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Report

Peroxisome proliferator-activated receptor γ augments tumor necrosis factor family-induced apoptosis in hepatocellular carcinoma

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Proliferator-activated receptor γ (PPARγ) is a nuclear receptor, which mainly associates with adipogenesis, but also appears to facilitate cell differentiation or apoptosis in certain malignant cells. This apoptosis induction by PPARγ is increased by co-stimulation with tumor necrosis factor (TNF)-α-related apoptosis-inducing ligand (TRAIL), a member of the TNF family. In this study, we investigated the effect of PPARγ on Fas-mediated apoptosis in hepatocellular carcinoma (HCC) cell lines. PPARγ was expressed on all seven HCC cell lines and located in their nuclei. 15-Deoxy-Δ-12,14-prostaglandin J_2 (15d-PGJ_2), a PPARγ ligand, inhibited cellular proliferation in HepG2, SK-Hep1 or HLE cells, unlike pioglitazone, another PPARγ ligand, which did not have a significant influence on proliferation of these cells. However, 15d-PGJ_2 facilitated Fas-mediated HCC apoptosis that could not be induced by Fas alone. These results suggest that PPARγ can augment TNF-family-induced apoptosis. [© 2002 Lippincott Williams & Wilkins.]

Key words: Apoptosis, hepatocellular carcinoma, peroxisome proliferator-activated receptor γ, tumor necrosis factor.

Introduction

Hepatocellular carcinoma (HCC) is becoming a leading cause of cancer death in Japan. Recent advances in understanding HCC and progressive treatments including hepatic resection have improved the prognosis of patients. However, it is difficult for most patients with HCC to achieve complete remission, especially with non-surgical treatment including transcatheter arterial embolization, percutaneous ethanol injection therapy or chemotherapies. Each approach has limitations and underscores the importance of research addressing the basic mechanisms leading to HCC to identify potentially new therapeutic approaches.

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the PPAR subfamily of nuclear receptors, which have an important regulatory role in adipogenesis and inflammation. PPARγ gene expression is abundant in adipose tissue, but it has become clear recently that this gene is also present in other tissues and specialized cells, including liver, colon, skeletal muscle, breast, prostate, type 2 pulmonary alveolar macrophages or activated macrophages and foam cells.[1] PPARγ has two isoforms, PPARγ1 and PPARγ2. PPARγ1 is encoded by eight exons and PPARγ2 is encoded by seven exons.[2] The extra-adipocyte expression of PPARγ is specifically the PPARγ1 isoform, whereas expression of the PPARγ2 isoform remains adipocyte specific.[1]

Ligands selective for PPARγ include prostaglandin J_2 (PGJ_2) derivatives, such as 15-deoxy-Δ-12,14-PGJ_2 (15d-PGJ_2),[3] and antidiabetic thiazolidinedione (TZD) compounds,[4] including pioglitazone or troglitazone. PPARγ ligand has been demonstrated to regulate adipocyte differentiation and glucose homeostasis, as well as inhibition of cellular proliferation and anchorage-independent growth of human colorectal cancer.[5] Furthermore, PPARγ activation by ligands induces apoptosis in carcinoma or normal cells.[6–10] In these studies, carcinoma cells were induced to undergo apoptosis by PPARγ ligand alone. Some carcinoma cell lines became apoptosis within

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24 h after treatment with PPARγ ligands and the cell viabilities were significant decreased,[6,7] but this apoptosis is not observed in other carcinoma cell lines.[9]

In liver tissue, PPARγ receptors play an important role in controlling the activation state of the stellate cells and their repression or inactivation may predispose to hepatic fibrosis.[11] In rodents, PPAR ligands are hepatocarcinogenetic[12] and the first approved drug that specifically activates PPARγ, troglitazone, has rarely been found to cause serious liver injury.[11] Troglitazone may not be appropriate for clinical treatment of tumors, even if treatment with PPAR ligands results in effective suppression or apoptosis of carcinoma cell, *in vitro*.

Members of the tumor necrosis factor (TNF) family, e.g. Fas ligand, TNF-α or TNF-related apoptosis-inducing ligand (TRAIL), can induce apoptosis of target cells by stimulation of specific receptors.[13] However, HCC is usually resistant to apoptosis induced solely by receptor stimulation. Numerous anti-apoptotic proteins in HCC interfere with the necessary signaling pathways of these receptors.[14,15] However, PPARγ ligand can induce apoptosis in malignant cells via its receptor stimulation. Although the mechanism is not known, this nuclear receptor stimulation may augment the effect of Fas stimulation and sensitize cells for Fas. Recently regulation of TRAIL-induced apoptosis by co-stimulation of PPARγ on Jurkat cells was reported.[16] TRAIL is a type II membrane protein, whose C-terminal extracellular domain shows clear homology to other TNF family members.[17] Hence, we speculate that PPARγ also may have similar effects on Fas-mediated apoptosis in HCC. Our studies suggest that PPARγ enhances Fas-mediated apoptosis of HCC cells and results in more effective suppression of HCC cellular proliferation.

Materials and methods

Cell lines and HCC tissues

The Jurkat T cell line, the human HCC cell lines, HepG2, Hep3B and SK-Hep1 cells were purchased from ATCC (Rockville, MD). The HCC cell lines, Huh7 (JCRB 0403), HLE (JCRB 0404) and PLC/PRF/5 (JCRB 0406), were all purchased from the Health Science Research Resource Bank (Osaka, Japan). The Jurkat cells were cultured in RPM1 1640 (Gibco/BRL, Grand Island, NY). The other cell lines were cultured in Dulbecco's modified Eagle's medium (Dainippon Pharmaceutical, Osaka, Japan) at 37°C. All media were supplemented with 1% penicillin/streptomycin (Gibco/BRL) and 10% heat-inactivated fetal calf serum (Gibco/BRL). Human HCC tissues and non-tumor tissues were obtained from surgical resection for immunohistochemical analysis. We obtained informed consent from patients for subsequent use of his resected tissues.

Detection of PPARγ

Expression of PPARγ in HCC cell lines was analyzed by Western blotting. HCC cells were harvested and lysed in lysis buffer (50 mmol/l Tris–HCl, pH 8, 150 mmol/l NaCl, 5 mmol/l EDTA, 1% NP-40 and 1 mmol/l phenylmethylsulfonyl fluoride) on ice. After centrifugation, supernatants were collected and their protein contents measured using a BioRad protein assay kit (BioRad, Hercules, CA). Equal amounts of protein from each extract were separated by 14% SDS–PAGE and transferred onto nitrocellulose membranes (Toyo Roshi, Tokyo, Japan) using the BioRad electrotransfer system (BioRad). Blots were blocked by incubation in 5% non-fat dried milk in phosphate-buffered saline (PBS) overnight at 4°C and probed for 2 h at room temperature with rabbit anti-PPARγ1,2 polyclonal antibody (Calbiochem, San Diego, CA). Antibodies were diluted 1:1000 with 0.05% Tween 20 in PBS. The immunoblots were then probed with horseradish peroxidase-conjugated anti-rabbit IgG (1:2000 diluted with 5% non-fat dried milk in Tris–HCl, pH 7.5 and 0.05% Tween 20). After the final washing, signal was detected with an ECL kit (Amersham Pharmacia Biotech, Little Chalfont, UK).

Immunohistochemical staining for PPARγ was performed on resected HCC tissues and non-tumor tissues. Deparaffinized sections were heated for 10 min at 120°C in a pressure cooker to reactive antigen and treated with 0.3% H_2O_2 in methanol for 20 min to abolish endogenous peroxidase activity. Sections were blocked with normal goat serum in PBS and incubated overnight with a 1:40 dilution (in PBS) of rabbit anti-PPARγ1,2 polyclonal antibody (Calbiochem) at 4°C. The sections were incubated with a second biotinylated anti-rabbit Ig diluted 1:200 in PBS, followed by a 1:200 dilution of avidin–biotin–peroxidase complex (Vectastain ABC kit; Vector, Burlingame, CA) diluted 1:200 in PBS for 30 min at room temperature. After each incubation step, the sections were carefully washed 3 times in PBS, for 5 min, each time. They were developed in a substrate solution of 0.01% 3,3′-diaminobenzidine–hydrogen peroxide for visualization and counter-stained with Mayer's hematoxylin. They were rehy-

drated in ethanol, cleared in xylene and mounted. The stain sections were examined under $\times 200$ magnification.

Assessment of viability of HCC cells

To assess the viability of HCC cells, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was performed. The HCC cells were plated at a density of 5×10^3 cells/well in 96-well microtiter plates (Corning Glass Works, Corning, NY) and each plate was incubated for 24 h at 37°C in 5% CO_2. 15d-PGJ_2 (Cayman Chemical, Ann Arbor, MI) (10 μM/l) or pioglitazone (Takeda, Osaka, Japan) (10 μM/l) were added in the absence or presence of 50 ng/ml Fas antibody (MBL, Nagoya, Japan), 50 ng/ml TRAIL (R & D System, Minneapolis, MN) or 50 ng/ml TNF-α (Cosmo Bio, Tokyo, Japan) and the plate were incubated for 48 h. The live-cell count was determined using a Cell Titer 96 assay kit (Promega, Madison, WI) according to the manufacturer's instructions. The absorbance of each well was measured at 570 nm with a microtiter plate reader (BioRad).

Detection of apoptosis

A total 2×10^4 HCC cells/well were cultured in an eight-well Lab-tek chamber slide (Nalge Nunc, Rochester, NY) for 24 h, followed up by addition of 15d-PGJ_2 (Cayman Chemical) (10 μM/l) or pioglitazone (Takeda) (10 μM/l) and 50 ng/ml Fas antibody (MBL). After incubation for 48 h, cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma, St Louis, MO) and observed with a fluorescence microscope (Zeiss, Guöttingen, Germany).

Results

PPARγ expression in HCC cell lines

We investigated the expression of PPARγ in seven human HCC cell lines (HepG2, HLE, SK-Hep1, Hep3B, Huh7, PLC/PRF/5 and Chang liver). Bands corresponding to PPARγ antigen were observed in all seven HCC cell lines examined (Figure 1).

PPARγ expression in human HCC tissues

To determine the *in situ* expression of PPARγ, nine HCC tissues and nine non-tumor liver sections were examined by immunochemistry. All non-tumor liver tissues showed negative staining for PPARγ, whereas all HCC tissues revealed positive staining for PPARγ (Figure 2A and B). At the cellular level, PPARγ expression was located in the nucleus of HCC cells. Taken together with the results from the HCC cell lines, it appears that these PPARγ appears to be prevalent in human HCC.

Effect of PPARγ ligand stimulation in human HCC cells

In order to elucidate the function of PPARγ in HCC cells, we investigated the effect of PPARγ ligands on human HCC cell line viability (Figure 3). Three HCC cell lines (HepG2, SK-Hep1 or HLE) were incubated with 15d-PGJ_2 at the indicated concentrations and cell viability was decreased by 50 μM with 15d-PGJ_2 in all cell lines after 48 h (Figure 3A). HLE cellular proliferation was suppressed at lower concentrations related to HepG2 and SK-Hep1 cells. We also investigated the effect of pioglitazone for cellular viabilities (Figure 3B), but did not observe changes in

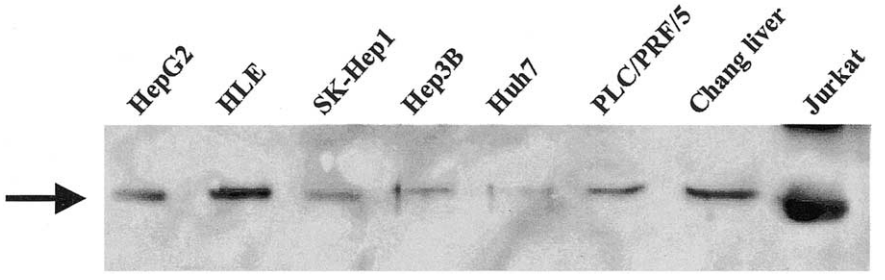

Figure 1. PPARγ expression in seven human HCC cell lines (HepG2, HLE, SK-Hep1, Hep3B, Huh7, PLC/PRF/5 and Chang liver) and a human T cell lymphoma cell line (Jurkat) by Western blotting. Arrow means the expression of PPARγ.

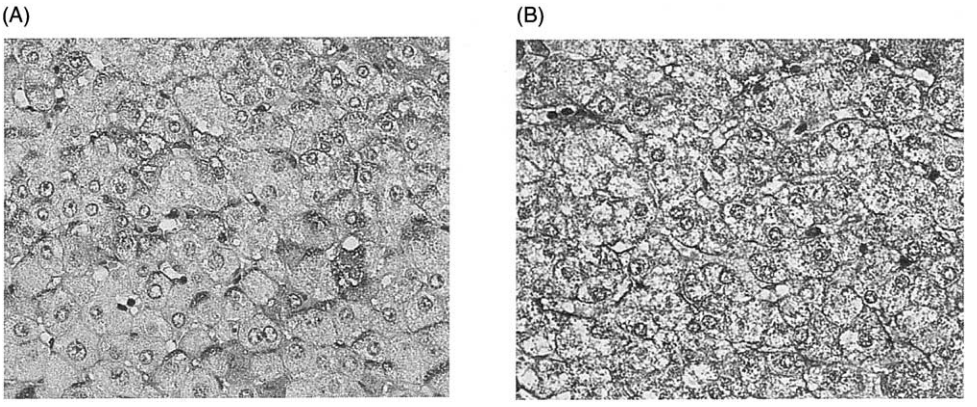

Figure 2. Indirect immunohistochemistry of non-tumor hepatic cells (A) and HCC cells (B) for PPARγ. Both preparations were stained with antibody for PPARγ and counterstained with hematoxylin ($\times$ 200).

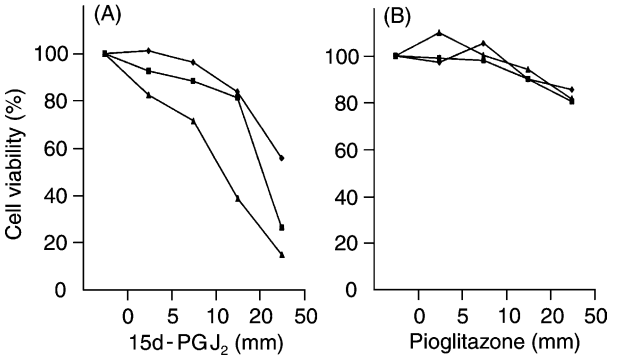

Figure 3. Effects of PPARγ ligand stimulation on viability of human HCC cells. HCC cells were incubated with the indicated concentrations of 15d-PGJ_2 (A) or pioglitazone (B) for 48 h at 37°C. Circles, HepG2; squares, SK-Hep1; triangles, HLE.

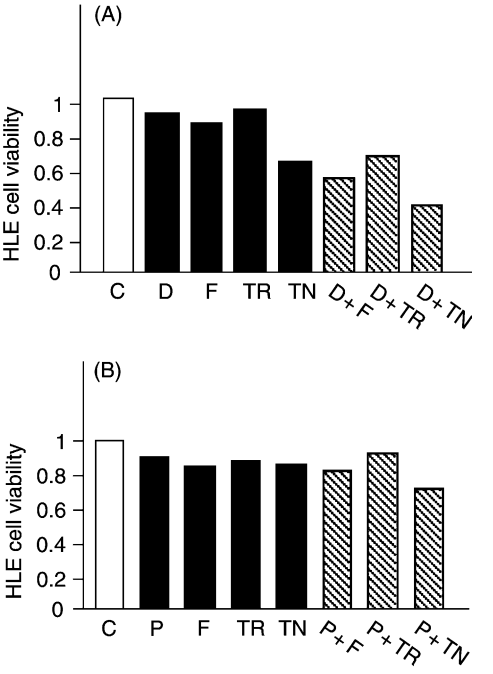

Figure 4. Effects of co-stimulation of PPARγ ligands and anti-Fas antibody (F), TRAIL (TR) or TNF-α (TN) on the viability of HCC cells. HCC cells were treated with anti-Fas antibody (50 ng/ml) in the presence or absence of 15d-PGJ_2 (D, 10 μM) or pioglitazone (P, 10 μM) for 48 h at 37°C. Instead of using anti-Fas antibody, TRAIL (50 ng/ml) or TNF-α (50 ng/ml) were also used in this experiment. C, control.

cell proliferation of these HCC cell lines with 48 h incubation at the indicated concentrations.

The inhibition of HCC cells proliferation and induction of apoptosis by PPARγ ligands and anti-Fas antibody

We evaluated the effects of PPARγ and Fas ligand, TNF-α and TRAIL on HCC cell viabilities. We incubated HCC cells with PPARγ ligands and TNF family members (anti-Fas antibody, TRAIL or TNF-α). After 48 h incubation, 15d-PGJ_2 in combination with anti-Fas antibody, TRAIL or TNF-α decreased cell viability greater that either alone (Figure 4A). In contrast, pioglitazone had little influence on HLE viability (Figure 4B). To evaluate induction of apoptosis by co-stimulation of PPARγ ligands and anti-Fas antibody, changes in nuclear morphology were studied using DAPI staining (Figure 5). HLE cells were incubated with or without the indicated reagents, for 48 h at 37°C. The cells that were co-stimulated by anti-Fas antibody and 15d-PGJ_2 showed nuclear fragmentation or condensation, as opposed to the other conditions which did not demonstrate morphological changes.

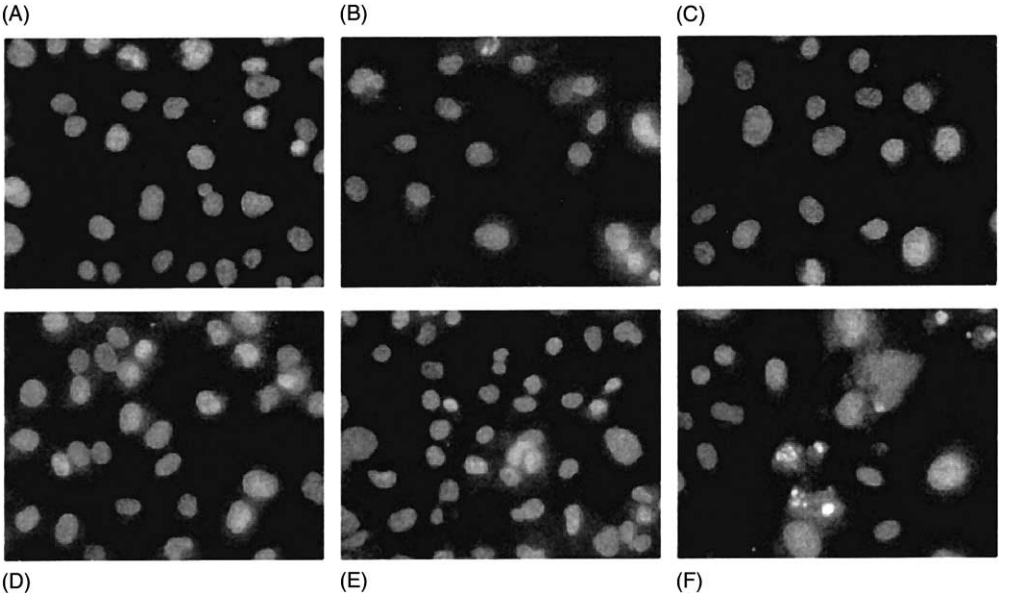

Figure 5. 15d-PGJ_2 and anti-Fas antibody-induced apoptosis in HLE cells. These cells were cultured for 48 h as follows: (A) no reagents, (B) 50 ng/ml of anti-Fas antibody, (C) 10 μM of pioglitazone, (D) 10 μM of 15d-PGJ_2, (E) 50 ng/ml of anti-Fas antibody and 10 μM pioglitazone or (F) 50 μg/ml of anti-Fas antibody and 10 μM 15d-PGJ_2. All cell nuclei were stained with DAPI.

Discussion

In this study, PPARγ was expressed on all seven HCC cell lines and located in their nuclei. In non-tumor tissues preparations, PPARγ was not detected in the cytoplasm or nuclei of hepatocytes, but it was observed in the inter-hepatocellular spaces of the same preparations. In accordance with a previous report,[11] this result reflects a phenomenon of PPARγ localized expression on Kupffer cells or stellate cells in normal liver tissues. With respect to protein expression levels of the HCC cell lines, PPARγ expression was observed in all HCC cell lines, but these expression levels varied among all cell lines. In particular, HLE cells and Chang liver cells showed higher expression than other cell lines.

Next, we evaluated the effects of PPARγ ligands for HCC cell lines using pioglitazone and 15d-PGJ_2. Pioglitazone is TZD derivative, which represents a novel class of oral drugs for the treatment of diabetes. However, troglitazone, also being a TZD derivative, causes severe hepatic dysfunction leading to hepatic failure;[18–20] the mechanism of hepatotoxicity is not known.[20] In this study, we use pioglitazone as a PPARγ ligand, instead of troglitazone, because there are no reports of hepatotoxicity with pioglitazone.[21] 15d-PGJ_2 is a naturally occurring compound and plays an important role for adipogenesis and inflammation as a homeostatic response. HLE cell proliferation was restricted strongly by 20–50 μM 15d-PGJ_2, affected at the lower concentration, whereas HepG2 cells and SK-Hep1 cells were not. This suggests that the PPARγ protein level between HLE cells and the other two cell lines may be related to sensitivities for 15d-PGJ_2. However, the effect of PPARγ may depend not only on the receptor density, but also on post-transcriptional or post-translational regulation, because the expression level of PPARγ protein did not necessarily correspond to the magnitude of the growth-inhibitory effects.[22] In this study, pioglitazone did not induce effective inhibition of cell proliferation on all three HCC cell lines, of up to the maximum 50 μM concentration. According to previous reports,[16,23] the inhibitory effect on cell proliferation was observed for 4 or 6 days when those cells were incubated with pioglitazone or other TZD derivatives. However, we examined the effect of PPARγ ligands on cell proliferation during 48-h culture using suitable concentrations exacted during clinical usage. The mechanism of the differing effects of 15d-PGJ_2 or pioglitazone on cell viabilities remains obscure in this study. We speculate that the most suitable conditions for suppression of cell proliferation may vary between these two PPARγ ligands or an unknown alternative signaling pathway may be present to influence cell

proliferation with PPARγ ligands. It should be noted as well that 15d-PGJ_2 is a natural product and pioglitazone is artificially synthesized. PPARγ is a nuclear receptor and PPARγ ligand must penetrate the cell-surface membrane transport through the cytoplasm to the nucleus. This process may be more difficult for pioglitazone than for 15d-PGJ_2.

The Fas system is an important component of cell apoptosis induction.[13] However, the Fas system is not significantly involved in apoptosis in human HCC,[24] although Fas is expressed on their surfaces. Consistent with previous reports, our study showed that anti-Fas antibody could not induce apoptosis of all three HCC cell lines, although they express Fas on their cell surfaces. However, a 48-h incubation with the 15d-PGJ_2 could augment Fas-mediated apoptosis of the HLE cells. In HCC cells, the Fas system is abnormal with soluble Fas expression,[14] a downstream inhibitor of the death signal from Fas.[15] We did not assess changes in Fas expression on HCC cell surfaces or proteins associated with inhibition of apoptosis when these HCC cells were cultured with 15d-PGJ_2 or pioglitazone. Because HCCs show one or more inhibitory proteins of the Fas signaling pathway, it may be difficult to induce apoptosis even if PPARγ affects Fas expression on the HCC cell surface. Although it is reported that PPARγ promotes development of malignancy under certain conditions,[25,26] it is already known that PPARγ induces G_1 arrest in malignant cells and suppresses cell proliferation.[22,27] Perhaps this nuclear receptor on malignant cell nuclei may induce transcriptional activities related to apoptosis signaling or down-regulation of apoptotic inhibitory proteins.

Recently, HCC cells were reported to be exposed to an environment containing Fas ligand.[14,24,28–30] This phenomenon implies that HCC cells receive Fas stimulation continuously. PPARγ stimulation induces apoptosis of HCC cells with continuous Fas stimulation, by release from Fas-induced death signaling pathway after a suitable period of incubation. In the current study, inhibition of cell proliferation by 15d-PGJ_2 was facilitated by co-stimulation with TNF-α or TRAIL. Further experiments are necessary to evaluate the interaction between PPARγ and Fas signaling pathways, which are competent to induce apoptosis. With respect to other malignant cell lines, e.g. colon or lung malignant cells, PPARγ ligands are already known to induce differentiation of tumor cells.[9,23] We did not assess the ability of inducing cell differentiation by PPARγ ligands in this study, but this characteristic is also interesting for application of HCC treatment and should be evaluated.

Conclusion

PPARγ can induce decrease of HCC cell viabilities by its ligand stimulation and PPARγ can enhance Fas-mediated apoptosis for HCC cells.

References

1. Wu GD, Lazar MA. A gut check of PPARγ. *Gastroenterology* 1998; **115**: 1283–94.
2. Fajas L, Auboeuf D, Raspe E, *et al*. The organization, promoter analysis, and expression of the human PPARγ gene. *J Biol Chem* 1997; **272**: 18779–89.
3. Forman BM, Tontonoz P, Chen J, Burn RP, Spiegelman BM, Evans RM. 15-Deoxy, delta 12,14-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. *Cell* 1995; **83**: 803–12.
4. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkinson WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedone is a high affinity ligand for the nuclear peroxisome proliferator-activated receptor γ (PPARγ). *J Biol Chem* 1995; **270**: 12953–6.
5. Brockman JA, Gupta RA, DuBois RN. Activation of PPARγ leads to inhibition of anchorage-independent growth of human colorectal cancer cells. *Gastroenterology* 1998; **115**: 1049–55.
6. Keelan JA, Sato TA, Marvin KW, Lander J, Gilmour RS, Mitchell MD. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2, a ligand for peroxisome proliferator-activated receptor-γ, induced apoptosis in JEG3 choriocarcinoma cells. *Biochem Biophys Res Commun* 1999; **262**: 579–85.
7. Tsubouchi Y, Sano H, Kawahito Y, *et al*. Inhibition of human lung cancer cell growth by the peroxisome proliferator-activated receptor-γ agonist through induction of apoptosis. *Biochem Biophys Res Commun* 2000; **270**: 400–5.
8. Harris SG, Phipps RP. The nuclear receptor PPAR gamma is expressed by mouse T lymphocyte and PPAR gamma agonists induce apoptosis. *Eur J Immunol* 2001; **31**: 1098–105.
9. Chang TH, Szabo E. Induction of differentiation and apoptosis by ligands of peroxisome proliferator-activated receptor γ in non-small cell lung cancer. *Cancer Res* 2000; **60**: 1129–38.
10. Rohn TT, Wong SM, Cotman CW, Cribbs DH. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2, a specific ligand for peroxisome proliferator-activated receptor-gamma, induces neuronal apoptosis. *Neuroreceptor* 2001; **12**: 839–43.
11. Everett L, Galli A, Crabb D. The role of hepatic peroxisome proliferator-activated receptors (PPARs) in health and disease. *Liver* 2000; **20**: 191–9.
12. Gonzalez FJ. The role of peroxisome proliferator activated receptor α in peroxisome proliferation, physiological homeostasis, and chemical carcinogenesis. *Adv Exp Med Biol* 1997; **422**: 109–25.
13. Nagata S. Review: apoptosis by death factor. *Cell* 1997; **88**: 355–65.
14. Lee SH, Shin MS, Lee HS, *et al*. Expression of Fas and Fas-related molecules in human hepatocellular carcinoma. *Hum Pathol* 2001; **32**: 250–6.

15. Takahashi M, Saito H, Okuyama T, *et al*. Overexpression of Bcl-2 protects human hepatoma cells from Fas-antibody-mediated apoptosis. *J Hepatol* 1999; **31**: 315–22.
16. Goke R, Goke A, Goke B, Chen Y. Regulation of TRAIL-induced apoptosis by transcription factors cellular. *Immunology* 2000; **201**: 77–82.
17. Wiley SR, Schooley K, Smolak PJ, *et al*. Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 1995; **3**: 673–82.
18. Watkins PB, Whitcomb RW. Hepatic dysfunction associated with troglitazone. *N Engl J Med* 1998; **338**: 916–7.
19. Li H, Heller DS, Leevy CB, Zierer KG, Klein KM. Troglitazone-induced fulminant hepatitis: report of a case with autopsy. *J Diabetes Complication* 2000; **14**: 175–7.
20. Shibuya A, Watanabe M, Fujita Y, *et al*. An autopsy case of troglitazone-induced fulminant hepatitis. *Diabetes Care* 1998; **21**: 2140–3.
21. Gillies PS, Dunn CJ. Pioglitazone. *Drug* 2000; **60**: 333–43.
22. Koga H, Sakisaka S, Harada M, *et al*. Involvement of $p21^{WAF1/Cip1}$, $p27^{Kip1}$, and $p18^{INK4c}$ in troglitazone-induced cell-cycle arrest in human hepatoma cell lines. *Hepatology* 2001; **33**: 1087–97.
23. Sarraf P, Mueller E, Jones D, *et al*. Differentiation and reversal of malignant changes in colon cancer through PPARγ. *Nat Med* 1998; **4**: 1046–52.
24. Kubo K, Matsuzaki Y, Okazaki M, Kato A, Kobayashi N, Okita K. The Fas system is not significantly involved in apoptosis in human hepatocellular carcinoma. *Liver* 1998; **18**: 117–23.
25. 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.
26. Saez E, Tontonoz P, Nelson MC, *et al*. Activation of the nuclear receptor PPAR gamma enhance colon polyp formation. *Nat Med* 1998; **4**: 1058–61.
27. Motomura W, Okumura T, Takahashi N, Obara T, Kohgo Y. Activation of peroxisome proliferator-activated receptor γ by troglitazone inhibits cell growth through the increase of $p27^{Kip1}$ in human pancreatic carcinoma cells. *Cancer Res* 2000; **60**: 5558–64.
28. Roskams T, Libbrecht L, Van Damme B, Desmet V. Fas and Fas ligand: strong co-expression in human hepatocytes surrounding hepatocellular carcinoma; can cancer induce suicide in peritumoural cells? *J Pathol* 2000; **191**: 150–3.
29. Ito Y, Monden M, Takeda T, *et al*. The status of Fas and Fas ligand expression can predict recurrence of hepatocellular carcinoma. *Br J Cancer* 2000; **82**: 1211–7.
30. Nagao M, Nakajima Y, Hisanaga M, *et al*. The alteration of Fas receptor and ligand system in hepatocellular carcinomas: how do hepatoma cells escape from the host immune surveillance *in vivo*? *Hepatology* 1999; **30**: 413–21.

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