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Original Article

Effects and mechanism of 15-deoxy-prostaglandin J_2 on proliferation and apoptosis of human HepG2 cells

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Abstract: Objective: To investigate the effects of 15-deoxy-prostaglandin J_2 (15d-PGJ_2), a peroxisome proliferator-activated receptor γ (PPARγ) endogenous ligand, on proliferation and apoptosis of human HepG2 cells and to explore the potential mechanism. Methods: 15d-PGJ_2 at different concentrations was used to treat the HepG2 cells cultured *in vitro*, MTT colorimetry was employed to measure proliferation of the cells, and ^{3}H-TdR uptake test was used to measure the synthetic rate of the cells. The expressions of PPARγ mRNA and protein were measured by reverse transcription-polymerase chain reaction (RT-PCR) and western blot. Cell apoptosis rate and cell cycle were analyzed by flow cytometry (FCM). And the effects of PPARγ-specific antagonist (GW9662) and/or transient transfection pSG5-PPARγ eukaryotic expression plasmid on proliferation of the HepG2 cells were also observed, and pGCsi-PPARγ was used to transfect the cells to observe the effects of 15d-PGJ_2 on proliferation of the HepG2 cells in the silence of PPARγ. The DNA binding activity of NF-κB was detected by electrophoretic mobility shift assay (EMSA). Results: 15d-PGJ_2 at different concentrations inhibited cell proliferation, DNA synthetic rate and induced apoptosis in a dose-dependent manner in HepG2 cells; in this process, the proportion of G0/G1 phase cells increased significantly while the proportion of S phase cells decreased significantly, and the expression levels of PPARγ mRNA and protein were not changed. GW9662 antagonized the proliferation inhibition effect of 15d-PGJ_2, but didn't block completely. However, transfection with a PPARγ-pSG5 expression plasmid restored the effect of 15d-PGJ_2 on cell growth in presence of GW9662. After transfection with pGCsi-PPARγ, 15d-PGJ_2 also inhibited cell proliferation at 20 μmol·L^{-1}. Furthermore, 15d-PGJ_2 inhibited the DNA binding activity of NF-κB at a higher concentration (50 μmol·L^{-1}). Conclusion: 15d-PGJ_2 inhibits cell growth, induces apoptosis and interferes with cell cycle of the HepG2 cells, which indicates that the activation of PPARγ is anti-neoplastic in HCC. This effect involves the PPARγ-dependent and PPARγ-independent pathways, which is related to the inhibition of NF-κB signaling pathway.

Keywords: Hepatocellular carcinoma, peroxisome proliferator-activated receptor γ, 15d-PGJ_2, cell proliferation, apoptosis, cell cycle, NF-κB

Introduction

Hepatocellular carcinoma (HCC) is a malignant tumor commonly seen in clinical practice and the third most fatal tumor-related disease [1]. Relevant research showed that peroxisome proliferator-activated receptor γ (PPARγ) ligand regulated proliferation, differentiation and apoptosis of multiple human tumor cells [2-7]. PPARγ is a ligand-dependent transcription factor, belonging to one of the nuclear hormone receptor superfamily. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) is one of the metabolites by the cyclooxygenase pathway of arachidonic acid and also the specific endogenous ligand of PPARγ *in vitro* [8, 9]. In the present study, the effects of treatment with 15d-PGJ_2 at different concentrations on proliferation and apoptosis of human HepG2 cells and the synthetic rate of DNA in the cells was observed to investigate the changes of cell cycle in these processes, and explore involvement of the PPARγ-dependent and -independent pathways in these processes and the detailed mechanism, so as to discuss the feasibility of PPARγ used as the new target in prevention and treatment of HCC.

Materials and methods

Materials

Reagents: GW9662 and pSG5-PPARγ was provided by Liu Yong-xue, research fellow from Beijing Academy of Military Medical Sciences; 15d-PGJ_2 was obtained from Oncogenel; the RPMI-1640 culture medium was from Hyclone; the newborn calf serum and trypsin was obtained from Gibco. ShRNA (pGCsi-PPARγ) for the CDS zone of PPARγ-encoded gene (NM 005037, GI: 116284367) was designed, synthesized and screened by Shanghai Genechem Co., Ltd., which also provided the negative control con siRNA. The RT-PC kit and DNA Marker DL2000 were from TaKaRa; TRIzol and LipofectamineTM 2000 (1 $g{\cdot}L^{-1}$) from Invitrogen; ^{3}H-TdR from Beijing Atom High-Tech Application Co., Ltd.; thiazolyl blue from Amersham LIFE SCIENCE; G418 Sulfate from Amresco; sheep anti-PPARγ polyclonal antibody, sheep anti-actin polyclonal antibody and the ECL kit from Santa Cruz; the Annexin V-FITC Kit, Coulter® DNA Prep™ Reagents Kit and IntraPrepTM Permeabilization Reagent from Backman Coulter; FITC-conjugated activated caspase 3 monoclonal antibody from BD; the nucleoprotein extraction kit from Beyotime Biotechnology; the EMSA kit from Pierce; and the others were analytically pure reagents.

Instruments: High-speed refrigerated centrifuge (Centrifuge 5417R, Eppendorf); PCR amplification device, vertical electrophoresis system and electrotransfer system (Bio-Rad); Microplate reader (Multiskan MK3, Labsystems Dragon); Gel imaging analysis system (Gis-2009 system, Tanon); DYY-III 2 constant-current/constant-voltage electrophoresis device (Beijing Liuyi Instrument Factory); DU®-640 nucleic acid and protein analyzer, LS-6500 liquid scintillation counter and EPICS-XL flow cytometer (Beckman Coulter, US).

Methods

Cell culture and treatment: The HepG2 cells were grown in a medium containing RPMI-1640 (10% calf serum) at 37°C, 5% CO_2 for 2 to 3 days. Following 60 to 70% confluence, the cells were washed with PBS for three times and the medium was replaced by serum-free medium. After 24 h, 15d-PGJ_2 was added to obtain individual final concentration, and the cells were cultured for 48 h before harvesting. GW9662 was added 1 h before the addition of 15d-PGJ_2 in order to observe the effect of GW9662 (thus ensuring a final concentration of 30 $\mu mol{\cdot}L^{-1}$).

Cell transfection: The HepG2 cells were digested with 0.2% trypsin and then seeded onto 24-well plates at 1×10^8 $cells{\cdot}L^{-1}$. Following 90% confluence, cells were transfected with LipofectamineTM 2000 mediated method, and the experimental method was conducted as exactly specified in the instruction strictly. After 48 h, the cells were harvested for subsequent studies.

Thiazolyl blue (MTT) colorimetric assay: The HepG2 cells were seeded onto 96-well plates at 1×10^8 $cell{\cdot}L^{-1}$ and then treated the same as 1.2.1 above. After 24 h of cultivation, 15 d-PGJ_2 was added to obtain required concentration, and the cells were cultured for 48 h before harvesting. 4 h before the culture was completed, MTT solution (5 $g{\cdot}L^{-1}$) was added at 20 μL/well. After 4 h, the supernatant was aspirated and discarded, and dimethyl sulfoxide was added (150 mL/well). After gently shaking for 10 min, absorbance at 492 nm was measured by a Multiskan MK3 microplate reader.

^{3}H-TdR incorporation assay: The HepG2 cells were seeded onto 96-well plates at 1×10^8 $cells{\cdot}L^{-1}$ and then treated the same as 1.2.1 above. 30 min after the addition of 15d-PGJ_2, 18.5 kBq 3H-thymidine (^{3}H-TdR) was added. After 48 h, the cells were washed twice with PBS, and 1 mL 10% trichloroacetic acid (TCA) was added. After standing at 4°C for 60 min, the precipitate was collected, washed with 95% ethanol, resuspended in 20 μL NaOH (0.15 $mol{\cdot}L^{-1}$) and collected on a glass fiber filter. The filter was dried, placed into a scintillation vial, and then detected by LS-6500 liquid scintillation counter after addition of 3 mL scintillation fluid.

Apoptosis assay: The control and treated HepG2 cells were collected, washed with PBS and then adjusted to a cell concentration of 1×10^8 $cells{\cdot}L^{-1}$ with 1 × binding buffer. The experimental method was conducted as exactly

Table 1. Effects of 15d-PGJ_2 on proliferation, ^{3}H-TdR uptake and apoptosis rate in human HepG2 cell lines ($\bar{x}$±s, n=3)

Concentration (μmol·L^{-1})	MTT colorimetry (OD well^{-1} values)	^{3}H-TdR uptake (Cpm·well^{-1})	Apoptosis rate (%) (D4 quadrant)
0 (con)	0.148±0.008	2342±162	9.10±0.46
10	0.115±0.005$^{**,\#}$	1712±121$^{**,\#}$	13.90±0.87$^{**,\#}$
20	0.096±0.003**	1219±83**	17.53±0.90**
50	0.070±0.002$^{**,\#}$	562±61$^{**,\#}$	31.03±1.50$^{**,\#}$

*P<0.05 *vs* con; **P<0.01 *vs* con; $^{\#}$P<0.05 *vs* 20 μmol·L^{-1}.

specified in the instruction of Annexin V-FITC Kit and the samples were detected by a flow cytometer within 30 min.

RT-PCR amplification of target gene: Total RNA was extracted with TRIzol reagent, purity and concentration were assayed by DU-640 nucleic acid spectrophotometer, and the experimental method was conducted as specified in the instruction of TaKaRa RT-PCR kit. The primer sequences are as follows: GAPDH: Upstream, 5'-ACG GAT TTG GTC GTA TTG GG-3'; Downstream, 5'-TGA TTT TGG AGG GAT CTC GC-3', the amplified fragment is 230 bp; PPARγ: Upstream, 5'-GCA TTC TGG CCC ACC AAC-3'; Downstream, 5'-CTG AAA CCG ACA GTA CTG-3', the amplified fragment is 484 bp. The PCR reaction procedures are as follows: Predenaturated at 95°C for 5 min; 95°C for 45 s, 48°C for 1 min, 72°C for 1 min, 28 cycles; extended at 72°C for 8 min. 2% agarose gel electrophoresis (containing 0.5 μg·ml^{-1} ethidium bromide) was performed for the amplified products, and the results were analysed by a gel imaging analysis system, wherein the relative expression of mRNA was measured as the optical density ratio of the gene to be tested and the internal control.

Western blot assay: The control and treated cells were collected, and 50 μL pre-cooled lysate was added to extract the intracellular proteins. Protein quantification was conducted by Bradford method, and electrophoretic separation was conducted using SDS-polyacrylamide gel (5%). PVDF membrane was transferred and blocked for 4 h, transferred to TTBS containing primary antibody (goat anti-PPARγ, 1: 100 diluted) and incubated for 2 h; the membrane was washed with TTBS for three times, and then transferred to horse anti-sheep secondary antibody (containing horseradish peroxidase conjugated, 1:2000 diluted) in TTBS and incubated for 40 min; the membrane was washed with TTBS for three times, developed with ECL and exposed to X-ray film, and the results were analysed by a gel imaging analysis system.

Cell cycle assay: The control and treated HepG2 cells were collected, washed twice with PBS and then adjusted to a cell concentration of 1 × 10^8 cells·L^{-1}. The experimental method was conducted as exactly specified in the instruction of Coulter® DNA Prep™ Reagents Kit and the samples were detected by a flow cytometer.

Electrophoretic mobility shift assay (EMSA): The control and treated cells were collected, and nucleoprotein was extracted as specified in the instruction of nucleoprotein extraction kit (Beyotime). The protein and probe reaction system is as follows: 10 × binding buffer 2 μL, 1 μg/μl poly (dI:dC) 1 μL, 50% glycerol 1 μL, 1% NP-40 1 μL, 1 M KCL 1 μL, 100 mM $MgCl_2$ 1 μL, 200 mM EDTA 1 μL, Protein extracts 10 μL, Biotin-DNA 0.2 μmol/μL 2 μL, and the total system 20 μL. pre-electrophoresis was conducted at 120 V for 1 h, 5 μL sample loading buffer was added to the sample mixture, and electrophoresis was conducted at 180 V for 30 to 45 min. The nylon film was placed into 0.5 × TBE and equilibrated for 10 min, and after the completion of electrophoresis, it was electrophoretically transferred at 380 mA for 30 min. After completion of transferring, the membrane was exposed to UV light for 20 min, and blocked for 20 min with blocking buffer. The antibody was diluted and reacted with the membrane for 30 min, washed with 1 × eluent for three times, equilibrated in equilibration buffer for 5 min, and finally ECL luminescence detection was conducted.

Statistical method

The experiment data were expressed in mean ± SD ($\bar{x}$±s). The differences among groups were analyzed by one-way ANOVA using the statistical software SPSS 11.5, and LSD test was used for comparison between groups. P<0.05 indicates statistically significant differences.

Results

Effect of 15d-PGJ_2 on proliferation of the HepG2 cells

As shown in **Table 1**, the MTT colorimetry experiment revealed that 15d-PGJ_2 significantly

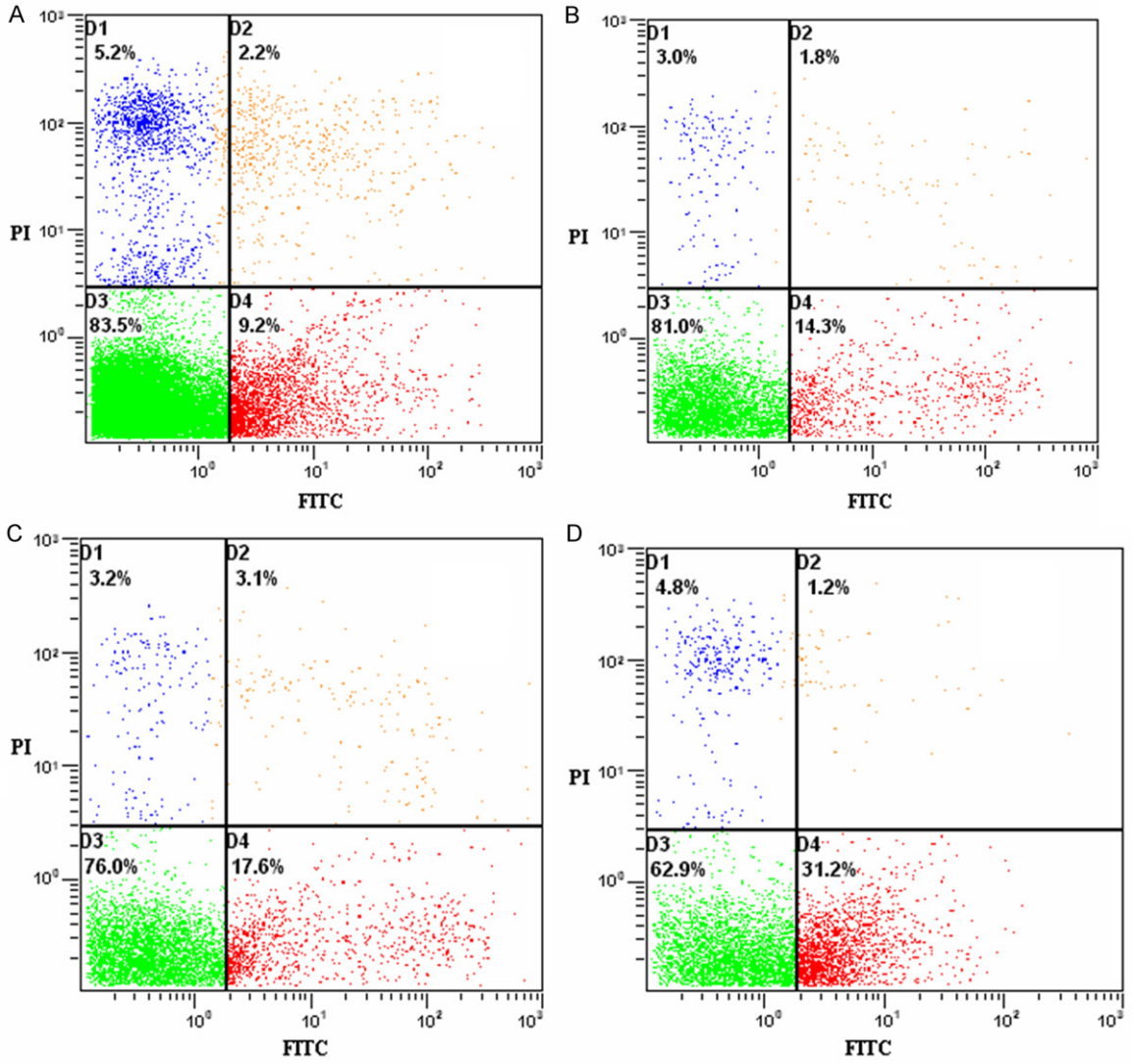

Figure 1. Effect of treatment with 15d-PGJ_2 at different concentrations on apoptosis of the HepG2 cells. A: Con, untreated cells; B: Cells treated with 15d-PGJ_2 10 μmol·L^{-1}; C: Cells treated with 15d-PGJ_2 20 μmol·L^{-1}; D: Cell treated with 15d-PGJ_2 50 μmol·L^{-1}.

inhibited proliferation of the HepG2 cells, and the effect enhanced gradually with its increasing concentration, showing a dose-dependent relationship.

Effect of 15d-PGJ_2 on DNA synthetic rate in the HepG2 cells

As shown in **Table 1**, the ^{3}H-TdR uptake experiment revealed that 15d-PGJ_2 significantly inhibited uptake of ^{3}H-TdR by the HepG2 cells, and the effect enhanced gradually with its increasing concentration, showing a dose-dependent relationship. It indicated that 15d-PGJ_2 was able to inhibit the DNA synthetic rate in the HepG2 cells.

Effect of 15d-PGJ_2 on apoptosis of the HepG2 cells

As shown in **Figure 1** and **Table 1**, the Annexin V-FITC results indicated that 15d-PGJ_2 induced apoptosis of the HepG2 cells, and the effect enhanced gradually with its increasing concentration, showing a dose-dependent relationship.

Effect of 15d-PGJ_2 on the expression of PPARγ mRNA and protein

As shown in **Table 2** and **Figure 2**, results from the RT-PCR and Western blot analysis revealed that treatment with 15d-PGJ_2 at different con-

Table 2. Effects of 15d-PGJ_2 on PPARγ mRNA, PPARγ protein, and cell cycle in the HepG2 cells ($\bar{x}$±s, n=3)

Concentration (μmol·L^{-1})	PPARγ mRNA	PPARγ protein	Cell cycle (%)		
			G0/G1 phase	S phase	G2/M phase
0 (con)	0.479±0.054	0.155±0.018	48.57±0.35	41.43±1.58	10.19±1.10
10	0.505±0.102	0.167±0.017	56. 60±1.23**	35. 00±0.78**	8.39±0.91
20	0.507±0.067	0.174±0.020	62.77±1.27**	28.73±1.00**	8.52±1.65
50	0.503±0.088	0.173±0.016	66.13±1.04**	26. 40±1.28**	7.46±1.66

*P<0.05 *vs* con; **P<0.01 *vs* con. The relative expression of PPARγ mRNA and protein was calculated by the densitometry value of PPARγ/GAPDH and PPARγ/β-actin.

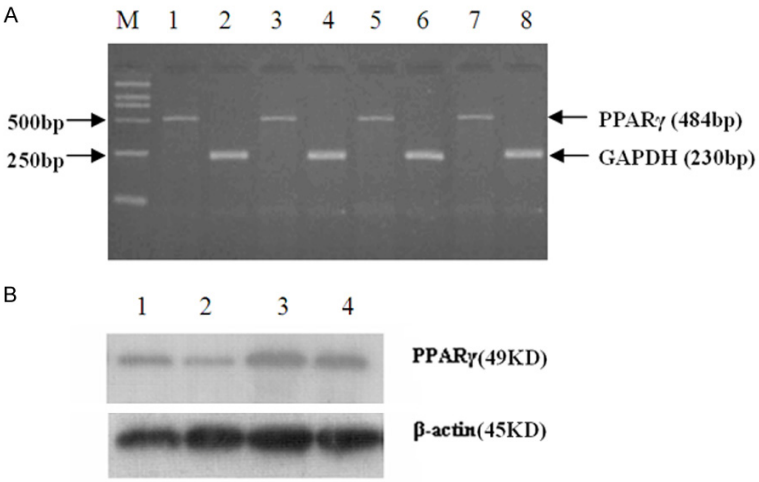

Figure 2. A: Effect of treatment with 15d-PGJ_2 at different concentrations on the expression of PPARγ mRNA in the HepG2 cells. M: Marker; 1, 2: con, control cells; 3, 4: cells treated with 15d-PGJ_2 10 μmol·L^{-1}; 5, 6: cells treated with 15d-PGJ_2 20 μmol·L^{-1}; 7, 8: cell treated with 15d-PGJ_2 50 μmol·L^{-1}. B: Effect of treatment with 15d-PGJ_2 at different concentrations on the expression of PPARγ protein in the HepG2 cells. 1: con, control cells; 2: cells treated with 15d-PGJ_2 10 μmol·L^{-1}; 3: cells treated with 15d-PGJ_2 20 μmol·L^{-1}; 4: cell treated with 15d-PGJ_2 50 μmol·L^{-1}.

centrations had no effect on the expression of PPARγ mRNA and protein in the HepG2 cells.

Effect of 15d-PGJ_2 on cell cycle of the HepG2 cells

As shown in **Table 2** and **Figure 3**, the results of flow cytometry revealed that 15d-PGJ_2 acting on the HepG2 cells for 48 h interrupted cell cycle. Compared with the control group, 15d-PGJ_2 led to a great increase in the G0/G1 phase cells and a great decrease in the S phase cells, showing a dose-dependent relationship, with no obvious changes in the G2/M phase cells.

PPARγ-dependent pathway involved in the inhibition of proliferation of the HepG2 cells

As shown in **Figure 4**, GW9662 at 30 μmol·L^{-1} antagonized remarkably the inhibition of proliferation of the HepG2 cells by 15d-PGJ_2 (at a concentration of 20 μmol·L^{-1}), but the effect was not wholly, and transient transfection with pSG5-PPARγ reversed these effects of GW9662. Transient transfection with GW9662 or pSG5-PPARγ alone had no effect on proliferation of the cells. It indicated that 15d-PGJ_2 inhibited proliferation of the cells through partly the PPARγ-dependent pathway, but GW9662 could not completely antagonize the effects of 15d-PGJ_2, indicating the plausible presence of the PPARγ-independent pathway.

PPARγ-independent pathway involved in the inhibition of proliferation of the HepG2 cells

PGCsi-PPARγ was taken to transfect the HepG2 cells to investigate the inhibition of proliferation of the cells by 15d-PGJ_2 in the silence of PPARγ. The results (**Figure 5**) showed that after treatment with 15d-PGJ_2 at 20 μmol·L^{-1}, proliferation inhibition of the cells in the transfection group was significantly lower than that in the non-transfection group using 15d-PGJ_2 at the same concentration (P<0.01), but compared with the control group, there were still significant effects (P<0.01), and transfection with con siRNA or pGCsi-PPARγ alone had no remarkable effects on proliferation of the cells. It demonstrated that the inhibition of proliferation of the cells by 15d-PGJ_2 was not dependent on the expression of PPARγ, and there was the PPARγ-nondependent pathway.

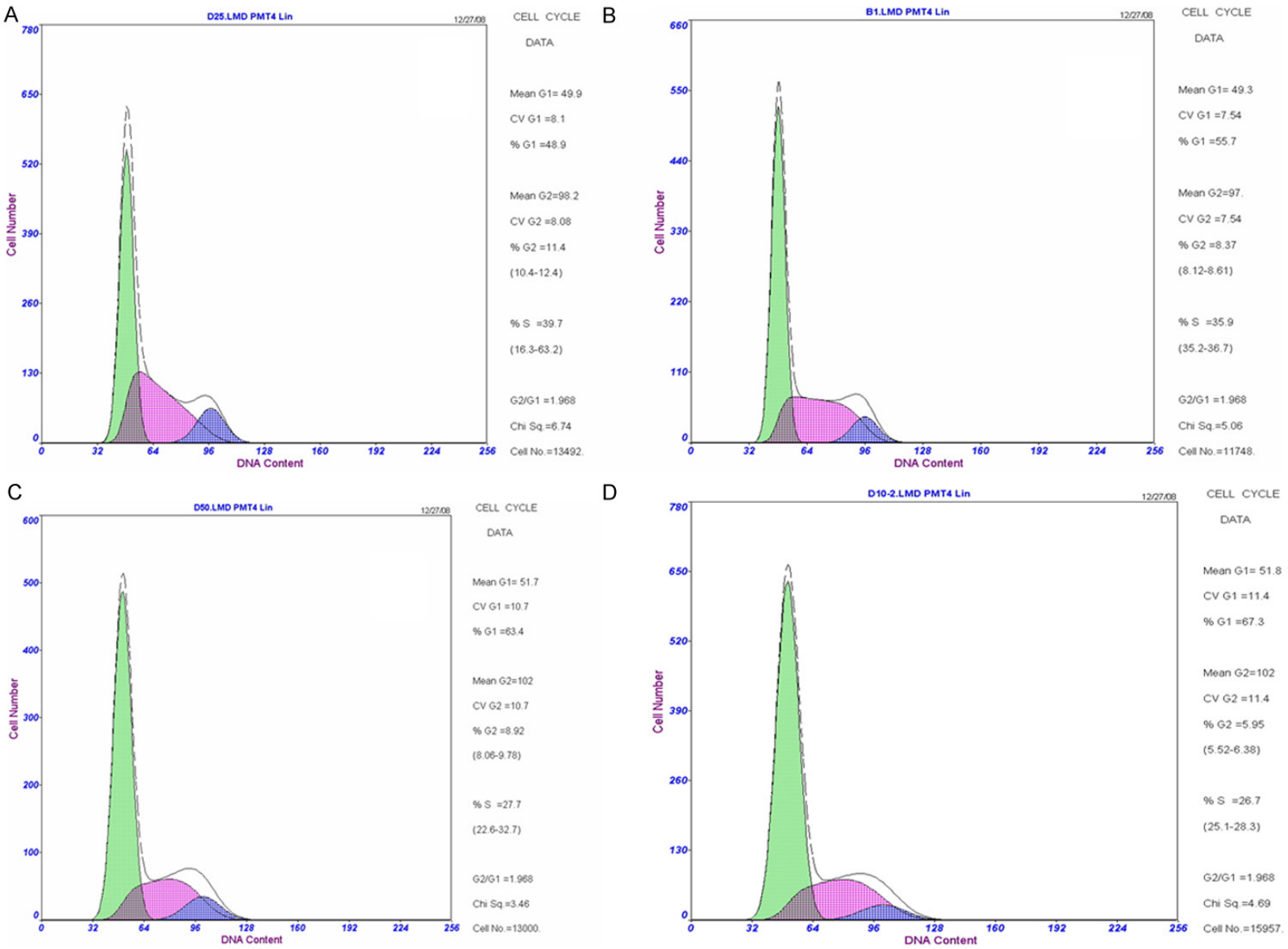

Figure 3. Effect of treatment with 15d-PGJ_2 at different concentrations on cell cycle of the HepG2 cells. A: Con, untreated cells; B: Cells treated with 15d-PGJ_2 10 μmol·L^{-1}; C: Cells treated with 15d-PGJ_2 20 μmol·L^{-1}; D: Cell treated with 15d-PGJ_2 50 μmol·L^{-1}.

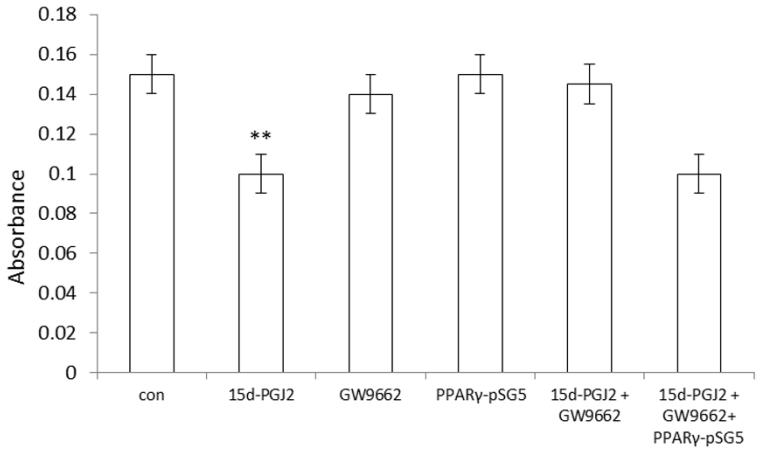

Figure 4. PPARγ-dependent pathway involved in proliferation inhibition of the HepG2 cells by 15d-PGJ_2. Con represents the control group; 15d-PGJ_2 represents the group treated with 15d-PGJ_2 20 μmol·L^{-1}; GW9662 represents the group treated with GW9662 (30 μmol·L^{-1}) alone; PPARγ-pSG5 represents the group transfected with PPARγ-pSG5 alone; 15d-PGJ_2 + GW9662 represents the group treated with both 15d-PGJ_2 20 μmol·L^{-1} and GW9662 (30 μmol·L^{-1}); 15d-PGJ_2 + GW9662+ PPARγ-pSG5 represents the group treated with the above two after transfection with PPARγ-pSG5. *P<0.05 vs con; **P<0.01 vs con.

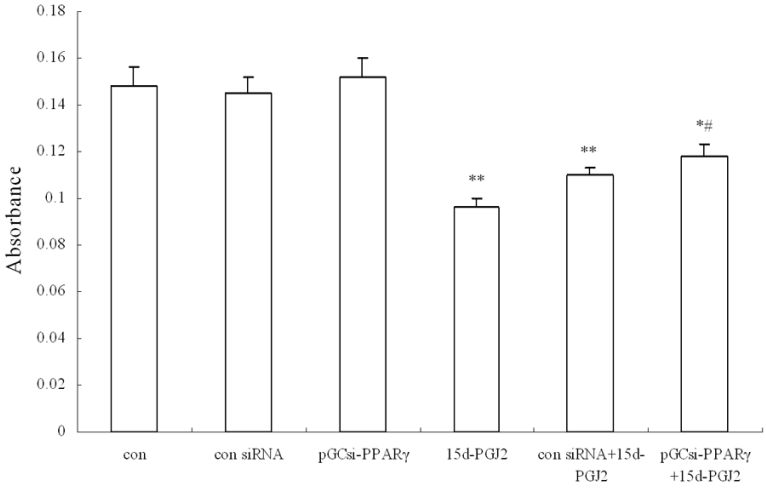

Figure 5. PPARγ-nondependent pathway involved in proliferation inhibition of the HepG2 cells by 15d-PGJ_2. Con represents the control group; con siRNA represents the group transfected with con siRNA alone; pGCsi-PPARγ represents the group transfected with pGCsi-PPARγ; 15d-PGJ_2 represents the group treated with 15d-PGJ_2 20 μmol·L^{-1}; con siRNA+15d-PGJ_2 represents the transfection control group for 15d-PGJ_2 20 μmol·L^{-1}; pGCsi-PPARγ+15d-PGJ_2 represents the pGCsi-PPARγ transfection group treated with 15d-PGJ_2 20 μmol·L^{-1}. *P<0.01 con; **P<0.01 con; #P<0.05 *vs* 15d-PGJ_2 (20 μmol·L^{-1}).

Effect of 15d-PGJ_2 on DNA binding activity of NF-κB

The EMSA experiment showed that at high concentration (50 μmol·L^{-1}), 15d-PGJ_2 significantly inhibited the DNA binding activity of the nuclear factor NF-κB, suggesting that the NF-κB signaling pathway was involved in the above effects on the HepG2 cells by 15d-PGJ_2, see **Figure 6**.

Discussions

Like other nuclear hormone receptors, PPARγ is activated after conjugation with its ligand, and then it forms heterodimer with 9-cis-retinoic acid receptor, which specifically conjugated with peroxisome proliferators response element (PPRE) at upstream of the promoter of target gene to regulate transcription of the gene [10, 11], which is the mode of action of the traditional PPARγ-dependent pathway. Recently, several studies also indicated that PPARγ ligand exerted its biological effects by the PPARγ-nondependent pathway [12-14]. The majority of studies available demonstrated that intervention of PPARγ ligand showed biological effects on multiple tumor cells, such as proliferation inhibition and apoptosis induction, indicating that PPARγ activation is anti-tumor in a certain degree [15-17]. However, there are also different opinions. It was demonstrated in study that PPARγ activation regulateed production of hepatocyte growth factor and accelerate tumor growth [18]. Therefore, further research is required on the effects of PPARγ in HCC. In addition, no current studies on human HCC cells have explained whether its effects of proliferation inhibition and/or apoptosis induction are via the PPARγ-dependent pathway, which is critical for investigating whether PPARγ can be the target for prevention and treatment of HCC.

It was demonstrated in the present study that 15d-PGJ_2 significantly inhibited proliferation, reduce the DNA synthetic rate and induce

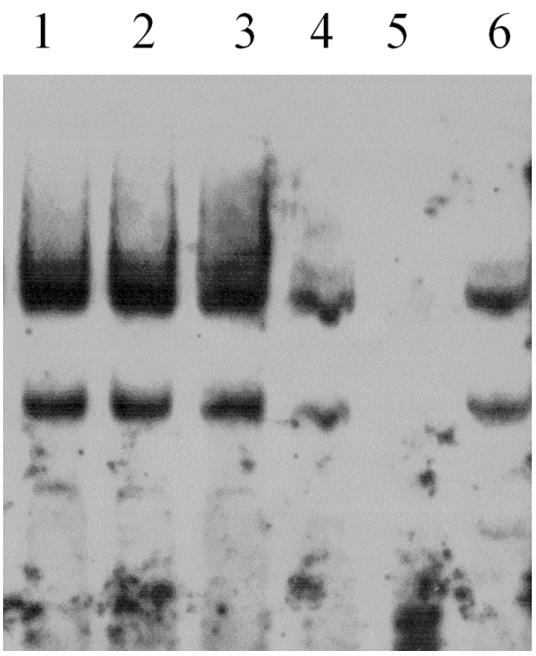

Figure 6. Effect of 15d-PGJ_2 on DNA binding activity of NF-κB. 1: control group (con); 2: 15d-PGJ_2 (10 μmol·L^{-1}) treatment group; 3: 15d-PGJ_2 (20 μmol·L^{-1}) treatment group; 4: 15d-PGJ_2 (50 μmol·L^{-1}) treatment group; 5: cold competitor; 6: positive control.

apoptosis of human HCC cells, suggesting that PPARγ activation in the HepG2 cells was antitumor, which is consistent with the latest study results [6, 7, 15-17]. Besides, the majority of current studies also support this opinion. Toyoda *et al.* found out that in the human HCC cells PLC/PRF/5 with high expression of PPARγ, after troglitazone intervention, inducing the expression of caspase3 led to cell apoptosis and inhibit growth of cancer cells [3]. Rumi *et al.* revealed that in the hepatocellular carcinoma cell lines like HepG2, Huh-7, KYN-1 and KYN-2, troglitazone, pioglitazone and 15d-PGJ_2 inhibited DNA synthesis, cell cycle progression and AFP expression in the hepatocellular carcinoma cells [4]; recently, Okano *et al.* found out in the human HCC cells like HepG2, SK-Hep1 and HLE that 15d-PGJ_2 inhibited growth of the cells through promoting cell apoptosis induced by Fas [5]. Recently, Koga [19, 20] *et al.* observed that in human HCC cell lines, pioglitazone down-regulated the expression of Skp2, leading to accumulation of P27Kip1 protein in the cells and arrest of cancer cell cycle, to inhibit growth of cancer cells. It was also demonstrated in the present study that in the above effects, 15d-PGJ_2 significantly interfered with cell cycle, lead to a great increase in the G0/G1 phase cells and decrease in the S phase cells, with no obvious changes in the G2/M phase cells.

However, in the above processes, 15d-PGJ_2 did not lead to increased expression of PPARγ mRNA and protein in the HepG2 cells, which may involve the PPARγ-nondependent pathway. To explore by which PPARγ pathway 15d-PGJ_2 exerts its effects described above, relevant research has been done in the present study using GW9662, PPARγ-pSG5 and pGCsi-PPARγ. The reaults showed that GW9662 antagonized partly proliferation inhibition of the HepG2 cells by 15d-PGJ_2, but in the cells transfected with PPARγ-pSG5 expression plasmid, 15d-PGJ_2 restored its above effect. It suggested tentatively that proliferation inhibition of the HepG2 cells by 15d-PGJ_2 was partly via the PPARγ-dependent pathway, but GW9662 could not wholly antagonize the effect of 15d-PGJ_2, indicating the plausible presence of the PPARγ-nondependent pathway. To demonstrate its presence, in the present study, pGCsi-PPARγ was taken to transfect the HepG2 cells, and it was observed that 15d-PGJ_2 at high concentration still showed inhibition on proliferation of the cells in silence of PPARγ, suggesting that in the above effects of 15d-PGJ_2, the PPARγ-nondependent pathway was surely involved. Studies on many other tumor cells also demonstrated presence of the PPARγ-nondependent pathway. Galli *et al.* found out that rosiglitazone and pioglitazone inhibited invasion of pancreatic cancer cells by regulating the expression of metalloproteinases 2 and plasminogen activator inhibitor 1 (PAI1), which is PPARγ-nondependent [21]. Shiau *et al.* revealed that in the two prostate cancer cell lines PC3 (expressing PPARγ) and LNCaP (PPARγ deficient) cells, troglitazone was able to induce cell apoptosis by inhibiting Bcl-xL and Bcl-2, which is unrelated to the expression of PPARγ and is realized through the PPARγ-nondependent pathway [22]. In studies applying PPARγ-/- embryonic stem cells, it was observed that troglitazone and ciglitazone inhibited cell proliferation by inhibiting translation initiation, and this effect is also PPARγ-nondependent [23]. In the study by Clay *et al.* [24], it was also demonstrated that in breast cancer cells, although 15d-PGJ_2 activated the PPRE-mediated transcriptional activity of multiple genes, PPARγ was not essential for apoptosis induction, and caspase inhibitors blocked cell apoptosis induced by 15d-PGJ_2.

There are many factors mediating apoptosis in tumor formation, such as nuclear factor-κB (NF-κB), caspase3, activating protein-1 (AP-1), signal transducer and activator of transcription (STAT) [25]. Evidences available showed that NF-κB was activated in multiple chronic liver diseases (eg. cholestasis, autoimmune liver disease, and hepatitis B and C) and was closely related to HCC. Deeper research revealed that NF-κB activation induced the expression of specific genes involved in regulating programmed cell death and inducing apoptosis [26]. The transcriptional activity of NF-κB depends on the DNA binding activity, structure of NF-κB dimer and its phosphorylation state. It was revealed in the present study that in the HepG2 cells, 15d-PGJ_2 at a high concentration of 50 μmol·L^{-1} significantly inhibited the DNA binding activity of NF-κB, suggesting that the NF-κB signaling pathway was involved in proliferation inhibition and apoptosis induction of the cells by 15d-PGJ_2, and played an important role in its anti-tumor effects. However, since it shows the above effects at high concentration only, it is speculated that the regulation of gene transcription is realized through influencing NF-κB by the PPARγ-nondependent pathway.

As suggested in current relevant studies on PPARγ, the same PPARγ ligand has different effects on different cancer cells, and different PPARγ ligands have different effects on the same cancer cells, which may involve the complicated physiological and pharmacological mechanisms of different PPARγ ligands. It has been demonstrated in the present study that 15d-PGJ_2 is able to inhibit proliferation of and induce apoptosis of the HepG2 cells, suggesting that PPARγ activation has anti-tumor effects, which involve both of the PPARγ-dependent and -nondependent pathways and are related to its inhibition of the NF-κB signaling pathway. Nevertheless, since the PPARγ-nondependent patyway is involved in its anti-tumor effects, further study *in vivo* is required to confirm whether PPARγ activation has the same effects in the body, which is also the topic of the next step in this research project.

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Disclosure of conflict of interest

None.

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