Control of COX-2 Gene Expression through Peroxisome Proliferator-Activated Receptor γ in Human Cervical Cancer Cells

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ABSTRACT

Purpose: The peroxisome proliferator-activated receptor-γ (PPARγ), a ligand-dependent transcription factor belonging to the family of nuclear receptors, has been implicated in the control of cyclooxygenase (COX) 2 expression in some tissues, although the exact mechanism(s) of this activity has not been elucidated. In this study we explored the possible mechanism(s) of control of COX-2 gene expression through PPARγ signaling in human cervical cancer.

Experimental Design: Using primary human cervical tissues and the CaSki human cervical cancer cell line, we assayed for PPARγ and COX-2 mRNA expression by reverse transcription-PCR. Nuclear protein binding activities to three response elements located in the COX-2 promoter [nuclear factor κB (NFκB), cyclic AMP response element, and activator protein (AP)-2] were measured by gel mobility shift assays. We used transient transfection assays with COX-2 promoter reporter gene constructs to determine the regulatory sites in this promoter, which mediates PPARγ regulation of COX-2 activity.

Results: We showed, for the first time, that primary human cervical cancer tissues express PPARγ. Using CaSki cells, we demonstrated that COX-2 and PPARγ mRNA levels were inversely regulated by PPARγ ligands in that these compounds up-regulated PPARγ but down-regulated COX-2. In contrast, epidermal growth factor (EGF), a potent activator of COX-2, decreased PPARγ mRNA levels. This down-regulation of PPARγ mRNA by EGF was blocked in the presence of NS-398, a selective COX-2 inhibitor. PPARγ ligands suppressed the binding activities of AP-1 (binding to CRE) and NFκB but not AP-2. Transient transfection results indicated that EGF stimulated whereas PPARγ ligands inhibited COX-2 promoter (−327/+59) activity. This effect by PPARγ ligands on the COX-2 promoter was blocked when the CRE, but not the NFκB, binding site was mutagenized.

Conclusion: Cervical cancer cells express readily detectable levels of PPARγ. There is reciprocal negative regulation between COX-2 and PPARγ signaling in human cervical cancer cells. The ability of PPARγ ligands to inhibit COX-2 appears to be mediated predominantly through inhibition of AP-1 protein binding to the CRE site in the COX-2 promoter.

INTRODUCTION

It has been generally accepted that there are two isoforms of COX-2. COX-1 is expressed constitutively in most tissues and appears to be responsible for various physiological functions. COX-2, which is a key enzyme in prostaglandin synthesis, is an immediate, early response gene that is rapidly induced by phorbol esters, growth factors, cytokines, and oncogenes. COX-2 overexpression has been observed in many tumor types including colon, lung, breast, and esophagus. Recent reports have indicated that COX-2 is also overexpressed in human cervical cancer, the second leading cause of cancer deaths in women worldwide and a leading cause of mortality among women of reproductive age in developing countries. Mechanistic studies have suggested that expression of COX-2 in stage IB cervical cancer may down-regulate apoptotic processes and thus enhance tumor invasion and metastasis.

PPARγs are ligand-dependent transcription factors belonging to the steroid hormone nuclear receptor superfamily. The expression of PPARγ has been reported in several organs and tissues, such as liver, lung, skeletal muscle, and monocyte/macrophages, and at high levels in adipose tissue. Although PPARγ is expressed at low levels in normal colonic and breast ductal epithelium, it is increased significantly in both colon and breast carcinoma. The expression of this receptor in cervical tissue has heretofore not been reported.

PPARγ has been implicated in the regulation of critical aspects of development and homeostasis, including cell cycle control and tumor growth inhibition. The PGD2 metabolite 15-deoxy-12, 14 PGJ2 (PGJ2) has been identified as a potent natural ligand for PPARγ and can be produced by overexpression of COX-2. This fact suggests that PPARγ...
signaling may be related to COX-2 gene expression. To this end, there are several reports suggesting a reciprocal interaction between COX-2 expression and PPARγ signaling. Thus, induction of COX-2 by 15d-PGJ2 was reported in immortalized epithelial and colorectal cancer cells (16, 17), although 15d-PGJ2 suppressed COX-2 expression in fetal hepatocytes (18). The molecular mechanism(s) that underlie PPARγ regulation of COX-2 expression remains to be elucidated. In this study, we investigated whether PPARγ signaling was involved in COX-2 regulation in human cervical cancer. Our data showed that PPARγ ligands can down-regulate COX-2 gene expression and that this regulation is mediated predominately through inhibition of the function of the AP-1 nuclear transcription factor.

MATERIALS AND METHODS

Patient Samples. Cervical cancer samples were collected for RT-PCR analysis from patients with different histological types: squamous cell carcinoma (n = 7) and adenocarcinoma (n = 1). Histologically normal cervical tissue samples were obtained from patients undergoing gynecological surgery at Grady Hospital/Emory University School of Medicine (Atlanta, GA). Samples were stored at −80°C until analysis. Informed consent was obtained from each patient. This study was approved by the Committees on Human Rights in Research at Emory University.

Cell Culture and Chemicals. The CaSki human cervical cancer cell line obtained from American Type Culture Collection (Manassas, VA) was grown in RPMI 1640 (Cellgro, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum, HEPES buffer, 50 IU/ml penicillin/streptomycin, and 1 μg amphotericin (complete medium) as described previously (19). Murine 3T3-L1 cells obtained from American Type Culture Collection were grown in complete medium and were induced to differentiate into adipocytes by standard procedures (20). These “adipocyte-differentiated” 3T3-L1 cells were used for comparing PPARγ expression in adipocytes versus cervical cancer cells. 15d-PGJ2 and Cig were purchased from Alexis Biochemical (San Diego, CA). Recombinant human EGF purchased from Genentech (South San Francisco, CA). The selective COX-2 inhibitors synthesized by Genosys (The Woodlands, Texas) were: for PPARγ sense (5′-TCTCTCCGTAAATGGAGACC-3′), antisense (5′-CGATTATGAGACATCCCCAC-3′); for COX-2 sense (5′-CTGTATCCGCCCTGCTGTT-3′), antisense (5′-ACTTCGTTGATGTTGGCTGCCTT-3′); and for GAPDH sense (5′-CCATGGAGAACGCTGAGG-3′), antisense (5′-CAAAGTTGTCATGGATGCC-3′) as according to the published data (24, 25). The RT-PCR was carried out as described (24). The samples were first denatured at 95°C for 30 s, followed by 32 PCR cycles, each with temperature variations as follows: 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The last cycle was followed by an additional extension incubation of 7 min at 72°C. Analysis of amplicons was accomplished on 1% agarose gel containing 0.2 μg/μl ethidium bromide and visualized under UV transiluminator. The densitometric analysis of PCR products was performed by the computer software (Bio-Rad Quantity One), GS-700 Imaging Densitometer (Bio-Rad, Hercules, CA), and standardized by the GAPDH product. Ratio of PPARγ/GAPDH or COX-2/GAPDH density bands in control group was considered as 100%. Values of treatment group PPARγ/GAPDH or COX-2/GAPDH ratios are given as a percentage of controls. A 100-bp ladder (Life Technologies, Inc.) was used as a size standard.

For real-time PCR, the treatment and total RNA preparations were the same as those for the RT-PCR procedures described above. All of the PCR reactions using LightCycler-FastStart DNA Master SYBR Green I kit were performed in the Cepheid Smart-Cycler real-time PCR cycler (Sunnyvale, CA). The cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 5 s, and 72°C for 10 s. Primers for PPARγ and GAPDH were the same as above. Primers used for detection of PPARγ in mouse 3T3-L1 cells were sense (5′-CGACTGCTGTGTTGACACAGAGATGC-3′) and antisense (5′-CGAGATCTGCCCATGAGGGAGTTAG-3′). Quantitative analysis for determining threshold cycle for each sample was performed according to the vendor guidelines. Experiments were performed in triplicate for each data point. For all of the experiments, controls without templates were included.

EMSA. Nuclear protein extracts from CaSki cells were prepared for EMSA as described earlier (26). The protein content of the nuclear extract was determined using the bicinechonic acid protein assay kit (Sigma). EMSA experiments were performed using double-stranded oligonucleotides comprising the consensus sequences (italized) for NFκB (5′-AGTTGAGGGGACTTTCCACGC-3′), CRE (5′-AGAGATGCCTGAGTGACGAGACT-3′), AP-1 (5′-GCTTGTGACGAGCCTAG-3′), and AP-2 (5′-GAAGAAGTACGCGCCGCGCTGAGGC-3′). The binding site for CRE comprised the cyclic AMP-responsive element. The NFκB, CRE, and AP-2 oligonucleotides were end labeled with [γ-32P]-dATP using T4 polynucleotide kinase as recommended by the manufacturer. In the mutated NFκB oligonucleotide, the consensus motif was changed to GGrGACCTTCCC. For mutated CRE and AP-2 oligonucleotides, the consensus motifs were changed to TGACTtg and CGCttGCGC, respectively. Five μg nuclear pro-
teins from control and treated cells were incubated with 32P-labeled oligonucleotide probe under binding conditions [10 mM HEPES, Tris-HCL (pH 7.9), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 12% (v/v) glycerol, and 2 μg poly(deoxyinosinic-deoxy- cytidylic acid)] for 20 min at room temperature in a final volume of 20 μl. For cold competition, a 100-fold excess of the respective unlabeled consensus oligonucleotides was added with the probe. The same amount of mutated oligonucleotides added with the probe was used as another control. All of these were in the same binding conditions as described before. After binding, protein-DNA complexes were electrophoresed on a native 4.5% polyacrylamide gel at 120 V using 1 Tris-Glycine buffer [10 Tris-glycine: Tris-base 30.28 g, glycine 142.7 g, EDTA 3.92 g, and H2O added up to 1 liter (pH 8.5)]. Each gel was then dried and subjected to autoradiography at −80°C for up to 72 h. All of the vehicle controls were considered as 100%. Value of treatment groups was given as percentage of controls.

**Plasmids.** The COX-2 promoter constructs ligated to luciferase (−327/+59, KBM, CRM, and KBM+CRM) has been reported previously (27). Among those, KBM represents the −327/+59 COX-2 promoter construct in which the NFκB site (−223/−214) was mutagenized; CRM refers to the −327/+59 COX-2 promoter construct in which the CRE site (−59/−53) was mutagenized; KBM+CRM represent the −327/+59 COX-2 promoter construct in which the both NFκB and CRE sites were mutagenized. Synthetic Renilla Luciferase Report Vector (phRL-TK) was obtained from Promega.

**Transient Transfection Assays.** CaSki cells were seeded at a density of 4 × 105 cells/well in six-well dishes and grown to 50–60% confluence. For each well, 2 μg of plasmid DNA of the above constructs and 0.3 μg of the internal control plasmid pRL-TK (renilla luciferase gene) were cotransfected into the cells using 3 μl of FUGENE 6 lipofection reagent (Roche Molecular Biochemicals, Indianapolis, IN) as performed in our earlier work (28). After 24 h of incubation, cells were treated with PPARγ ligands (GW1929 and Cig), EGF, or solvent control for another 24 h. The preparation of cell extracts and measurement of luciferase activities were carried out using the Dual-Luciferase Reporter kit according to recommendations of the manufacturer (Promega). The assays for firefly luciferase activity and renilla luciferase activity were performed sequentially using one reaction tube in a luminometer with one injector. Changes in firefly luciferase activity were calculated and plotted after normalization with changes in renilla luciferase activity in the same sample. All of the vehicle controls were considered as 100%. Values of treatment group firefly luciferase:renilla luciferase ratio were given as percentage of controls.

**Statistical Analysis.** All of the experiments were repeated a minimum of three times. All of the gel shift assays, luciferase activity assays, and RT-PCR data were expressed as a mean ± SE. The data in some figures are from a representative experiment, which was qualitatively similar in the replicate experiments. Statistical significance was determined with Student’s t test (two-tailed) comparison between two groups of data set. Asterisks shown in the figures indicate significant differences of experimental groups in comparison with the corresponding control condition (P ≤ 0.05; see figure legends).

**RESULTS**

**Expression of COX-2 and PPARγ Genes in Human Primary Cervical Cancer Cells and Cell Lines.** Although recent reports have demonstrated overexpression of COX-2 activity in human cervical cancer cells, there are presently no reported studies showing the presence of PPARγ in this malignancy. As exemplified in Figs. 1 and 2, RT-PCR analysis indicated that PPARγ was readily detected in both human primary cervical cancer as well as the CaSki cervical cancer cell line. In addition, Fig. 1 shows relatively high expression of both COX-2 and PPARγ in malignancies, whereas COX-2 expression was low in nonmalignant samples. Similar results were obtained in a total of 8 cervical cancer and 13 normal tissue samples. To compare PPARγ mRNA levels in cervical cancer to that in adipose tissue, which are known to express very high levels of PPARγ (29), real-time RT-PCR was performed on CaSki cells and adipocyte-differentiated mouse 3T3-L1 cells (30). Results showed that the steady-state levels of PPARγ mRNA in adipocyte-differentiated 3T3-L1 was ~1000-fold greater than that found in CaSki. These findings represent the first report that PPARγ is expressed in either normal or malignant cells of the cervix.

**Regulation of COX-2 Expression and Its Link to PPARγ Signaling.** In previous dose-response studies by one of us (H. I.) as well as by others (31, 32), it was shown that significant modulation of COX-2 levels in a variety of cell systems can be consistently induced by PPARγ ligands at concentrations of 10–50 μM. Preliminary experiments demonstrated that modulation of COX-2 by PPARγ ligands in CaSki cervical cancer cells showed a similar dose dependency (data not shown). Therefore, 20 μM of PPARγ ligands were used in many of the following experiments. Fig. 2A shows that treatment of CaSki cells with 15d-PGJ2 and GW1929 (20 μM each) for 5 h significantly induced PPARγ mRNA levels, whereas EGF (20 ng/ml) remarkably inhibited the expression of PPARγ as compared with the controls. In contrast, the PPARγ ligands decreased COX-2 mRNA levels, whereas EGF, a known activator of COX-2 (4), enhanced COX-2 expression (Fig. 2). Next we determined whether COX-2 signaling was directly involved in the inhibition of PPARγ expression caused by EGF treatment. As shown in Fig. 2B, this was indeed the case; down-regulation of PPARγ mRNA levels induced by EGF was blocked in the presence of NS-398, a selective COX-2 inhibitor, at a concen-
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GW1929 and 15d-PGJ2 at a concentration of 20 μM. In other experiments, PPARγ ligands GW1929 and 15d-PGJ2 at a concentration of 20 μM blocked EGF induction of COX-2 mRNA and suppression of PPARγ mRNA (Fig. 3), additionally suggesting that suppression of COX-2 could be mediated by PPARγ activation. Taken together, these findings suggest a reciprocal interaction between PPARγ and COX-2 regulation in cervical cancer cells.

AP-1 and NFκB Binding Activities Were Inhibited by PPARγ Ligands. To additionally elucidate the mechanisms responsible for the changes in amounts of COX-2 mRNA, EMSA was performed to identify the nuclear transcription factors that mediated the regulation of COX-2 mRNA by PPARγ ligands and EGF. The human COX-2 promoter region contains multiple transcription factor binding sites including a cyclic AMP response element (CRE), and binding motifs for NFκB and nuclear factor interleukin-6 (27, 33). Reports showed that AP-1 protein could bind to the CRE site located in the COX-2 promoter region (31). In agreement with other studies (34–36), CaSki cells treated with EGF (20 ng/ml) for 24 h slightly increased AP-1 and NFκB binding activity as compared with solvent controls. There was no binding activity when the CRE or NFκB sites were mutagenized (Figs. 4 and 5). In contrast, 15d-PGJ2 and GW1929 (20 μM each) inhibited AP-1 and NFκB binding as compared with controls. Inhibitory effects on AP-1 by these ligands were blocked in the presence of the PPARγ antagonist GW9662 (20 μM), whereas GW9662 alone had no effect on AP-1 binding activity (Fig. 6). These findings additionally indicated that the inhibitory effects of 15d-PGJ2 and GW1929 were mediated through the PPARγ signaling pathway. Additional experiments were performed to investigate the specificity of these PPARγ ligands on AP-1 and NFκB binding by assessing their effects on AP-2 binding activity. An AP-2 binding motif is also known to be located in the COX-2 promoter (37). Fig. 7 shows that 15d-PGJ2 and GW1929 had little effect on AP-2 binding. As a result of competition assays, specific bands for AP-1 (Fig. 6), NFκB (Fig. 5), and AP-2 (Fig. 7) were attenuated by a 100-fold molar excess of unlabeled oligonucleotides. These results confirmed that the nuclear protein binding to CRE is AP-1 and that the PPARγ ligands (15d-PGJ2 and GW1929) specifically inhibited the binding levels of both AP-1 and NFκB transcription factor complexes in human cervical cancer cells.

The CRE Binding Site in the COX-2 Promoter Plays a Major Role in the Effects of PPARγ Ligands. To define the regions of the COX-2 promoter that responded to EGF and PPARγ ligands, transient transfections were performed with human COX-2 promoter constructs. As shown in Fig. 8, EGF (20 ng/ml) treatment caused 4–5-fold increase in COX-2 promoter (−327/+59) activity, whereas the activity was suppressed
to 35–40% of control in the presence of GW1929 or Cig. To additionally test for specific elements that are responsible for mediating the effect of EGF and PPARγ ligands, transient transfections were performed using COX-2 promoter constructs in which the CRE, NFκB, or both binding sites were mutagenized. As shown in Fig. 9, mutagenizing the NFκB site (KBM construct) caused some decrease in responsiveness to EGF, but no significant effect on GW1929 or Cig inhibitory responses. However, mutagenizing the CRE site (CRM) caused a marked inhibition of responses to GW1929 and Cig, as well as to induction by EGF. Mutations of both the CRE and NFκB binding sites almost completely blocked the EGF-dependent promoter activation and totally blocked the PPARγ ligand-suppressive effect on COX-2 promoter activity.

Fig. 4 Regulation of AP-1 binding activity in cervical cancer cells. Nuclear extracts were prepared from CaSki cells treated for 24 h with the compounds as indicated: solvent control (Con), 20 ng/ml EGF, 20 μM 15d-PGJ2 or GW1929, or 20 μM 15d-PGJ2 + 20 ng/ml EGF. Nuclear extracts were subjected to EMSA using a CRE consensus site radiolabeled probe and protein-DNA complexes were visualized by autoradiography (A). NIH 3T3 cells were used as a positive control (3T3); the CRE probe was mutagenized to show specificity of binding (Mut); FP indicates free probe. The bar graph (B) represents the mean of relative AP-1 binding as quantified by densitometry of at least three independent experiments for each treatment condition; bars, ±SD. * indicates significant difference as compared with the vehicle control. ** indicates significance of combination treatment as compared with single treatment values.

Fig. 5 Regulation of NFκB binding activity in cervical cancer cells. Nuclear extracts were prepared from CaSki cells treated for 24 h with the indicated compounds as described in Fig. 4 with the additional condition of 20 μM GW1929 + 20 ng/ml EGF. The extracts were then subjected to EMSA using a NFκB consensus site radiolabeled probe and protein-DNA complexes were visualized by autoradiography (A). A 100-fold molar excess of unlabeled (Cold) NFκB oligonucleotide was incubated with the radiolabeled probe/extract mixture for competition. The NFκB probe was mutagenized to show specificity of binding (Mut); FP indicates free probe; NS indicates a nonspecific band. The bar graph (B) represents the mean of relative NFκB binding as quantified by densitometry of at least three independent experiments for each treatment condition; bars, ±SD. * indicates significant difference as compared with the vehicle control. ** indicates significance of combination treatments as compared with the corresponding single treatment values.
DISCUSSION

In this study, we demonstrated that COX-2 and PPARγ genes are expressed in both primary human cervical cancer tissues and the cervical cancer cell line CaSki. The expression of PPARγ in cells derived from the cervix has heretofore not been reported. Our results demonstrated reciprocal negative regulation between PPARγ and COX-2 gene expression, because up-regulation of COX-2 was coincident with down-regulation of PPARγ, and vice versa. The ability of PPARγ ligands to down-regulate COX-2 has also been reported in breast cancer cells and macrophages (31, 32), but was shown to up-regulate COX-2 expression in monocytes (38), synovial fibroblasts (39), and some colon cancer cells (17, 40). Taken together, these divergent results indicate that the qualitative nature of the interaction(s) between PPARγ and COX-2 (negative or positive) is tissue-specific and extrapolation of findings to different cell types cannot be made.

In light of our demonstration that PPARγ ligands negatively regulate COX-2 in cervical cancer cells, the strong expression of both PPARγ and COX-2 in primary cervical cancer
is surprising and suggests aberrant interaction between these pathways in malignant versus normal tissue. However, it should be remembered that down-regulation of COX-2 by PPARγ was dependent on activation with PPARγ ligands, and it is not known whether the availability of endogenous ligands (e.g. certain polyunsaturated fatty acids as well as 15d-PGJ2) is different between normal and cancerous tissue. In addition, the fact that the activity of PPARγ is also dependent on its phosphorylation status (41) could provide another potential mechanism for the aberrant functioning of this receptor. Similar conflicting observations have also been reported in the case of colon cancer; that is high tissue expression of both PPARγ and COX-2, in the face of their inverse regulation by PPARγ and COX-2 activators (42). The cause(s) for the simultaneous high expression of PPARγ and COX-2 in colon cancer, as in cervical cancer, remains unknown.

In experiments to shed light on the mechanisms(s) responsible for the PPARγ-induced inhibition of COX-2 mRNA, electrophoretic mobility shift assays were performed to identify the binding activities in CaSkis cells of the nuclear transcription factors AP-1 and NFκB. The human COX-2 promoter contains multiple transcription factor binding sites including NFκB, CRE, and NF-IL6. Recent reports demonstrated that AP-1 nuclear protein can bind to the CRE site located in the COX-2 promoter region in human mammary cells (31). Our results showing that CRE binding activity was attenuated in the presence of 100-fold excess of unlabeled AP-1 oligonucleotides demonstrated that this phenomenon is also true in cervical cancer cells. We showed that PPARγ ligand treatment of CaSki cervical cancer cells reduced the binding activities of both AP-1 and NFκB nuclear proteins. On the other hand, PPARγ ligands had little effect on AP-2, another transcription factor binding motif located in the COX-2 promoter, indicating that PPARγ signaling affects only certain transcription factors involved in COX-2 regulation. The blocking effect on AP-1 binding was inhibited in the presence of GW9662, a specific PPARγ antagonist, confirming that PPARγ signaling was involved in this activity. To determine whether these effects were responsible for PPARγ-mediated inhibition of COX-2, transient transfection experiments were performed with a series of COX-2 promoter reporter constructs in which the binding sites for NFκB and/or AP-1 nuclear protein were mutagenized. The results demonstrated that down-regulation of COX-2 by PPARγ ligands was predominantly mediated by antagonizing the transactivating activity of AP-1 nuclear proteins on the COX-2 promoter. This finding is consistent with that found in normal and malignant human mammary cells where this AP-1 binding to CRE site was shown to play a major role in mediating PPARγ ligand regulation of COX-2 expression (31). In contrast, it was found that modulation of COX-2 by PPARγ ligands in macrophages was largely mediated by NFκB (32). It is clear that regulation of COX-2 through PPARγ signaling is cell-type specific and may also be dependent on developmental processes.

The present data and previous findings have demonstrated that alteration of COX-2 levels can be mediated by a diverse group of seemingly unrelated natural, dietary, and synthetic compounds that have been shown to bind to and activate PPARγ (43–45). These include long chain PUFA such as that found in fish oil (e.g. ω-3 PUFA), aromatic small chain fatty acids (e.g. phenylacetate), various eicosanoids (e.g. 15d-PGJ2), lipid hydroperoxides (e.g. 9(s)-HODE), oxidatively modified lipoproteins (e.g. oxidized low-density lipoprotein), linoleic acid, thiazolidinediones (e.g. rosiglitazone), and a variety of recently synthesized compounds (e.g. GW1929). Studies in our laboratory have used a number of these agents in showing that 15d-PGJ2, GW1929, fish oil, and linoleic acid increased PPARγ while decreasing COX-2 mRNA levels. No differences have been seen between oleic acid, which is not a PPARγ ligand, and vehicle-treated control groups.3 Because routine screening and diagnostic procedures for cervical cancer have become so well developed, this disease may represent the ideal cancer for chemopreventive intervention by dietary means or long-term pharmaceutical administration. As such, routine screening by Pap smear can detect dysplasia long before micro or frank invasive cancer develops. Thus, it is conceivable that dietary modification that includes high PPARγ ligand-containing foods might be able to prevent certain dysplastic progressions and cervical cancer occurrence. This hypothesis is supported by reports showing that populations having frequent intake of foods rich in ω-3 and ω-6 PUFA, such as found in boiled or broiled fish, show decreased risk of cervical cancer (46, 47).

In conclusion, our study indicates that there is cross-regulation between COX-2 and PPARγ gene expression in human cervical cancer cells. The ability of PPARγ ligands to inhibit COX-2 appears to be mediated predominantly through inhibi-

Fig. 9 Inhibition of COX-2 promoter activity by PPARγ ligands is mediated by the CRE binding site. CaSki cells were transfected with the −327/+59 COX-2 promoter construct or the mutant promoter constructs as indicated and immediately treated for 24 h with vehicle control (Control), 20 ng/ml EGF, 20 μM GW1929, or 30 μM Cig as designated in the figure key. After normalizing the results using an internal control plasmid as described in “Materials and Methods,” the vehicle control value for each reporter construct was set as 100% activity. The bars represent the mean ± SD of at least four independent experiments for each condition. * indicates significant inhibition of activity as compared with vehicle controls. The CRM and CRM+KMB mutants showed no significant inhibition of activity after treatment of cells with the PPARγ activators.

3 Unpublished observations.
tion of AP-1 protein binding to the CRE binding site in the COX-2 promoter. Because COX-2 can act as a promoter of several cancers, the ability of PPARγ activators to inhibit COX-2 expression in human cervical cancer suggests that PPARγ signaling may be a useful target for therapeutic intervention and/or chemoprevention of this disease.

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