**STOMACH**

Effect of peroxisome proliferator activated receptor \( \gamma \) ligands on growth and gene expression profiles of gastric cancer cells

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Background and aims: Although peroxisome proliferator activated receptor \( \gamma \) (PPAR\( \gamma \)) agonists have been implicated in differentiation and growth inhibition of cancer cells, the potential therapeutic and chemopreventive effects on gastric cancer are poorly defined. We examined the in vitro and in vivo effects of PPAR\( \gamma \) ligands on growth of gastric cancer, and the effect of PPAR\( \gamma \) activation on expression of cyclooxygenase 2 (COX-2) and cancer related genes.

Methods: Gastric cell lines (MKN28 and MKN45) were treated with two specific PPAR\( \gamma \) ligands: ciglitazone and 15-deoxy-\( \Delta^{12,14} \)-prostaglandin \( J_2 \). Cell growth was determined by bromodeoxyuridine incorporation assay and apoptosis was measured by DNA fragmentation. Expression of COX-2 was determined by western blot and real time quantitative polymerase chain reaction (PCR). Expression profiles of cancer related genes were screened with cDNA array. In vivo growth of implanted MKN45 cells in nude mice was monitored after oral treatment with rosiglitazone.

Results: PPAR\( \gamma \) ligands suppressed the in vitro growth of MKN45 cells in a dose dependent manner whereas prostacyclin, a PPAR\( \delta \) agonist, had no growth inhibitory effect. Growth inhibition was more pronounced in MKN45 cells, which was accompanied by DNA fragmentation and downregulation of COX-2. Screening by cDNA microarray showed that PPAR\( \gamma \) ligand treatment was associated with upregulation of bad and p53, and downregulation of bcl-2, bcl-xl, and cyclin E1 in MKN45 cells, which was confirmed by quantitative real time PCR. In contrast, MKN28 cells with lower PPAR\( \gamma \) and COX-2 expression levels had lower growth inhibitory responses to PPAR\( \gamma \) ligands. Microarray experiments only showed induction of the bad gene in MKN28 cells. In vivo growth of MKN45 cells in nude mice was retarded by rosiglitazone. Mean tumour volume in rosiglitazone treated mice was significantly lower than controls at six weeks \((p = 0.019)\) and seven weeks \((p = 0.001)\) after treatment.

Conclusions: PPAR\( \gamma \) ligands suppress both in vitro and in vivo growth of gastric cancer and may play a major role in cancer therapy and prevention.

Peroxisome proliferator activated receptors (PPAR), ligand dependent transcription factors, are members of the nuclear receptor superfamily that are involved in cellular differentiation and proliferation. There are at least three isoforms that have been identified: PPAR\( \alpha \), PPAR\( \delta \), and PPAR\( \gamma \). Of particular interest is the PPAR\( \gamma \) isoform which initially was found to be expressed at high levels in adipocytes and to be responsible for central regulation of adipocyte differentiation. Subsequently, it was detected in other organs, including the colon, stomach, small intestine, liver, and pancreas. In common with other members of the PPAR family, PPAR\( \gamma \) forms heterodimers with the retinoid X receptor. This heterodimer preferentially binds to the PPAR responsive element of target genes and regulates gene expression. In addition to its role in cellular differentiation, ligand activation of PPAR\( \gamma \) has been linked to apoptosis and tumorigenesis. In primary colon cancers, there is aberrant expression of PPAR\( \gamma \). Activation of PPAR\( \gamma \) by synthetic ligands resulted in growth inhibition and induction of differentiation in colonic cell lines. Several in vitro experiments showed that treatment with PPAR\( \gamma \) ligands resulted in growth inhibition of other tumours, including sarcoma and carcinoma cells. However, different tumour cell lines, including bladder, breast, and thyroid cancers, may have different antiproliferative responses to PPAR\( \gamma \) ligands. More interestingly, these in vitro findings may not be directly relevant to the in vivo effects. Conflicting results were obtained when thiazolidinediones were applied in different animal models of colon cancer. PPAR\( \gamma \) ligands inhibited growth of tumours transplanted into nude mice and reduced aberrant crypt foci formation by azoxymethane in rats. On the other hand, treatment with PPAR\( \gamma \) ligands was shown to promote tumorigenesis in the Apc\( ^{-/min} \) mouse model with an increase in the number and size of colon polyps. The reasons for these discrepant findings remain elusive and it is unclear whether they are related to the use of different mouse models or the intrinsic pro-tumorigenic properties of PPAR\( \gamma \) ligands.

Gastric cancer is the second most common cause of cancer death in the world. Apart from surgery, the role of adjuvant therapy remains unproven. Thus the need to identify potential novel therapeutic and chemopreventive agents is obvious. Recent in vitro studies showed that treatment with PPAR\( \gamma \) ligands resulted in growth inhibition and induction of apoptosis in gastric cell lines but whether PPAR\( \gamma \) ligands

Abbreviations: PPAR\( \gamma \), peroxisome proliferator activated receptor \( \gamma \); COX, cyclooxygenase; PGJ\( _2 \), prostaglandin \( J_2 \); PGE\( _2 \), carboprostanoyclind; FBS, fetal bovine serum; BrDU, bromodeoxyuridine; RT-PCR, reverse transcription-polymerase chain reaction
have antitumorigenic effects on gastric cancer in vivo is unknown. In this study, we examined the in vitro and in vivo effects of PPARγ ligand activation on the growth of gastric cancer cells. Moreover, to elucidate the mechanisms underlying growth inhibition of PPARγ ligands, we determined expression of cyclooxygenase 2 (COX-2) and other tumour related genes in two gastric cell lines after treatment with PPARγ ligands.

**METHODS**

**Chemicals**

The PPARγ agonists ciglitazone and 15-deoxy-Δ12,14-prostaglandin J2 (PGJ2), the PPARγ agonist carbaprostacyclin (PGI2), and the COX-2 specific inhibitor NS398, were all obtained from Cayman Chemical (Ann Arbor, Michigan, USA). Rosiglitazone was kindly provided by Glaxo SmithKline (Hong Kong).

**Cell cultures**

The human gastric cancer cell lines MKN45 and MKN28 were obtained from Riken Gene Bank (Japan). They were cultured in RPMI medium 1640 (Invitrogen Co., Carlsbad, California, USA) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Invitrogen Co.) at 37°C in a humidified atmosphere of 95% room air and 5% CO₂.

**Cell proliferation and apoptosis**

Cell proliferation was detected by a quantitative ELISA kit (Cell Proliferation ELISA; Roche, Germany) which measures bromodeoxyuridine (BrdU) incorporation during DNA synthesis. Cells were grown in 96 well culture dishes (2 x 10⁴ cells/well), incubated with PPAR ligands for 72 hours, and labelled with BrdU for another six hours. BrdU incorporation was measured colorimetrically.

Apoptosis was measured by the Cell Death Detection ELISA (Roche) which detects the amount of cytoplasmic histone associated DNA fragments. Similar to the proliferation assay, cells were grown in 96 well plates (2 x 10⁴ cells/well) with test agents for 72 hours. The amount of fragmented DNA was then measured colorimetrically, as suggested by the manufacturer.

**RNA extraction and cDNA synthesis**

Total RNA was extracted using RNA Trizol reagent (Invitrogen Co.). Total RNA (1 μg) was reverse transcribed into complementary DNA using dNTPs (1 mM), 5x reverse transcription buffer (500 mM Tris HCl, pH 8.3, 250 mM KCl, 50 mM MgCl₂, and 50 mM DTT), 16 units of RNasin ribonuclease inhibitor (Promega), and 2.5 units of AMV reverse transcriptase (GibcoBRL, Rockville, Maryland, USA). Total RNA was isolated after 48 hours of treatment.

**Real time quantitative reverse transcription-polymerase chain reaction (RT-PCR)**

Quantitation of PPARγ and COX-2 RNA expression was performed using the TaqMan real time PCR assay (ABI PRISM 7700 Sequence Detection System: PE Applied Biosystems, Foster City, California, USA), as described previously by Humar and colleagues. A gene specific PCR oligonucleotide primer pair and an oligonucleotide probe labelled with a reporter fluorescent dye at the 5’ end and a quencher fluorescent dye at the 3’ end were used. For primers and probes were:

- PPARγ forward primer 5’-CAGGAGCGGCGTGAACTCA-3’;
- PPARγ reverse primer 5’-ACTGCACTGTACAAAGACGGAAGCAG-3’;
- β-actin forward primer 5’-CTAATGGGCAACCCAGCACAAGTCG-3’;
- β-actin reverse primer 5’-GCGGATCCACACGAGGATCT-3’;
- PPARγ reverse primer 5’-TCAGAGATTTGCCTCCTCTGAGGC-3’.

Each amplification consisted of a 24 μl reaction mixture with 100 nM of each probe, 300 nM of each primer (or 25 nm of β-actin primer), 2.5 U AmpliTaq gold polymerase, 200 μM each of dATP, dCTP, and dGTP, 400 μM dUTP, 5 mM MgCl₂, 0.5 U AmpErase uracil N-glycosylase, and 1× Taqman buffer (Roche Diagnostics). Complementary DNA (cDNA 1 μl) was added into each reaction. Reaction tubes were sealed with optical caps, and the PCR reaction was run on an ABI Prism 7700. Cycling conditions were 50°C for two minutes, 95°C for 10 minutes, followed by 40 cycles at 95°C for 20 seconds and 58°C for 45 seconds. Each sample was added in triplicate. A template free negative control was included in all amplifications. The intensity of the fluorescent dye was determined and expression of COX-2 was normalised to cDNA loading for each individual sample using β-actin as an internal standard. Changes in expression of COX-2 in treated cell lines were compared with vehicle treated cells.

**Western blot**

Total protein was extracted by Trizol (Invitrogen) and the concentration was determined by Lowry assay (Bio-Rad, Hercules, California, USA). Total protein (25 μg) was resolved onto a 10% sodium dodecyl sulphate-polyacrylamide gel. After electrophoresis, proteins were transferred to polyvinylidene difluoride blotting membranes (Amersham, Buckinghamshire, UK). Membranes were blocked in Tris buffered saline-T buffer with 10% skim milk at 4°C, and incubated with corresponding secondary antibody, goat polyclonal IgG anti-COX-2 (Santa Cruz Biotechnology, California, USA) at 1:1000 dilution. Membranes were then washed by Tris buffered saline-T buffer and incubated with corresponding secondary antibody, Enhanced chemiluminescence was detected by SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, Illinois, USA). Membranes were stripped and probed with 1:2500 diluted mouse monoclonal anti-β-actin (Sigma-Aldrich, St Louis, Missouri, USA) as the expression control. Images were scanned by GS-700 Imaging Densitometer and analysed by Quantity One software version 4.2.1 (Bio-Rad). Expression of COX-2 protein in vehicle treated cells after standardisation with β-actin was compared with PPARγ ligand treated cells.

**cDNA microarray experiments**

Gene expression profiles of MKN28 and MKN45 gastric cell lines after treatment with PPARγ ligands were analysed by a commercially available gene expression array system (GEArray Q series Human Cancer Pathway Finder Gene Array; SuperArray, Bethesda, Maryland, USA). This array consists of 96 specific cDNA fragments of genes involved in apoptosis and cell senescence, cell cycle control and DNA damage repair, signal transduction molecules and transcription factors, angiogenesis, adhesion, invasion, and metastasis, as well as control sequences. We compared the expression profiles of these genes in vehicle control treated gastric cells and cells treated with PGJ2 (10 μM) or ciglitazone (20 μM). Total RNA was isolated after 48 hours of treatment. Subsequent procedures were performed according to the manufacturer’s suggestion. The arrays were visualised using a non-radioactive method and the intensity of the hybridisa-
tion signals was quantitated by scanning densitometry after adjustment to housekeeping genes within the same array using the GE ArrayAnalyzer software (version 1.35; SuperArray Biosciences). Each experiment was performed in triplicate to ensure reproducibility of the results. Those genes with concordant twofold changes in expression levels by the two distinct PPARγ ligands, when compared with vehicle treated cells, were selected for examination by real time PCR.

Real time PCR was performed to confirm the results of the cDNA expression array experiments. The reaction was carried out in an ABI PRISM 7700 sequence detection system using SYBR green PCR reagents (Molecular Probes, Eugene, Oregon, USA). Detection of PCR products by agarose gel electrophoresis confirmed the homogeneity of the DNA products. Template free water was used as a negative control. Expression levels of target genes in PPARγ ligand treated cells were standardised against β-actin mRNA levels. Relative quantitation was expressed as fold induction or repression of the genes of interest compared with controls.

In vivo tumour growth assay
Eight week old male athymic nude mice (BALB/c nu/nu) were obtained from the Laboratory Animal Service Center of the Chinese University of Hong Kong. All mice were kept under specific pathogen free conditions using a laminar airflow rack and had free access to sterilised food and autoclaved water. All experiments were performed under license from the government of the Hong Kong SAR and endorsed by the Animal Experimentation Ethics Committee of the Chinese University of Hong Kong.

A MKN45 cell suspension (5 x 10⁴ cells suspended in 0.1 ml of media) was injected subcutaneously into the flank of each mouse on day 0. Animals were randomly divided into two groups (eight animals per group): one group received rosiglitazone (6 mg/kg/day) and the other received vehicle only. All study medications were given by oral gavage five times per week starting from day 1 of injection through to the end of week 7. Tumour size was measured weekly with callipers in three dimensions: that is, length (L), width (W), and height (H). Tumour volume was calculated using the formula \( (\pi/6) \times L \times W \times H \). At the end of week 7, all mice were sacrificed by cervical dislocation. Tumours were dissected from the body and fixed in buffered formalin for subsequent histological examination. Paraffin embedded tissues were deparaffinized and stained with haematoxylin-eosin for histological examination. Proliferation of tumour cells was determined by Ki-67 immunostaining (Zymed, California, USA), as described previously. The proliferation index was expressed as the percentage of Ki-67 positive nuclei to total nuclei counted.

Statistics
Data are expressed as mean (SD). ANOVA with Bonferroni’s multiple comparison correction was performed when more than two groups were compared. Differences in tumour volume between animals fed with rosiglitazone and vehicle controls were determined by the Student’s t test. A p value of <0.05 was considered statistically significant.

RESULTS
PPARγ and COX-2 expression
Both MKN28 and MKN45 cell lines expressed PPARγ and COX-2 by ordinary RT-PCR (fig 1). COX-2 expression was higher in MKN45 than in MKN28 cell lines whereas PPARγ expression levels appeared to be comparable. However, by quantitative PCR, expression levels of PPARγ and COX-2 were, respectively, 10-fold and 18-fold higher in MKN45 than in MKN28 cell lines.

In contrast, the growth inhibitory effect of PPARγ ligands was less prominent in MKN28 cells (fig 2C, 2D). There was approximately 1.5-fold and 4.6-fold increase in DNA fragmentation after treatment with PGJ2 (10 μM) and ciglitazone (20 μM), respectively. A dose dependent reduction in proliferation with 10 μM PGJ2 (p<0.01 v control) but treatment with ciglitazone was not associated with any growth suppressive effect. Notably, addition of 10% FBS in the culture medium ameliorated the growth inhibitory effect of PGJ2 and ciglitazone. After addition of FBS, there was only approximately 40% growth inhibition with PGJ2 (10 μM) and 20% inhibition with ciglitazone (20 μM). In contrast, treatment with PGJ2, the PPARγ agonist, has no effect on proliferation of MKN28 and MKN45 cells, either in the presence or absence of FBS.

Induction of apoptosis by PPARγ ligands
After treatment with PGJ2 or ciglitazone in MKN45 cells, there was an increase in the amount of fragmented DNA in the cytoplasm, as detected by ELISA (fig 3). There was approximately 1.5-fold and 4.6-fold increase in DNA fragmentation after 72 hours of treatment with PGJ2 (10 μM) and ciglitazone (20 μM), respectively.

PPARγ ligand activation and COX-2 expression
Treatment with PGJ2 1 μM and 10 μM for 72 hours resulted in 54% (p<0.05 v control) and 66% (p<0.01 v control) reduction in COX-2 protein expression (fig 4A). Moreover, there was a 46% (p<0.05 v control) and 67% (p<0.01 v control) reduction in COX-2 protein expression after treatment with ciglitazone 3 μM and 10 μM, respectively. A dose dependent reduction in COX-2 mRNA level after treatment with PGJ2 was also shown by real time quantitative PCR (p=0.041; fig 4B). However, treatment with PPARγ ligands
had no effect on COX-2 expression on MKN28 cells (data not shown).

Although the use of NS398 (100 µM) or PPARγ ligands (10 µM PGJ2 and 20 µM ciglitazone) alone suppressed growth of MKN45 cells, the combined use of these two agents did not have any synergistic effect on growth suppression (fig 5).

**Gene expression profiles**

Screening by cDNA microarray showed that there was concordant upregulation of bad and downregulation of bcl-2 and bcl-xL in MKN45 cells after treatment with the two PPARγ ligands (ciglitazone and PGJ2). In addition, there was repression of cyclin E1 and activation of p53 in MKN45 cells after treatment. Quantitative PCR confirmed that treatment with PPARγ ligands was associated with upregulation of bad (p<0.01 v control) and p53 (p<0.01 v control) whereas expression of bcl-xL (p<0.01 v control), bcl-2 (p>0.01 v control), and cyclin E1 (p<0.05 v control) was suppressed in treated cells (fig 6). In contrast, only the bad gene was induced by the two PPARγ ligands whereas expression of the other four genes remained unchanged in MKN28 cells. Furthermore, not all genes showed concordant alteration by the two PPARγ ligands. The following genes were only repressed by ciglitazone but not by PGJ2 in MKN45 cells: angiopoietin 2, epidermal growth factor, vascular endothelial growth factor receptor, and integrin alpha-1, alpha-4, and beta-3. On the other hand, PGJ2, but not ciglitazone, appeared to suppress tumour necrosis factor α and induce p16 expression in MKN45 cells.

**Effect of PPARγ ligand on tumour growth in vivo**

Subcutaneous tumour growth was first palpable four weeks after injection of MKN45 cells (fig 7A). At week 6, there was a significant difference in tumour volume between rosiglitazone treated mice and control mice (48.8 (54.9) mm³ v 166.3 (73.3) mm³; p = 0.019) (fig 7D). At week 7, there was mild shrinkage of tumour volume in rosiglitazone treated group whereas tumours continued to grow in the vehicle control group. Hence the difference between the two groups was more pronounced at week 7 (33.9 (33.7) mm³ v 226.2 (107.4) mm³; p = 0.001). Body weight in the two treatment

![Figure 2](http://gut.bmj.com/)  
**Figure 2**  
Effect of peroxisome proliferator activated receptor (PPAR) ligands on proliferation of MKN45 (A, B) and MKN28 (C, D) cells. Cell lines were treated with various doses of the PPARγ agonist (carbaprostacyclin (PGJ2) 1 and 10 µM) and PPARγ agonists (prostaglandin J2 (PGJ2) 1 and 10 µM or ciglitazone 5–50 µM (B, D)). Cell proliferation was determined by bromodeoxyuridine incorporation in serum free condition. The y axis indicates the percentage of proliferation compared with controls (100%). (A) Treatment with PGJ2 (10 µM) in serum free condition was associated with 80% growth suppression (p<0.05 v control) in MKN45 cells but there was negligible growth inhibitory effects with PGJ2 treatment. (B) Treatment with ciglitazone at doses >10 µM were associated with marked growth inhibition on MKN45 cells (p<0.01 v control). (C) Treatment with different doses of PGJ2 (1 and 10 µM) or low dose PGJ2 (1 µM) had no suppressive effect on MKN28 cells, except when PGJ2 10 µM was used (p<0.05 v control). (D) Treatment with different doses of ciglitazone (5–50 µM) was not associated with any growth inhibitory effects on MKN28 cells.

![Figure 3](http://gut.bmj.com/)  
**Figure 3**  
Induction of apoptosis by peroxisome proliferator activated receptor γ (PPARγ) ligands. Treatment with PPARγ ligands was associated with an increase in the amount of DNA fragmentation. (A) Treatment with prostaglandin J2 (PGJ2) at doses >5 µM was associated with an increase in DNA fragmentation (p<0.05 v control). (B) A similar increase in DNA fragmentation was observed with high dose ciglitazone treatment (20 µM, p<0.05 v control).
groups was comparable and none of the study animals died during the study period. Histological examination of the tumour confirmed the presence of cancer cells within the tumour and there was no apparent difference in the morphology of tumour cells between the two groups (fig 7B, 7C). The proliferation index of the tumour tended to be lower in animals treated with rosiglitazone than in controls (0.48 (0.07)\% vs 0.57 (0.09)\%; p = 0.16).

**DISCUSSION**

In this study we have demonstrated both the in vivo and in vitro growth suppressive effects of PPARγ ligands on gastric cancer cells. The in vitro antiproliferative effect of PPARγ ligands was dose dependent and was more prominent in MKN45 cells. Treatment with a PPARδ agonist, carbaprostacyclin, had no effect on the growth of gastric cells. This is in agreement with a recent report by Kojima et al which also failed to show any effect of PPARδ agonists on the growth of gastric cells. The presence of serum however ameliorated the growth suppressive effect of the two PPARγ ligands. This antagonistic effect of serum is attributed to the presence of polypeptide growth factors and other cell protective substances in serum.22 23

Despite the promising in vitro data, the in vivo antitumorigenic effect of PPARγ ligands is controversial.4 5 14 15 In this study, we demonstrated a significant reduction in the in vivo growth of MKN45 cells in nude mice after treatment with rosiglitazone. At a modest dose of rosiglitazone (6 mg/kg/day) there was a significant reduction in tumour volume compared with those treated with vehicle alone. While tumour development was noticed four weeks after implantation, there was a significant difference in tumour size from six weeks onwards (fig 7). Notably, the dose of rosiglitazone used in our study was much lower than in a recent in vivo study that examined the antiangiogenic effect of PPARγ ligands.11 Panigrahy et al showed that neovascularisation and tumour metastasis were suppressed by rosiglitazone 50–100 mg/kg/day. This discrepancy may be related to the differential antiproliferative responses of different tissue types to PPARγ ligands. In keeping with this speculation, some cancer cells such as bladder, breast, and thyroid may require higher doses of PPARγ ligands for growth inhibition in vivo.20 21 22 23

In MKN45 gastric cancer cells, the growth suppressive effect of high dose PGJ2 (10 \( \mu \)M) and ciglitazone (20 \( \mu \)M) was accompanied by apoptosis induction with a modest increase in DNA fragmentation. Additionally, we determined expression of COX-2 mRNA and protein expression after treatment with PPARγ ligands. There was approximately 50% reduction in COX-2 expression with PGJ2 (10 \( \mu \)M) or ciglitazone (10 \( \mu \)M) treatment. In keeping with this, downregulation of COX-2...
has also been demonstrated in colonic cells after treatment with ciglitzone.27 A recent study on a mammary cell line suggested that PPARγ ligands inhibit activator protein 1 mediated transcriptional activation of COX-2.28 Although the use of PPARγ ligands or a specific COX-2 inhibitor (NS398) alone inhibits gastric cancer cell growth, the combined use of these two agents had no additional or synergistic effect on growth. Intuitively, this finding may suggest that COX-2 inhibitors and PPARγ ligands act through a common pathway on growth suppression.

In addition to differences in COX-2 expression, there are other possible explanations to account for the differential responses of the two cell lines to PPARγ ligands. Firstly, MKN45 cells have higher PPARγ expression, as demonstrated by quantitative PCR. Secondly, recent data showed that mice with pre-existing damage to the Apc gene developed colon tumours in a manner insensitive to the status of PPARγ,29 suggesting that PPARγ suppress β-catenin levels and colon carcinogenesis only before damage to the APC/β-catenin pathway. In this regard, the MKN28 cell line, which has a mutation in the Apc gene,30 may have a reduced response to ciglitzone and PGJ2. Intriguingly, although PPARγ ligands have been shown to induce apoptosis in a number of gastric cell lines, activation of the PPARγ pathway attenuates the ability of Helicobacter pylori to induce nuclear factor κB mediated apoptosis in gastric epithelial cells.31 Hence activation of the PPARγ receptor may result in varying levels of cellular turnover depending on the intrinsic properties of the cells.

By screening the expression profiles of cancer related genes after treatment with PPARγ ligands, we demonstrated upregulation of bad and p53 and downregulation of bcl-xl, bcl-2, and cyclin E1 in MKN45 cells. Both bcl-2 and bcl-xl have an antiapoptotic function by preventing cytochrome c release and hence caspase-9 activation. In line with this finding, there was induction of bad, a proapoptotic gene, by PPARγ ligands. A similar finding was demonstrated in breast cancer cells in which treatment with PPARγ ligands was associated with downregulation of bcl-2.32 Cyclin E1 is responsible for G1/S progression in mammalian cells and overexpression is implicated for neoplasia, including gastric cancer.33 To this end, downregulation of cyclin E1 by PPARγ ligands may offer another explanation for the growth inhibition in gastric cancer cells. Previous studies on other cell types have demonstrated that treatment with PPARγ ligands is associated with reduced cyclin D1 expression in MKN45 cells. In contrast, we demonstrated activation of p53 by PPARγ ligands in MKN45 cells with wild type p53.34 Similar activation of p53 was demonstrated in vascular smooth muscle cells after treatment with troglitazone.35 By comparing the gene expression profiles of the two gastric cell lines, we found that the bad gene was the only gene altered by the two PPARγ ligands in MKN28 cells. This finding may further explain the reduced growth inhibitory response of this cell line to PPARγ ligand activation. Interestingly, a recent report on colonic cells showed that

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Figure 6  Quantitative polymerase chain reaction (PCR) in the determination of expression levels of selected genes in MKN45 cells. Genes with altered expression, as detected by cDNA microarray, were selected for examination by quantitative PCR. mRNA expression levels of (A) bad, (B) bcl-xl, (C) bcl-2, (D) cyclin E1, and (E) p53 after treatment with prostaglandin J2 (PGJ2, 10 μM) or ciglitzone (Cig, 20 μM) were determined. Results are shown as mean (SD) after standardisation to β-actin in three separate experiments. *p<0.05, **p<0.01 versus controls.
treatment with PPARγ ligands was associated with down-regulation of c-myc and upregulation of c-jun and gadd153 in colonic cells. Thus the investigators speculated that PPARγ ligands compensate for dysregulated c-myc expression caused by a mutated Apc gene in colon cancer cells. However, our array experiments failed to show alterations in these loci in MKN45 gastric cells after treatment with PPARγ ligands, which may be related to the presence of the wild-type Apc gene in this cell line.

In conclusion, PPARγ ligands inhibit the growth of gastric cancer both in vitro and in vivo. The antineoplastic effects are mediated by multiple pathways, including suppression of COX-2, inhibition of the antiapoptotic bcl-2/bcl-xl family and cyclin E1, and activation of p53. While we and others have previously demonstrated the ubiquitous expression of PPARγ in primary gastric cancer tissues, the role of PPARγ ligands in the chemoprevention and therapy of human gastric cancer warrants further evaluation.

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Figure 7  In vivo growth of MKN45 cells in nude mice. (A) Representative tumour growth in nude mice fed with vehicle control (left) and rosiglitazone (right). (B, C) Haematoxylin-eosin sections of MKN45 tumours from the control group (B) and rosiglitazone group (C), with yellow arrows showing sheets of gastric cancer cells. There was no obvious difference in the degree of tumour differentiation and necrosis between the two groups. (D) The tumour growth rate was significantly lower in the rosiglitazone treated group compared with controls at week 6 (*p = 0.02) and week 7 (**p = 0.0014). The solid line represents control mice whereas the broken line represents rosiglitazone treated mice.

 GI SNAPSHOT

Answer

From question on page 330

These histological findings are characteristic of Menetrier’s disease; a rare and acquired disorder of the stomach. Criteria for diagnosis include giant gastric folds, histological features of marked foveolar hyperplasia, atrophy of glands, and increase in mucosal thickness. There is excess mucus production, decreased acid secretion, and hypoproteinemia. The differential diagnosis includes Zollinger-Ellison syndrome, Helicobacter pylori infection, gastric lymphoma, cytomegalovirus infection, gastric cancer, and eosinophilic gastritis. The pathogenesis involves elevated levels of transforming growth factor α (TGF-α) in gastric mucous cells, which exerts its effect by binding to the epidermal growth factor receptor. TGF-α increases gastric mucous production, cell renewal, and inhibits acid secretion. Hypoproteinemia occurs due to selective loss of serum proteins across the gastric mucosa. The patient underwent total gastrectomy due to ongoing blood loss. That was difficult to manage, with iron and blood supplementation without complications. The patient continues to do well one year after surgery with no anaemia, gastrointestinal bleeding, or abdominal pain.