream) (17). RT-PCR efficiency was confirmed in all samples by amplifying human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using the primers (sense) 5′-AACGTGTCAGTGGTGGTGGACCT-3′ (nucleotides 775–794) and (antisense) 5′-ATGGCCCACATGGCCTCCAA-3′ (nucleotides 1042–1061) (18). The primers using for amplification of PPARγ and GAPDH were designed to span the 2nd intron and 8th intron, respectively. PCR products were sequenced using a Cycle Sequencing Kit (Takara Shuzo Co.).

[^{3}H]Thymidine incorporation

Subconfluent cells in 24-well tissue culture plates were washed twice with phosphate-buffered saline (PBS) and incubated with the indicated concentrations of test substances in 1 mL RPMI 1640 medium supplemented with 0.1% or 10% FBS for 4 h. The cells were then incubated with 0.5 μCi [^{3}H]thymidine per well for 4 h, washed twice with 1 mL ice-cold PBS, harvested with 1 mL PBS containing 100 mg trypsin and 1 mmol/L EDTA, and sonicated. The amount of trichloroacetic acid-precipitable (10%) radioactivity was measured by liquid scintillation counting. Thymidine radioactivity was normalized by DNA content.

Cell counting

Cells were seeded at a density of 1×10^4 cells per well in 24-well tissue culture plates in 1.0 mL RPMI 1640 medium supplemented with 10% FBS with or without the indicated factors. The medium was changed every other day. After 72 h in an atmosphere of 5% CO_2-95% air at 37 C, cells were detached from plates by incubation with 500 μL PBS containing 100 mg trypsin and 1 mmol/L EDTA. Cells were counted using a hemocytometer.

Measurement of apoptosis

BHP18–21 thyroid carcinoma cells cultured on cover slips were incubated with or without 10 μmol/L troglitazone in RPMI 1640 medium supplemented with 0.1% FBS for 24 h. The cells were fixed with 1% glutaraldehyde and stained with 1 mmol/L bisBenzimide (Hoechst 33342; Sigma). Apoptotic bodies were observed by fluorescent microscopy.

DNA fragmentation was visualized by autoradiography. Confluent cells in culture dishes were incubated with indicated concentration of troglitazone in RPMI 1640 medium supplemented with 0.1% or 10% FBS, then harvested at various times. DNA was extracted, 3′-end labeled, and resolved by electrophoresis as described (19).

Northern blotting

Total RNA (20 μg) was denatured by incubation with 50% formamide and resolved by electrophoresis through a 1% agarose gel containing 6.0% formaldehyde and 20 mmol/L morpholinepropane sulfonic acid buffer. RNA was then blotted onto nitrocellulose membranes. Equal loading per lane was verified by ethdium bromide staining of 18S and 28S ribosomal RNA, which was visualized and photographed under ultraviolet illumination. After prehybridization for 4 h at 42 C in 50% deionized formamide containing 5× SSC, 50 mmol/L sodium phosphate (pH 6.7), 40 mg/mL denatured salmon sperm DNA, and 4× Denhardt's solution (50× Denhardt's solution, 10 g/L polyvinylpyrrolidone, 10 g/L Ficoll, and 10 g/L BSA), the membranes were hybridized with ^{32}P-labeled complementary DNA (cDNA) overnight at 42 C. cDNAs for human c-*myc*, *bax*, and *bcl*-2 were labeled with [α-^{32}P]deoxy-cytidine triphosphate using a random primer labeling kit (Takara Shuzo Co.). Blots were washed in 2× SSC, 0.1% SDS three times at room temperature for 10 min, then in 0.1× SSC, 0.1% SDS three times at 50 C for 20 min. Northern analysis was repeated three times. The membranes were exposed to an imaging plate, and densitometric analysis of results was performed using a Bas 2000 image analyzer (Fuji Photo Film Co., Ltd., Tokyo, Japan).

Murine studies in vivo

BHP18–21 thyroid carcinoma cells (5×10^6) in 0.1 mL PBS were injected sc into the trunk of 8 male BALB/c nude mice at 6 weeks of age. Treatment began on the day after injection and was continued for 8 weeks. Control mice (4 mice per group) received solvent (dimethylsulfoxide) only or troglitazone (500 mg/kg·day) orally by gavage. Tumors were measured every week, and size was calculated using the following formula: A (length) × B (width) × C (height) × 0.5236. After 8 weeks, nude mice were killed and distant metastasis was searched macroscopically. Tumor, liver, lung, and spleen were fixed and stained for histological analysis. All experiments were conducted in accordance with the *NIH Guide for the Care and Use of Laboratory Animals*. The experimental protocol was approved by the Animal Care and Use Committees of Yamanashi Medical University.

Statistical analysis

Samples were examined in triplicate wells. Data are presented as means ± SD. Statistical analysis was performed by one-way ANOVA. Statistical significance was set at P less than 0.05. Scheffé's F *post hoc* method detected significant differences between group means.

Results

Expression of PPARγ in thyroid carcinoma cells

We studied the expression of PPARγ in six human papillary thyroid carcinoma cells using RT-PCR and specific primers. Amplification by RT-PCR yielded a DNA fragment of the predicted size and revealed PPARγ messenger RNA (mRNA) in four of six papillary thyroid carcinoma cell lines, namely BHP2–7, BHP7–13, BHP10–3, and BHP18–21 (Fig. 1). In contrast, the human papillary carcinoma cell lines BHP14–9 and BHP19–24 did not express PPARγ mRNA.

The expression of PPARγ mRNA in human thyroid tissues was also investigated. We analyzed cDNAs prepared from 12 human thyroid tissues (6 papillary carcinoma, 4 normal thyroid, and 2 adenomas) using RT-PCR. Of the 6 papillary carcinoma tissues, 3 expressed PPARγ mRNA. In these samples, PPARγ mRNA was not expressed in adjacent normal thyroid tissues (Fig. 2). Furthermore, PPARγ mRNA was not expressed in any other normal or adenoma thyroid tissue investigated.

Incorporation of [^{3}H]thymidine into thyroid carcinoma cells

Troglitazone decreased [^{3}H]thymidine incorporation into all thyroid carcinoma cell lines that expressed PPARγ

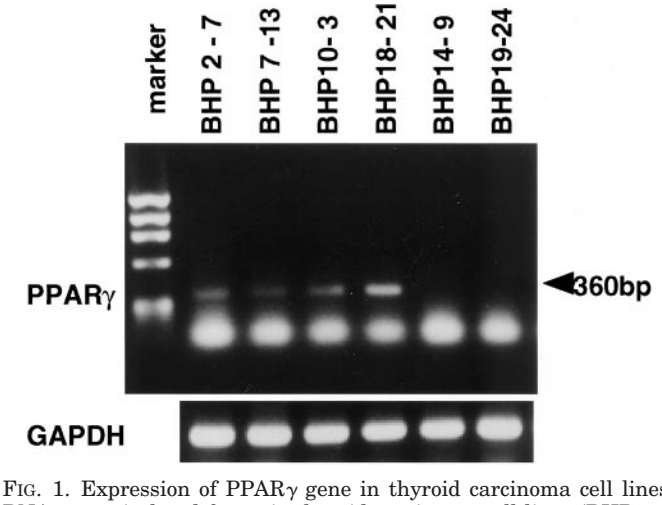

FIG. 1. Expression of PPARγ gene in thyroid carcinoma cell lines. RNAs were isolated from six thyroid carcinoma cell lines (BHP2–7, 7–13, 10–3, 18–21, 14–9, and 19–24), reverse transcribed using random primers, and amplified by PCR using specific primers for PPARγ and GAPDH. The PCR products were resolved by electrophoresis and stained with ethidium bromide.

mRNA. Troglitazone (10 μmol/L) in 10% FCS significantly decreased [^{3}H]thymidine incorporation into BHP2–7, 7–13, 10–3, and 18–21 cell lines by 52, 47, 55, and 44%, respectively ($P < 0.05$). In contrast, [^{3}H]thymidine incorporation into BHP14–9 and BHP19–24 cells was not inhibited by 10 μmol/L troglitazone in 10% FCS (Fig. 3). Under low serum conditions (0.1% FCS), troglitazone (10 μmol/L) reduced [^{3}H]thymidine incorporation into BHP2–7, 7–13, 10–3, and 18–21 cells to a level below 1% of the control. In the presence of troglitazone (10 μmol/L) in 0.1% FCS, cells became deformed and detached within 24 h. Wy14,643 (10 μmol/L) and all-*trans*-retinoic acid (10 μmol/L) did not reduce [^{3}H]thymidine incorporation into BHP2–7, 14–9, and 18–21 cells (data not shown).

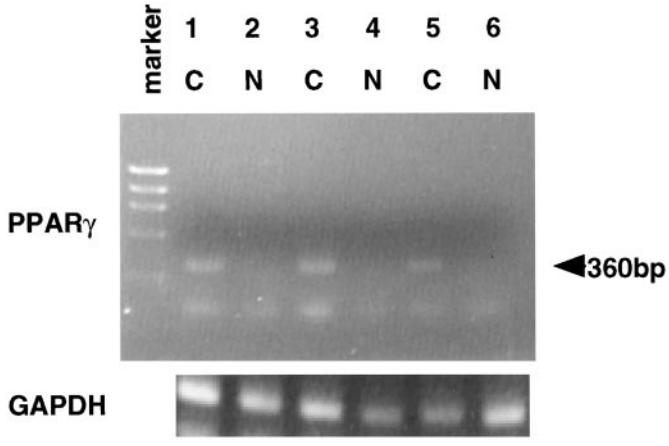

FIG. 2. Expression of PPARγ gene in human thyroid tissues. PCR products were resolved by electrophoresis and stained with ethidium bromide. cDNA samples on *lanes 1* and *2*, *3* and *4*, and *5* and *6* originated from the same patients. C, Carcinoma tissue; N, adjacent normal thyroid tissue.

Effects of PPARγ agonists on the growth of human thyroid carcinoma cell lines

Troglitazone (10 μmol/L) and BRL 49653 (10 μmol/L) significantly decreased numbers of BHP2–7, 7–13, 10–3, and 18–21 thyroid carcinoma cells compared with control values ($P < 0.05$) (Table 1). In contrast, these agents did not affect the number of BHP14–9 and 19–24 thyroid carcinoma cells that did not express PPARγ. 15-deoxy-Δ12,14-prostaglandin J2 (prostaglandin J2; 1 μg/mL) significantly inhibited the growth of BHP7–13 and BHP18–21 cells, but not BHP14–9 cells. Wy14,643 (10 μmol/L), a PPARα agonist, did not influence the growth of any thyroid carcinoma cell lines examined.

Detection of apoptotic cells

Figure 4, A and B, shows the morphological changes induced by troglitazone. BHP18–21 cells in logarithmic growth phase were transferred into 0.1% FCS RPMI 1640 medium with or without 10 μmol/L troglitazone. Cells incubated with troglitazone lost cell-to-cell contact. Fluorescent staining revealed fragmentation and condensation of the nucleus (Fig. 4, C and D).

DNA extracted from BHP18–21 cells treated for 4 h with 10 μmol/L troglitazone in 0.1% FCS showed evidence of degradation, with internucleosomal DNA laddering, which is associated with apoptotic cell death (Fig. 5A). In contrast, 10 μmol/L troglitazone in 0.1% FCS did not induce DNA fragmentation on BHP14–9 thyroid carcinoma cells that did not express PPARγ. Troglitazone in 0.1% FCS induced DNA fragmentation on BHP18–21 cell in a dose-dependent manner. However, under 10% FCS conditions, even 10 μmol/L troglitazone did not apparently induce DNA fragmentation (Fig. 5B). To induce DNA fragmentation on BHP18–21 cells under 10% FCS condition, more than 100 μmol/L troglitazone was required (data not shown).

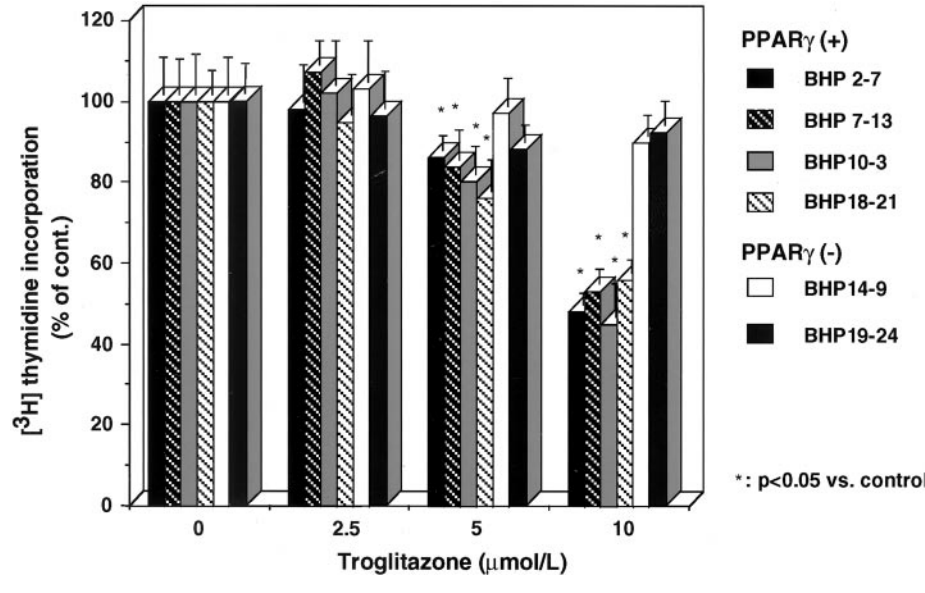

FIG. 3. Dose-response studies of troglitazone on [^{3}H]thymidine incorporation into thyroid carcinoma cells lines. Data represent means ± SD of triplicate determinations. Troglitazone (10 μmol/L) in 10% FCS significantly reduced [^{3}H]-thymidine incorporation into BHP2–7, 7–13, 10–3, and 18–21 cells. In contrast, [^{3}H]thymidine incorporation into BHP14–9 and 17–10 cells was not decreased by 10 μmol/L of troglitazone. *, $P < 0.05$. *vs.* control.

TABLE 1. Effects of PPARγ agonists on cell number

Substance added	Cell number (× 10,000/well)					
	BHP2-7	BHP7-13	BHP10-3	BHP18-21	BHP14-9	BHP19-24
None	46.5 ± 3.5	43.5 ± 3.6	36.8 ± 4.0	54.5 ± 4.0	33.5 ± 3.0	29.8 ± 3.8
Troglitazone (10 μmol/L)	27.5 ± 3.5[a]	24.5 ± 3.6[a]	20.8 ± 4.0[a]	35.5 ± 2.6[a]	30.5 ± 2.0	25.8 ± 2.8
BRL 49653 (10 μmol/L)	34.5 ± 2.0[a]	27.5 ± 1.5[a]	28.5 ± 3.5[a]	40.3 ± 3.6[a]	29.5 ± 2.0	26.8 ± 3.8
Prostaglandin J2 (1 μg/mL)	ND	26.5 ± 1.5[a]	ND	30.5 ± 2.2[a]	28.5 ± 2.5	ND
Wy14,643 (10 μmol/L)	ND	43.7 ± 4.7	ND	50.0 ± 4.9	31.5 ± 1.5	ND

ND, Not done.
[a] $P < 0.05$ *vs.* control.

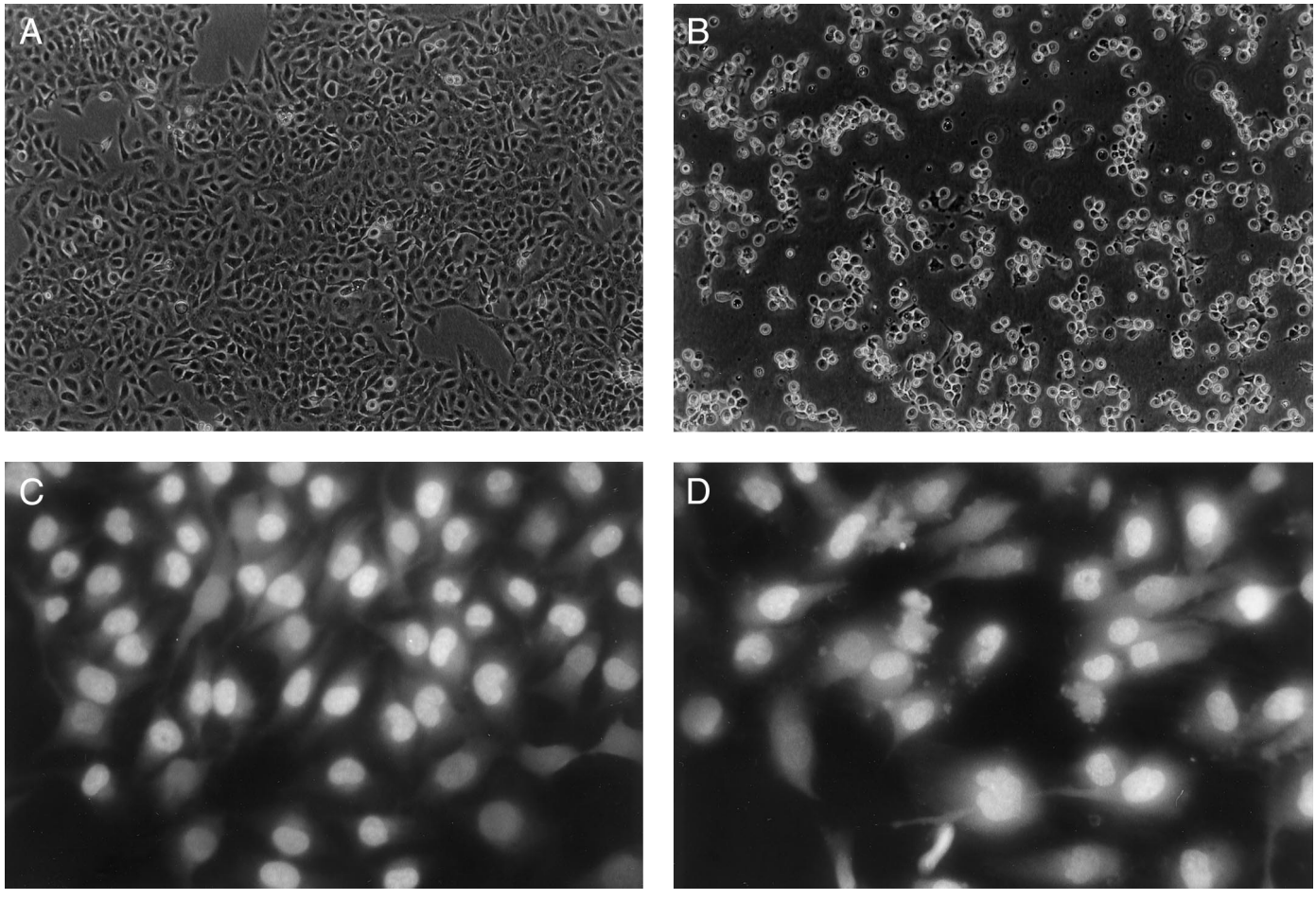

FIG. 4. Morphological changes of BHP18–21 induced by 10 μmol/L troglitazone. BHP18–21 papillary carcinoma cells were transferred to medium containing 0.1% FCS with or without 10 μmol/L troglitazone. After 24 h, cells were examined by phase-contrast microscopy. A, Control; B, 10 μmol/L troglitazone. BHP18–21 cells stained with bisBenzimide. C, Control; D, 10 μmol/L troglitazone. The apoptotic body was observed.

Northern blots

To investigate the molecular mechanism of apoptosis, we evaluated the expression levels of apoptosis-regulated genes in BHP18–21 cells using c-*myc*, *bcl-2*, and *bax* cDNA as probes. Troglitazone in 0.1% FCS significantly and dose-dependently increased c-*myc* mRNA levels in BHP18–21 cells. Densitometric data showed 10 μmol/L troglitazone in 0.1% FCS increased c-*myc* mRNA levels by 3.0-fold compared with the control (Fig. 6A). However, in 10% FCS 10 μmol/L troglitazone increased c-*myc* mRNA level only by 1.3-fold of control. Figure 6B shows the time course of c-*myc* mRNA expression. Troglitazone (10 μmol/L) in 0.1% FCS increased levels of c-*myc* mRNA within 2 h and continued to do so for 12 h. In contrast, expression levels of *bcl-2* and *bax* genes were not affected by 10 μmol/L troglitazone.

Antitumor effect in vivo

We evaluated the antitumor effect of troglitazone *in vivo* using BHP18–21 thyroid carcinoma cells in BALB/c nude mice. Histological analysis of BHP18–21 tumors from untreated mice revealed undifferentiated thyroid carcinoma tissue and distant metastasis of tumor in the liver (Fig. 7, A and B). Troglitazone visibly inhibited the growth of BHP18–21 tumors (Fig. 7, C and D).

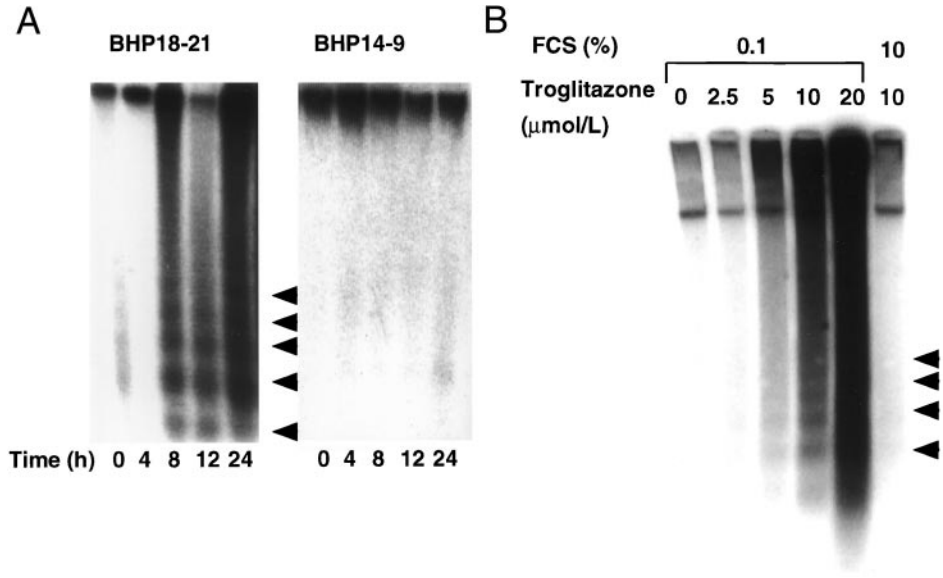

FIG. 5. A, BHP18–21 and BHP14–9 cells were incubated with 10 μmol/L troglitazone in 0.1% FCS. DNAs harvested at indicated time were resolved by electrophoresis after 3′-end labeling. *Arrows* indicate DNA fragmentation. B, BHP18–21 cells were incubated with indicated concentration of troglitazone in 0.1% or 10% FCS. DNAs harvested at 12 h were resolved by electrophoresis after 3′-end labeling.

Discussion

We studied six human papillary thyroid carcinoma cell lines. The RT-PCR data showed that four of six thyroid carcinoma cell lines expressed PPARγ mRNA. Interestingly, four PPARγ-positive thyroid carcinoma cells could form a tumor, if these cells were transplanted to nude mice. In contrast, PPARγ-negative cells could not form a tumor in nude mice. These observations suggest that PPARγ expression might be related to tumor progression. We also demonstrated expression of PPARγ mRNA in three of six human papillary carcinoma tissues. It is noteworthy that PPARγ mRNA was undetectable in the adjacent normal thyroid tissues. Additionally, PPARγ mRNA was not expressed in any thyroid adenoma tissues examined. These findings suggest that the prominent expression of PPARγ mRNA is a feature of thyroid carcinoma. Elstner *et al.* (10) reported that breast cancer cells express a higher level of PPARγ protein than normal breast epithelial cells. Our data agree with these findings. However, we could not find any histological or clinical difference between PPARγ-positive and -negative thyroid papillary carcinoma. Further study is required to understand the relationship between PPARγ expression and the growth and tumorigenecity of thyroid neoplasms.

Because PPARγ was expressed in four thyroid carcinoma cell lines, we examined the effects of PPARγ agonists on these lines. The growth of these four thyroid papillary carcinoma cells was inhibited by troglitazone or BRL 49653, synthetic ligands for PPARγ, and prostaglandin J2, a natural ligand for PPARγ. In contrast, PPARγ agonists did not inhibit the growth of PPARγ-negative thyroid carcinoma cells. Furthermore, Wy14,643 or all-*trans*-retinoic acid, a PPARα agonist or retinoic acid receptor agonist, did not affect the growth of any thyroid carcinoma cells examined. These results suggest that the inhibition of thyroid carcinoma cells is specifically mediated through PPARγ.

Troglitazone inhibits growth by inducing apoptosis in several carcinoma cells (10, 11, 13). We examined the notion that apoptosis is induced during the inhibition of thyroid carcinoma cell growth. We demonstrated that DNA from BHP18–21 cells undergoing death induced by troglitazone in 0.1% FCS showed chromatin fragmentation into nucleosome ladders. Furthermore, morphological observation revealed that troglitazone caused nuclear condensation and fragmentation. These findings indicate that troglitazone induced apoptosis and decreased the number of thyroid carcinoma cells.

The mechanism by which PPARγ agonists induce apoptosis remains unknown. Many genes are involved in the progression of apoptosis. To investigate the molecular mechanism underlying the apoptosis of thyroid carcinoma cells, we evaluated apoptosis-related gene expression. We found that troglitazone dose-dependently increased c-*myc* protooncogene mRNA. Furthermore, serum deprivation potentiated the effect of troglitazone on the induction of c-*myc* mRNA and apoptosis. In marked contrast, the expression levels of *bcl-2* or *bax*, which are survival and apoptotic genes, respectively (20, 21), were not affected by troglitazone. The results of dose-response studies lead us to conclude that apoptosis by troglitazone may be, at least in part, due to increase of c-*myc*. The inappropriate expression of c-Myc induces or sensitizes cells to apoptosis (22). Evan *et al.* (23) reported that the overexpression of c-*myc* rendered fibrobrasts prone to cell death on serum deprivation and led to the induction of apoptosis by various means. Our findings are in agreement with these observations. However, further study is required to reveal the role of c-*myc* in apoptosis.

In 10% FCS condition, 10 μmol/L troglitazone reduced [^{3}H]thymidine incorporation and cell number of PPARγ-positive thyroid carcinoma cells. However, DNA fragmentation was not clearly observed. One of the possibilities of these inconsistencies might be due to the sensitivity of DNA fragmentation assay. Another possibility is that troglitazone might inhibit the growth of thyroid carcinoma cells by other mechanism than apoptosis. To clarify this subject, we are trying to identify the gene, which is responsible for the growth inhibition by troglitazone in 10% FCS using differential display technique.

Our animal studies revealed that troglitazone also inhibits the growth of thyroid carcinoma cells *in vivo*. Mice inoculated

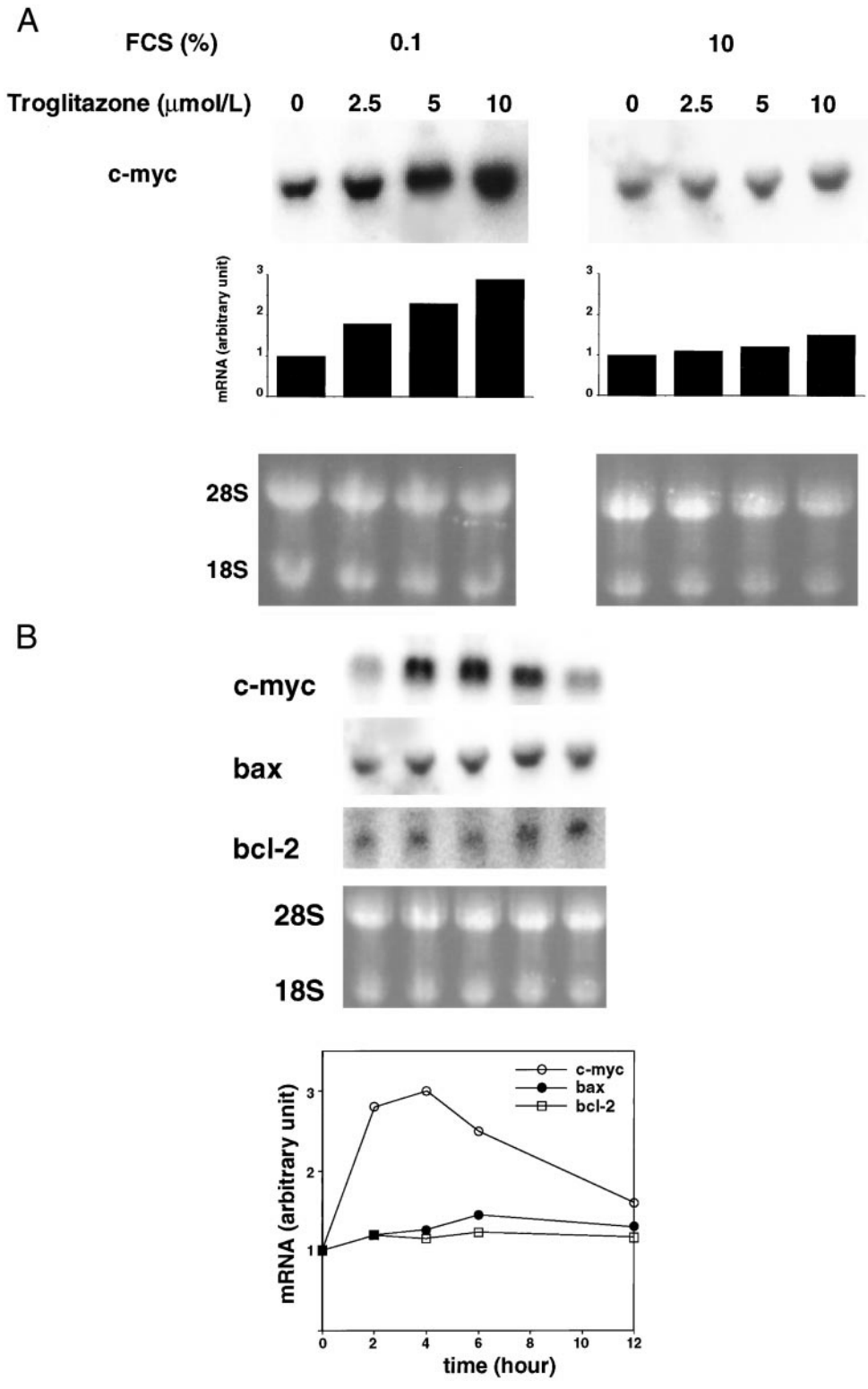

FIG. 6. A, Expression levels of apoptosis-regulated genes in BHP18–21 thyroid carcinoma cells. Cells were cultured in 0.1% or 10% FCS for 24 h and exposed to indicated concentration of troglitazone for 4 h. B, Time course of c-*myc*, *bax*, and *bcl-2* mRNA expression induced by 10 μmol/L troglitazone in 0.1% FCS.

with tumor cells and given only solvent developed sc tumors with distant metastasis in the liver. In contrast, mice treated with troglitazone did not develop tumors and distant metastasis. The doses of troglitazone applied in the present study (500 mg/kg·day) is higher than human dose and dose titration experiment must be required. However, it is noteworthy that troglitazone inhibits tumor growth without affecting the mean blood chemistries and hematopoietic values (11). Pioglitazone, another synthetic thiazolidinedione, also exerts growth inhibitory effect on BHP18–21 cells (data not shown). Troglitazone is no longer widely used, but Pioglitazone is used to treat patients with type 2 diabetes mellitus.

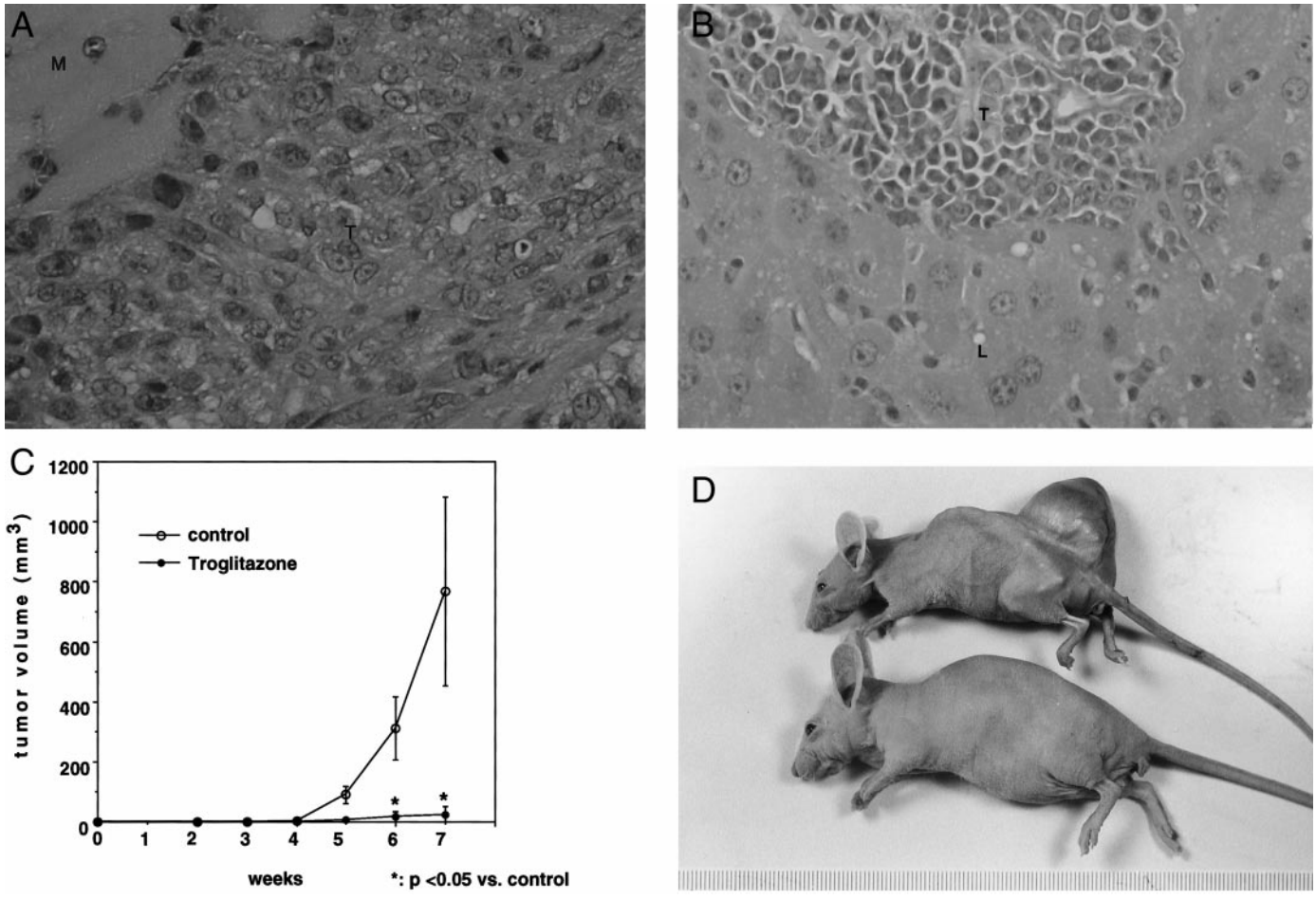

FIG. 7. Effects of troglitazone on the growth of BHP18–21 tumors in nude mice. Troglitazone (500 mg/kg·day) was administered po for 5 days per week. A, Histological analysis of BHP18–21 tumor. T, Tumor; M, muscle. B, Liver metastasis. T, Tumor; L, liver. C, Time course of tumor volume measured every week. Each point represents the mean ± SD. D, *Upper*, control mouse; *lower*, treated by troglitazone.

Although further study using different animal models is required to clarify antitumor effects, thiazolidinedione compounds might be useful as therapeutic agents in treating thyroid carcinoma that expresses PPARγ.

The present study examined four thyroid carcinoma cell lines that expressed PPARγ mRNA. These PPARγ-positive thyroid carcinoma cell lines express Pax8 and/or TTF1, but not thyroid-specific proteins such as thyroglobulin, thyroid peroxidase, TSH receptor, and Na/I symporter (14, 15). Therefore, these cell lines might be considered models of dedifferentiated papillary carcinoma. PPARγ agonists exerted antiproliferative effects on these dedifferentiated cells. Thus, PPARγ agonists might have therapeutic benefit even in the treatment of undifferentiated thyroid carcinoma that does not express thyroid-specific proteins.

In summary, we showed that PPARγ is expressed by four thyroid carcinoma cell lines and several thyroid carcinoma tissues. We demonstrated that PPARγ agonists inhibit growth and induce apoptosis in these cell lines and that troglitazone inhibits the growth of thyroid carcinoma cells *in vivo*. These findings suggest that PPARγ agonists may be useful agents with which to treat thyroid cancer.

References

1. **Mazzaferri EL.** 1996 Carcinoma of follicular epithelium. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid, ed 7. Philadelphia: Lippincott; 922–945.
2. **Simpson WJ, Mckinney SE, Carruthers JS, Gospodarowicz MK, Sutcliffe SB, Panzarella T.** 1987 Papillary and follicular thyroid cancer-prognostic factors in 1578 patients. Am J Med. 83:479–488.
3. **Mazzaferri EL, Robyn J.** 1996 Postsurgical management of differentiated thyroid carcinoma. Otolaryngol Clin North Am. 29:637–663.
4. **Samaan NA, Schltz PN, Hickey RC, et al.** 1992 The results of various modalities of treatment of well-differentiated thyroid carcinoms: a retrospective review of 1599 patients. J Clin Endocrinol Metab. 75:714–720.
5. **McHenry CR, Rosen IB, Walfish PG.** 1991 Prospective management of nodal metastasis in differentiated thyroid cancer. Am J Surg. 162:353–356.
6. **Ohta K, Endo T, Onaya T.** 1990 The mRNA levels of thyrotropin receptor, thyroglobulin and thyroid peroxidase in neoplastic human thyroid tissues. Biochem Biophys Res Commun. 165:1148–1153.
7. **Fabbro D, DiLoreto C, Beltrami CA, DiLauro R, Damante G.** 1994 Expression of thyroid specific transcription factors TTF-1 and PAX-8 in human thyroid neoplasms. Cancer Res. 54:4744–4749.
8. **Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA.** 1994 Peroxisone proliferator-activated receptor (PPAR)γ: adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology. 135:798–800.
9. **Tontonoz P, Hu E, Spiegelman BM.** 1994 Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor. Cell. 79:1147–1156.
10. **Elstner E, Muller C, Koshizuka K, et al.** 1998 Ligands for peroxisome proliferator-activated receptorγ and retinoid acid receptor inhibit growth and induce apoptosis of human breast cancer cells *in vitro* and in BNX mice. Proc Natl Acad Sci USA. 95:8806–8811.
11. **Kubota T, Koshizuka K, Williamson EA, et al.** 1998 Ligand for peroxisome

proliferator-activated receptor γ (Troglitazone) has potent antitumor effect against human prostate cancer both *in vitro* and *in vivo*. Cancer Res. 58:3344–3352.
12. **Tontonoz P, Singer S, Forman BM, et al.** 1997 Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor. Proc Natl Acad Sci USA. 94:237–241.
13. **Takahashi N, Okumura T, Motomura W, et al.** 1999 Activation of PPARγ inhibits cell growth and induces apoptosis in human gastric cancer cells. FEBS Lett. 455:135–139.
14. **Ohta K, Pang XP, Berg L, et al.** 1996 Antitumor actions of cytokines on new human thyroid papillary carcinoma cell lines. J Clin Endocrinol Metab. 81:2607–2612.
15. **Ohta K, Pang XP, Berg L, et al.** 1997 Growth inhibition of new human thyroid carcinoma cell lines by activation of adenylate cyclase through the β-adrenergic receptor. J Clin Endocrinol Metab. 82:2633–2638.
16. **Chomczynski P, Sacchi N.** 1987 Single-step method of RNA by acid guanidium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162:156–159.
17. **Elbrecht A, Chen Y, Cullinan C, et al.** 1996 Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors γ1 and γ2. Biochem Biophys Res Commun. 224:431–437.
18. **Tokunaga K, Nakamura Y, Sakata K, et al.** 1987 Enhanced expression of a glyceraldehyde-3-phoshate dehydrogenase gene in human lung cancers. Cancer Res. 47:5616–5619.
19. **Tilly JL, Hsueh AJW.** 1993 Microscale autoradiographic method for the qualitative and quantitative analysis of apoptotic DNA fragmentation. J Cell Physiol. 154:519–526.
20. **Hale AJ, Smith CA, Sutherland LC, et al.** 1996 Apoptosis: molecular regulation of cell death. Eur J Biochem. 236:1–26.
21. **Brambilla E, Negoescu A, Gazzeri S, et al.** 1996 Apoptosis-related factors p53, Bcl2, and Bax in neuroendocrine lung tumors. Am J Pathol. 149:1941–1952.
22. **Hoffman B, Liebermann DA.** 1998 The proto-oncogene c-*myc* and apoptosis. Oncogene. 17:3351–3357.
23. **Evan IG, Wyllie AH, Gilbert CS, et al.** 1992 Induction of apoptosis in fibroblasts by c-*myc* protein. Cell. 69:119–128.