Peroxisome proliferator-activated receptor-γ is essential in the pathogenesis of gastric carcinoma

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AIM: To investigate whether peroxisome proliferator-activated receptor γ (PPAR-γ) is expressed in human gastric carcinoma and whether PPAR-γ is a potential target for gastric carcinoma therapy.

METHODS: PPAR-γ protein in gastric carcinoma was examined by immunohistochemistry. In the gastric carcinoma cell line MGC803, PPAR-γ, survivin, Skp2 and p27 protein and mRNA were examined by Western blotting and real-time reverse transcription-polymerase chain reaction, respectively; proliferation was examined by MTT; apoptosis was examined by chromatin staining with Hoechst 33342 and fluorescence activated cell sorting (FACS); and cell cycle was examined by FACS; the knockdown of PPAR-γ was done by RNA interference.

RESULTS: A high level of expression of PPAR-γ was observed in human gastric carcinoma and in a human gastric carcinoma cell line MGC803. The PPAR-γ agonist 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) inhibited growth, and induced apoptosis and G0/G1 cell cycle arrest in MGC803 cells in a concentration-dependent and time-dependent manner. The effect of 15d-PGJ2 on MGC803 cells was not reversed by the selective and irreversible antagonist GW9662 for PPAR-γ. Furthermore, survivin and Skp2 expression were decreased, whereas p27 expression was enhanced following 15d-PGJ2 treatment in a dose-dependent manner in MGC803 cells. Interestingly, we also found that small interfering RNA for PPAR-γ inhibited growth and induced apoptosis in MGC803 cells. The inhibition of PPAR-γ function may be a potentially important and novel modality for treatment and prevention of gastric carcinoma.

CONCLUSION: A PPAR-γ agonist inhibited growth of human gastric carcinoma MGC803 cells by inducing apoptosis and G0/G1 cell cycle arrest with the involvement of survivin, Skp2 and p27 and not via PPAR-γ.

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Key words: Peroxisome proliferator-activated receptor γ; Gastric cancer; Apoptosis; Cell cycle

INTRODUCTION

Gastric carcinoma is the second most common cancer in the world and kills more than 600 000 people annually[1]. At present, the management of gastric carcinoma mainly includes surgery and chemotherapy, but the curative effect of the existing chemotherapeutic drugs is not effective and they have numerous side effects. Many studies have been performed to search for therapeutic targets and drugs capable of preventing and treating gastric carcinoma and other malignancies.

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors, initially described as molecular targets for compounds, which induce peroxisomal proliferation[2]. Up to now, 3 different isotypes of PPARs have been identified in various species, being the products of distinct genes and generally designated as PPAR-α, PPAR-β/δ, and PPAR-γ[2,3]. PPAR-γ has been extensively studied. Similarly to other
members of the nuclear receptor gene family, PPAR-γ is an agonist-activated transcription factor[9,10]. PPAR-γ heterodimerizes with retinoid X receptor to bind to the PPAR response element, leading to the transcription of downstream genes[9]. Several specific agonists have been found, such as the thiazolidinediones (including pioglitazone, rosiglitazone, and troglitazone), 15-deoxy-

\[12,14\text{-prostaglandin-J}_2\] (15d-PGJ\(_2\)), and certain polyunsaturated fatty acids. GW9662 has been shown to be a selective and irreversible antagonist of PPAR-γ, and irreversibly binds within the agonist binding domain through covalent modification of a cysteine residue[9].

PPAR-γ is predominately expressed in adipose tissue and plays a central role in adipocyte differentiation and insulin sensitivity[11]. Recent studies have shown that, in addition to its classic role, PPAR-γ is implicated as a putative therapeutic target for cancer in a variety of tumors as several observations suggest that stimulation of PPAR-γ function may inhibit carcinogenesis and tumor cell growth[9,12]. However, the exact role of PPAR-γ on carcinogenesis and tumor cell growth is still unclear[10-14]. Recent investigations by Morita et al[15] and Konturek et al[16] have shown that PPAR-γ is implicated in Helicobacter pylori-related gastric carcinogenesis, and that PPAR-γ agonists may have potential in a cancer therapeutic role.

We here demonstrate that PPAR-γ may be involved in gastric carcinogenesis, and that the inhibition of PPAR-γ may be of benefit in the treatment of gastric carcinoma.

**MATERIALS AND METHODS**

**Chemicals**

PPAR-γ agonist, 15d-PGJ\(_2\) and antagonist GW9662 were purchased from Cayman chemical (Ann Arbor, MI). Other drugs were reagent grade.

**Tissue samples**

One hundred and thirty eight samples of surgically-resected primary gastric carcinoma tissues, 138 samples of paired adjacent mucosa (2.5 cm from the margin of the gastric carcinoma) and 138 samples of paired normal mucosa (histologically proven) at the surgical margin (at least 5 cm from the margin of the gastric carcinoma) tissues were obtained from the First Affiliated Hospital of Inner Mongolia Medical College in China from October 2004 to January 2005. All gastric carcinoma patients underwent total or subtotal gastrectomy, and no patient received any treatment for cancer before surgery. The patient series included 111 males and 27 females with a mean age of 58.5 years and a median age of 60 years (range, 36-78). After surgery, the gastric specimens were fixed in 10% neutral buffered formalin and embedded in paraffin for immunohistochemistry. The clinical stage according to new TNM criteria published by the Union International Centre Cancer in 1997. All pathological diagnoses were performed by 2 independent pathologists.

**Immunohistochemical staining of PPAR-γ**

Sections of paraffin-embedded tissues (4 μm) were mounted on glass slides. The intracellular expression of PPAR-γ was detected using a PPAR-γ specific mAb (sc-7372, E-8, Santa Cruz Biotechnology, CA) with standard non-biotin horseradish peroxidase (HRP) 2-step immunostaining (Zymed) according to the manufacturer’s instructions. The immunoreactive products were visualized using 3,3’-diaminobenzidine (DAB)/H\(_2\)O\(_2\).

**Cell culture and treatment with PPAR-γ agonists and antagonists**

We used the human gastric carcinoma cell line MGC803 from Beijing Tumor Institute. It was maintained in RPMI1640 containing 10% fetal bovine serum (FBS) at 37°C under 5% CO\(_2\). A PPAR-γ agonist or antagonist were added at the time of replating.

**Cell survival assay**

Gastric carcinoma cells were treated with a PPAR-γ agonist or antagonist dissolved in DMSO for 8, 18 and 24 h in culture medium in 96-well plates. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma] solution was then added to the well with gentle pipetting. After 4 h, the supernatant was removed after centrifugation at 1000 r/min for 5 min at room temperature, and 180 μL DMSO was added to each well to solubilize the crystal products by shaking for 10 min. Absorbance at 570 nm was measured with an ELISA reader. The negative control had medium without serum and cells, and was used as the zero point of absorbance. Cell viability of the control group was 100%. The inhibitory rate (IR) of cells was calculated according to the equation as follows: IR (%) = \[
\frac{[1-(A_{570} \text{nm absorbance in 15d-PGJ}_2 \text{treated group}/A_{570} \text{nm absorbance in control group})] \times 100\%}
\]

**Apoptosis analysis**

Apoptosis was analyzed by 2 different methods. First, chromatin staining with Hoechst 33342 was carried out. Briefly, MGC803 cells treated with 15d-PGJ\(_2\) were cultured on Laboratory-Tek Chamber Slides for 24 h. The cells were then fixed with methanol for 10 min and rinsed. Chromatin staining was done with Hoechst 33342 (Sigma) to detect nuclear condensation. Secondly, cell apoptosis was measured by fluorescence activated cell sorting (FACS) using the Annexin-FITC Apoptosis Detection Kit according to the manufacturer’s protocol. The apoptotic rate was calculated as the percentage of annexin V-positive and propidium iodide (PI)-negative cells divided by the total number of cells in the gated region. The data was pooled from 3 independent experiments.

**Cell cycle examination**

The effect of 15d-PGJ\(_2\) on the cell cycle was examined with PI staining by FACS using Epics-XL (Beckman Coulter).

**Western blotting analysis**

Cell total protein was extracted from MGC803 cells using
We used shRNA™ vector (provided by Wuhan Genesil Biotechnology Co., Ltd.) in which shRNA expression is driven by the U6 RNA promoter to produce small and hairless RNA transcripts. We generated 3 different shRNA™ vectors and an empty vector used as a control. The oligonucleotides 5'GATCCGACAGATCCGTCGTTCAAGACGTCGTAACAGC-3', 3'GCTTGTCTAGGTCACCAACGAAGTTCTGC-5' were used to generate anti-heparanase shRNA™ pSi1, and oligonucleotides 5'-GATCCGCTAAA-GTGGAGCCCTCTTCCTCAGAACGGATGCAGGCTCCACCTTTGTTCTGAACAGG-3', 3'GAGATTCTGCCCACATGCTTGAAG-5' were used to generate anti-heparanase shRNA™ pSi2. Oligonucleotides 5'-GATCCGACACCTCTCAAGGGG-TGTAGCAGGTTGTCTTTTTG-3', 3'GCTTGTCTAGGTCACCAACGAAGTTCTGC-5' were used to generate anti-heparanase shRNA™ pSi3.

Transfection
For the transfection, pSi1, pSi2 and pSi3 (1.0 µg) were added to RPMI1640 medium (without FBS) containing cation liposome vector (2.0 µL) and allowed to incubate for 20 min at room temperature to produce the transfection mixture. The transfection mixture was then added to the cells (1 × 10⁶ per dish) in the serum-free medium. Six hours after the start of transfection, the medium was changed to RPMI1640 medium containing 10% FBS. RNA interference effect was examined by RT-PCR, Western blotting and MTI assay. Cell apoptosis determination was carried out at the final step.

Statistical analysis
The results were expressed as mean ± SD. Statistical analysis was performed using the Student's t-test, χ² test or one-way ANOVA and subsequent Fisher's LSD test. P < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS11.0 software.

RESULTS
Enhanced PPAR-γ expression in gastric carcinoma tissue and MGC803 cells
The positive rate of PPAR-γ protein expression was significantly higher in gastric carcinoma (75.0%) than in paired mucosa adjacent to gastric carcinoma (27.5%) and in normal gastric mucosa at the surgical margin of gastric carcinoma (13.0%) (P < 0.001). Mucosa adjacent to gastric carcinoma had significantly higher expression than normal gastric mucosa at the surgical margin of gastric carcinoma (P < 0.05 and P < 0.001) (Figure 1). The rate of PPAR-γ protein expression was positively associated with histologic type and negatively associated with invasive depth, vessel invasion, lymph node metastasis, distant metastasis and clinical stage.
(P < 0.05) of gastric carcinoma. The rate of PPAR-γ protein expression was significantly higher in intestinal-type gastric carcinoma (95.2%) than in diffuse-type gastric carcinoma (58.7%) (P < 0.001) (Table 1). Both PPAR-γ mRNA and protein showed positivity in the human gastric carcinoma cell line MGC803 (Figure 2).

| Clinicopathological features | n   | PPAR-γ protein | χ² | P     |
|------------------------------|-----|----------------|----|-------|
| Histologic subtype           |     |                |    |       |
| Intestinal type              | 63  | 60             | 3  | 24.663 | 0.000 |
| Diffuse type                 | 75  | 44             | 31 |       |
| Depth of invasion            | 138 | 1.183          | 0.277 |       |
| Serosal                      | 24  | 16             | 8  |       |
| Out of serosa                | 114 | 88             | 26 |       |
| Vessel invasion              | 138 | 0.065          | 0.799 |       |
| Absent                       | 120 | 90            | 30 |       |
| Present                      | 18  | 14             | 4  |       |
| Lymph node metastasis        | 138 | 0.309          | 0.578 |       |
| Absent                       | 36  | 28             | 8  |       |
| Present                      | 102 | 76             | 28 |       |
| Distant metastasis           | 138 | 0.535          | 0.464 |       |
| Absent                       | 126 | 96            | 30 |       |
| Present                      | 12  | 8             | 4  |       |
| Clinical stagea              | 126 | 0.017          | 0.897 |       |
| I - II                       | 39  | 30             | 9  |       |
| III-IV                       | 87  | 61             | 26 |       |

aBecause clinical staging could not be performed because of the lack of accurate records about lymph nodes, data of 12 cases were not included in the statistical analysis.

Effect of 15d-PGJ₂, GW9662 and 15d-PGJ₂ + GW9662 on growth of MGC803 cells

Treatment with a concentration of 0.01, 0.1 and 1 μmol/L 15d-PGJ₂ did not affect MGC803 cell growth. The inhibition of proliferation of MGC803 cells by 5, 10, 20, 30, and 40 μmol/L 15d-PGJ₂ was significantly higher than that of 0 μmol/L (P < 0.001) (Table 2). Correspondingly, inhibition was also enhanced with increasing 15d-PGJ₂ concentrations. Inhibition was 5.98% ± 1.41%, 13.64% ± 0.69%, and 34.64% ± 0.99% after 8, 18, and 24 h, respectively, following 30 μmol/L 15d-PGJ₂ addition. It was significantly higher at 18 h than 8 h (P < 0.001), and it was significantly higher at 24 h than 18 h (P < 0.001); it was enhanced over a prolonged time. Thus 15d-PGJ₂ inhibited proliferation of MGC803 cells in a concentration-dependent and time-dependent manner (Figure 3A). The proliferation inhibition rate of MGC803 cells by GW9662 treatment for 24 h, showed no difference between 0.01, 0.1 and 1 μmol/L and 0 μmol/L concentrations (all P > 0.05). Inhibition of proliferation was significantly higher at 2.5 and 5 μmol/L compared to 0 μmol/L (all P > 0.05). The proliferation inhibition rate of MGC803 cells by 15d-PGJ₂ and GW9662 combination treatment for 24 h showed no difference between the 0.01, 0.1 and 1 μmol/L GW9662 and 5, 10, 20, 30 and 40 μmol/L 15d-PGJ₂ combination groups and the 5, 10, 20, 30 and 40 μmol/L/15d-PGJ₂ group (Table 3).

Effect of 15d-PGJ₂ on apoptosis of MGC803 cells

15d-PGJ₂ is capable of inducing MGC803 cell apoptosis as seen by morphological observation (Figure 4) and flow cytometry (Figure 5). The apoptosis rate of MGC803 cells of 5, 10, 20, 30, and 40 μmol/L 15d-PGJ₂ was significantly higher than that of 0 μmol/L (P < 0.001) (Table 1); it was enhanced with increasing concentrations of 15d-PGJ₂. The apoptosis rate was 6.58% ± 0.62%, 15.99% ± 1.73%, and 38.23% ± 1.36% at 8, 18 and 24 h, respectively, following 30 μmol/L 15d-PGJ₂ addition, and increased with time. Thus, 15d-PGJ₂ induced...
MGC803 cell apoptosis in a concentration-dependent and time-dependent manner (Figure 3B).

**Effect of 15d-PGJ2 on cell cycle of MGC803 cells**

15d-PGJ2 at 30 μmol/L significantly increased the proportion of MGC803 cells in the G0/G1 phase (P < 0.001) and significantly decreased the proportion in the S and G2/M phases (P < 0.001 and P < 0.01, respectively) (Figure 6).

**Effect of 15d-PGJ2 on survivin, Skp2 and p27 expression in MGC803 cells**

Survivin and Skp2 protein were decreased and p27 protein was increased as the concentration of 15d-PGJ2 increased as shown by Western blotting (Figure 7).

**Effect of knockdown of PPAR-γ by RNAi**

To examine whether the inhibitory effect of PPAR-γ agonist 15d-PGJ2 on the growth of MGC803 cells was via a PPAR-γ specific pathway and influenced PPAR-γ expression in the tumor cells, we used the RNAi approach for PPAR-γ. Efficiency of transfection approximated 80% was evaluated by fluorescence microscopy 48 h after transfection of a vector containing the gene encoding green fluorescent protein (Figure 8). MGC803 cells were transiently transfected with pSi1, pSi2, and pSi3 or empty vectors by cation liposome vector, and the cells were tested for PPAR-γ expression 48 h later. Of the cells transfected with pSi1, pSi2 and pSi3, pSi1 was most effective in silencing PPAR-γ mRNA and protein expression in MGC803 cells, as determined by semiquantitative RT-PCR (Figure 9A) and Western blotting (Figure 9B). PPAR-γ specific RNAi effectively decreased PPAR-γ mRNA and protein level in the MGC803 cells and showed inhibition of cell growth (Figure 10A) and induction of cell apoptosis (Figure 10B).

**DISCUSSION**

Recently, the potential of PPAR-γ as a target for the prevention and treatment of cancer has been widely studied[19-22]. However, the potential therapeutic role of PPAR-γ agonists has been questioned, based on contradictory results. In experiments using animal models of colon cancer, PPAR-γ agonists increased the development of colon tumors[10]. This contradictory result was supplemented by a recent report using transgenic mice that expressed a constitutive active form of PPAR-γ in mammary glands, and showed that PPAR-γ signaling accelerated tumor development in mammary glands[18]. The actual role of PPAR-γ in cancer has been complicated by recent findings that PPAR-γ agonists affect cancer cells independent of PPAR-γ[19-22], and silencing of PPAR-γ and PPAR-γ antagonists have been shown to inhibit cancer cell growth[13,14]. To date, the role of PPAR-γ in gastric carcinogenesis remains unclear.

In the present study, PPAR-γ was found to be expressed at higher levels in gastric carcinoma than in paired mucosa adjacent to gastric carcinoma, and in both tissues the PPAR-γ levels were higher than in their paired mucosa adjacent to gastric carcinoma.
paired normal gastric mucosa at the surgical margin of the gastric carcinoma. Similar effects were also observed in gastric cancer in another study. In addition, we used the siRNA approach to confirm the effect of PPAR-\(\gamma\) on gastric carcinoma. The result showed that silencing PPAR-\(\gamma\) clearly inhibited the growth and induced apoptosis of MGC803 cells. Similar effects were also observed in other cancers such as hepatocellular carcinoma. These results indicated that PPAR-\(\gamma\) may be involved in gastric carcinogenesis.

Here, we clearly showed that the PPAR-\(\gamma\) agonist 15d-PGJ\(_2\) inhibited growth of cultured gastric cancer MGC803 cells, and our study also demonstrated that the PPAR-\(\gamma\) antagonist GW9662 did not interfere with this effect and that 2.5 \(\mu\)mol/L GW9662 inhibited growth of MGC803 cells. Furthermore, PPAR-\(\gamma\) siRNA markedly inhibited the growth of MGC803 cells in our study. These results indicated that 15d-PGJ\(_2\) inhibited growth of cultured gastric carcinoma MGC803 cells by a PPAR-\(\gamma\)-independent pathway. These results also suggested that PPAR agonists may be useful in the chemoprevention or chemotherapy of gastric malignancies. Additionally, some recent studies reported that the biological effect of PPAR-\(\gamma\) agonists is independent of PPAR-\(\gamma\)[19,23-28], but other research concluded that 15d-PGJ\(_2\) inhibited growth of cancer cells and gastric cancer cells by activating the PPAR-\(\gamma\) pathway[29-32]. What is the reason for the discrepancies between our data and the data found in other reports? This may be attributed to the fact that some investigators did not study the effect of PPAR antagonists on tumor cell growth and on the effect of PPAR agonists, as they only used PPAR agonists.

The inhibition of growth of cultured gastric cancer MGC803 cells by PPAR-\(\gamma\) agonists is mainly a result of apoptosis, as evidenced by the data for nuclear condensation by chromatin staining with Hoechst 33342 and annexin V-FITC staining through FACS analysis. Mechanisms may exist by which apoptosis is induced by PPAR agonists. Furthermore, the inhibition of cell growth and induction of apoptosis by PPAR-\(\gamma\) agonists were time- and concentration-dependent.

Survivin is an inhibitor of apoptosis, and is expressed during embryonal development but lacks expression in terminally differentiated adult tissues. Interestingly, it becomes re-expressed in transformed cell lines and in a variety of human tumors, and deserves growing attention as “an ideal target” for cancer therapy. Survivin is involved in the control of apoptosis and directly inhibits caspase 3 and 7 activities. Survivin is mostly
Survivin may have a direct effect on survivin expression. In eukaryotes, progression of the cell cycle is controlled by interactions between cell cycle control proteins (cyclins) and their catalytically active cyclin-dependent kinases (CDKs). The activity of each cyclin-CDK complex is in turn regulated by several different mechanisms, the most important being negative regulation by CDK inhibitors[39]. p27 is an inhibitor of cyclin E-CDK2 and cyclin A-CDK2, which drive cells from the G1 phase to the S phase of the cell division cycle[36,37], and p27 is expressed at its highest level in the G1/G0 phase. The amount of p27 is mainly regulated by posttranslational

**Figure 5** Apoptosis rate of MGC803 cells detected by flow cytometry 24 h after treatment with 15d-PGJ$_2$ at various concentrations. A: 0 μmol/L; B: 5 μmol/L; C: 10 μmol/L; D: 20 μmol/L; E: 30 μmol/L; F: 40 μmol/L.

**Figure 6** Cell cycle distribution of MGC803 cells by flow cytometry 24 h after treatment with 15d-PGJ$_2$ at various concentrations. 15d-PGJ$_2$ at 30 μmol/L could significantly increase the proportion of cells in the G0/G1 phase (P < 0.001) and decrease the proportion in the S and G2/M phases in MGC803 cells (P < 0.001 and P < 0.01).

induced MGC803 cell arrest in the G1/G0 cell cycle phase and reduced the percentage of cells in the S and G2/M cell cycle phases. This may be another reason for inhibition of the growth of gastric carcinoma MGC803 cells by 15d-PGJ$_2$. Additionally, this may be in part the reason that 15d-PGJ$_2$ reduced the level of survivin, although we cannot exclude the possibility that 15d-PGJ$_2$ may have a direct effect on survivin expression. In eukaryotes, progression of the cell cycle is controlled by interactions between cell cycle control proteins (cyclins) and their catalytically active cyclin-dependent kinases (CDKs). The activity of each cyclin-CDK complex is in turn regulated by several different mechanisms, the most important being negative regulation by CDK inhibitors[39]. p27 is an inhibitor of cyclin E-CDK2 and cyclin A-CDK2, which drive cells from the G1 phase to the S phase of the cell division cycle[36,37], and p27 is expressed at its highest level in the G1/G0 phase. The amount of p27 is mainly regulated by posttranslational
ubiquitin–proteasome-mediated proteolysis[38]. The cell cycle-dependent degradation of p27 is dependent on phosphorylation at Thr187 in the late G1 phase by CDK2, and Thr187 phosphorylation is a necessary prerequisite for the sequential addition of ubiquitin molecules by a ubiquitin ligase complex, SCFskp2, composed of Skp1, Cull, Rbx1, and the F-box protein Skp2[37]. Polyubiquitination of p27 then targets p27 for degradation in the proteasome, thus removing the p27 cell cycle “brake”, allowing cells to transition from G1 to the S phase[39]. Some investigations have shown that Skp2 is a specific substrate-recognition subunit of SCF[37], the expression of Skp2 is required for the ubiquitination and subsequent degradation of p27 in vitro and in vivo[40–42], and Skp2 knockout cells exhibit p27 at high levels[43]. Skp2 levels are cell cycle regulated, and Skp2 accumulates during the S phase. Inappropriate expression of Skp2 in G0 cells can promote S phase entry concomitant with loss of p27. Recently, the targeted disruption of Skp2 resulted in an accumulation of p27, and cell cycle arrest in the G1 cycle. The level of Skp2 protein was decreased, whereas the level of p27 protein was increased with increasing concentration of 15d-PGJ2 treatment when examined by Western blotting. These data suggested that 15d-PGJ2 induced MGC803 cell arrest in G1/G0 by Down-regulating Skp2 expression and upregulating p27 expression. Furthermore, these results suggested that 15d-PGJ2 may interfere with ubiquitin-proteasome-mediated proteolysis in gastric carcinoma cells.

In conclusion, our study suggests that PPAR-γ may be involved in gastric carcinogenesis, and that 15d-PGJ2 may inhibit the growth of human gastric carcinoma MGC803 cells by inducing apoptosis and G1/G0 arrest, with the involvement of survivin, Skp2 and p27, but via a PPAR-γ-independent pathway.

**COMMENTS**

**Background**

Many efforts have been made to search for therapeutic targets and drugs capable of preventing and treating gastric carcinoma and other malignancies. Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors, initially described as molecular targets for compounds, which induce peroxisomal proliferation.

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Figure 8 Human gastric carcinoma MGC803 cells observed by fluorescence microscopy 48 h after pSi1 transfection.

Figure 9 PPARγ after pSi transfection. A: PPARγ mRNA expression in various cells detected by RT-PCR 48 h after transfection; B: PPARγ protein expression in various cells 48 h after transfection as detected by Western blotting. Lane M: 100 bp DNA marker, 500 bp was the brightest; Lane 1: MGC803-N (normal MGC803 cells); Lane 2: MGC803-HK (MGC803 cells transfected by empty vector); Lane 3: MGC803-pSi3 (MGC803 cells transfected by pSi3 vector); Lane 4: MGC803-pSi2 (MGC803 cells transfected by pSi2 vector); Lane 5: MGC803-pSi1 (MGC803 cells transfected by pSi1 vector). The effect of pSi1 was best in silencing PPAR-γ mRNA and protein expression in MGC803 cells.

Figure 10 Proliferative inhibition and apoptosis rate of MGC803-Y1 and of MGC803-HK cells after transfection. The proliferative inhibition (A) and apoptosis (B) rate of MGC803-Y1 cells was higher than that of MGC803-HK cells at 12-72 h after transfection.
Research frontiers
Three different isotypes of PPARs have been identified in various species, being the products of distinct genes and generally designated as PPAR-α, PPAR-β/δ, and PPAR-γ. PPAR-γ is one that has been extensively studied. As with other members of the nuclear receptor gene family, PPAR-γ is an agonist-activated transcription factor. PPAR-γ heterodimerizes with retinoid X receptor to binding to the PPAR response element, leading to the transcription of downstream genes.

Innovations and breakthroughs
Several specific agonists have been found, such as the thiazolidinediones (including pioglitazone, rosiglitazone, and troglitazone), 15-deoxy-12,14-prostaglandin-J₉, and certain polyunsaturated fatty acids. GW9662 has been shown to be a selective and irreversible antagonist of PPAR-γ, and irreversibly binds within the agonist binding domain through covalent modification of a cysteine residue.

Applications
By understanding how PPAR-γ affects the growth of cells, this study may represent a future strategy for therapeutic intervention in the treatment of patients with gastric carcinoma.

Terminology
PPAR-γ receptors are nuclear hormone receptors, which induce peroxisomal proliferation. 15d-PGJ₂ is a specific agonist. Peroxisomal proliferation is thought to be crucial in gastric carcinoma.

Peer review
PPAR-γ receptors are nuclear hormone receptors. The authors examined whether PPAR-γ may affect gastric carcinogenesis, and 15d-PGJ₂ may inhibit the growth of MGC803 cells by inducing apoptosis and G₀/G₁ arrest, but through a PPAR-γ-independent pathway. The result may be useful in treatment of gastric carcinoma.

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