Peroxisome proliferator-activated receptor ligand MCC-555 suppresses intestinal polyps in \( Apc^{Min/+}\) mice via extracellular signal-regulated kinase and peroxisome proliferator-activated receptor-dependent pathways

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Abstract

A large body of studies has suggested that peroxisome proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) ligands, such as thiazolidinediones, are potent candidates for chemopreventive agents. MCC-555 is a PPAR\( \gamma \)/\( \alpha \) dual agonist and has been shown previously to induce apoptosis in \textit{vitro}; however, the molecular mechanisms by which MCC-555 affects antitumorigenesis \textit{in vivo} are poorly understood. In this study, we explored the antitumorigenic effects of MCC-555 both in cell culture and in \( Apc\)-deficient mice, an animal model for human familial adenomatous polyposis. MCC-555 increased \( MUC2 \) expression in colorectal and lung cancer cells, and treatment with the PPAR\( \gamma \) antagonist GW9662 revealed that \( MUC2 \) induction by MCC-555 was mediated in a PPAR\( \gamma \)-dependent manner. Moreover, MCC-555 increased transcriptional activity of human and mouse \( MUC2 \) promoters. Subsequently, treatment with MCC-555 (30 mg/kg/d) for 4 weeks reduced the number of small intestinal polyps to 54.8% of that in control mice. In agreement with \textit{in vitro} studies, enhanced \( Muc2 \) expression was observed in the small intestinal tumors of \( Min \) mice treated with MCC-555, suggesting that \( MUC2 \) expression may be associated at least in part with the antitumorigenic action of MCC-555. In addition, highly phosphorylated extracellular signal-regulated kinase (ERK) was found in the intestinal tumors of MCC-555-treated \( Min \) mice, and inhibition of the ERK pathway by a specific inhibitor markedly suppressed MCC-555-induced \( Muc2 \) expression \textit{in vitro}. Overall, these results indicate that MCC-555 has a potent tumor suppressor activity in intestinal tumorigenesis, likely involving \( MUC2 \) up-regulation by ERK and PPAR\( \gamma \) pathways. [Mol Cancer Ther 2008;7(9):2779–87]

Introduction

Thiazolidinediones, synthetic peroxisome proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) ligands, are a novel class of antidiabetic drugs for patients with type 2 diabetes, and two of these, rosiglitazone and pioglitazone, are currently available for clinical use (1). In addition, thiazolidinediones have recently been found to have antitumorigenic activity in a wide variety of cancer cells. As a transcription factor, PPAR\( \gamma \) targets genes associated with cell cycle, differentiation, and apoptosis (2), implying that PPAR\( \gamma \) ligands can be potent candidates for cancer prevention and/or therapy. Some of the most extensive studies have been done in the colon, where PPAR\( \gamma \) is highly expressed in both adenocarcinomas and normal colonic mucosa (3, 4). For example, PPAR\( \gamma \) ligands alter the expression of apoptotic and cell proliferation genes, thereby enhancing their antitumorigenic activity through a PPAR\( \gamma \)-dependent mechanism in colorectal cancer cells (2, 5). Somatic PPAR\( \gamma \) mutations were also found in sporadic colon cancers, and these mutations cause deletion of the entire ligand-binding domain and loss of transactivation ability, resulting in incomplete function of the protein (6). In addition, several reports showed new PPAR\( \gamma \)-dependent and PPAR\( \gamma \)-independent target genes of thiazolidinediones, resulting in modulation of cell proliferation and apoptosis in cancer cells (7–11). For example, the thiazolidinedione troglitazone was found not only to induce cell death in colon cancer cells (12, 13) but also to reduce the clonogenic capacity of all human colorectal cancer cells tested (3). Subsequently, the cDNA microarray analysis from colon cancer cells treated by PPAR\( \gamma \) agonists identified many target genes linked to the cell growth regulatory pathway (14, 15).

Several animal models for human colorectal cancer have been used to study whether thiazolidinediones possess...
these antitumorigenic activities in vivo. APC mutations, occurring early in the transformation process, are found in the majority of sporadic colorectal tumors as well as in familial adenomatous polyposis. Several promising chemopreventive agents, such as nonsteroidal anti-inflammatory drugs, have been reported to strongly suppress tumor formation or growth in the small intestine of Min mice (16, 17). In fact, treatment of Apc<sup>16309</sup> mice with pioglitazone (100 and 200 ppm) for 6 weeks significantly reduced the total number of intestinal polyps to 67% of control (18). Results from another animal model, using the colonic carcinogen azoxymethane, support the antitumorigenic activity of troglitazone and pioglitazone with significant suppression of azoxymethane-induced aberrant crypt foci (precursor lesions for colon carcinoma) formation (19). Although most studies indicate that thiazolidinediones suppress tumors in animal models, troglitazone has also been reported to enhance poly formation in the intestinal tract of Min mice (20). In addition, it has been recently revealed that troglitazone is hepatotoxic (21). This variety of actions of thiazolidinediones might be due to a multitarget property of PPAR<sub>γ</sub> ligands that remains to be elucidated. Therefore, better thiazolidinedione compounds are needed to achieve antitumorigenic activity with less liver toxicity.

Mucus in the gastrointestinal tract plays an important role as a physiologic barrier between the intestinal contents and underlying epithelial cells. Alteration of the expression of mucins, the major glycoprotein constituents in mucus, is a common feature of colonic neoplasia (22). Mucin 2 (MUC2), secreted by goblet cells of the small and large intestines, is the major structural component of the mucus gel. Levels of MUC2 mRNA expression are often decreased in colon cancer, although that expression depends on the type of colon cancer and its progression (23–25). Furthermore, Muc2-deficient mice developed adenomas in the small intestine, along with increased proliferation, decreased apoptosis, and increased migration of intestinal adenocarcinoma cells, suggesting MUC2 is linked to suppression of colorectal cancer (26).

In this study, the novel synthetic PPAR ligand MCC-555 was investigated to determine the effect on MUC2 expression in vitro and in vivo. MCC-555, a novel thiazolidinedione (also known as netoglitazone), was found to have a great effect on decreasing blood glucose levels in animal models of type 2 diabetes and to possess characteristic binding to PPARs (27). We reported previously that MCC-555 induces apoptosis in human colorectal cancer cells (28). In this study, we showed that MCC-555 increased MUC2 expression and suppressed intestinal polyposis in Min mice. In addition, we showed a possible mechanism of MUC2 up-regulation via the extracellular signal-regulated kinase (ERK) pathway. This is the first report suggesting that MUC2 is a novel PPAR<sub>γ</sub> target gene and that its expression plays a role in colorectal tumorigenesis.

**Materials and Methods**

**Cell Lines and Reagents**

Human colorectal cancer SW480 cells, mouse rectal cancer CMT-93 cells, and human lung cancer NCI-H292 cells were purchased from The American Type Culture Collection. MCC-555 (Fig. 1A) was obtained from Mitsubishi Pharma. Ciglitazone, rosiglitazone, prostaglandin...
15-deoxy-Δ12,14-prostaglandin J2, and GW9662 were purchased from Cayman Chemical. Troglitazone was obtained from Calbiochem. Anti-MUC2 and anti-actin antibodies were purchased from Santa Cruz Biotechnology, whereas anti-phospho-ERK1/2 (Thr202/Tyr204) and anti-ERK1/2 mitogen-activated protein kinases were obtained from Cell Signaling Technology.

RNA Purification and Reverse Transcription-PCR

Normal and tumor tissues isolated from the small and large intestine and liver tissues were kept in RNA later solution (Ambion) and stored at −80 °C. Cells were treated with different PPARγ ligands at the indicated doses and time points. Total RNA was extracted from these tissues and cells using Perfect RNA Eukaryotic Mini (Eppendorf) or TRIzol (Invitrogen), and cDNA was synthesized from 1 μg total RNA using an iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer’s protocol. PCR was done with specific primers: MUC2 (sense 5′-GACCTCCAGCACGTTTTATCAACAGGAAGAACTTT-3′ and antisense 5′-GCCAGCGCAATTGACAGCTTACT-3′), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sense 5′-GACCCAGCGTACTGGCATC-3′ and antisense 5′-TCCACCACTTGTGGTGCTG-3′), β2-microglobulin (sense 5′-CTGGCCGCTACTCTTTTCTGG-3′ and antisense 5′-GCTTACATGTCTCAGG-3′), and 2-microglobulin (sense 5′-GACCACAGTCCATGCAGGAAGA-3′ and antisense 5′-TGGGATGAAGAGGAGACTGAA-3′).

Immunohistochemistry

Small intestine and colon tissues were formalin-fixed, embedded in paraffin, and sectioned at 4 μm thickness. Tissue sections were then heated, deparaffinized in xylene, rehydrated in graded alcohol to PBS, and pretreated with 10 mmol/L citrate buffer (pH 6.0) for 10 min at just below boiling. Endogenous peroxidase activity was quenched by incubation in 3% H2O2 in PBS for 15 min at room temperature, and tissues were incubated with protein block (Biogenex USA) for 30 min at room temperature. Slides were then incubated for 1 h with anti-MUC2 antibody (1:200) followed by biotinylated anti-rabbit IgG (30 min at room temperature) and streptavidin/biotin-horseradish peroxidase complex (20 min at room temperature), which was visualized by 3,3′-diaminobenzidine tetrahydrochloride (0.7 g/L; Biogenex) for 10 min. Slides were lightly counterstained with Mayer’s hematoxylin.

Animals and Experimental Design

All animal research procedures were approved by the University of Tennessee Animal Care and Use Committee and were in accordance with NIH guidelines. C57BL/6j ApcMin/+ mice (The Jackson Laboratory) were randomly assigned to their respective experimental groups (n = 7 per each group). Min mice were maintained at 22 ± 2 °C on a 12 h light/dark cycle and with free access to standard rodent chow and water. MCC-555 was suspended in 1.5% carboxymethylcellulose with 0.2% Tween 20. At age 10 weeks, the experimental group (3 males and 4 females) received the MCC-555 suspension (30 mg/kg/d, 5 days/wk) for 4 weeks by gavage. The control group (4 males and 3 females) was gavaged with the suspending vehicle solution alone. Twenty-four hours after final treatment, the mice were euthanized, and the intestinal tract was isolated and washed with PBS. Tumor numbers and sizes in the small intestine and colon were assessed with a stereoscopic microscope as described previously (32).

Statistical Analysis

Statistical analyses were done with the Mann-Whitney’s U test or Student’s t test. Results were considered statistically significant at P < 0.05.

Results

Enhanced MUC2 mRNA Expression by PPARγ Ligands in Colorectal Cancer Cells

We and others have reported that PPARγ ligands display antitumorigenic activity in colorectal cancer (7, 28, 33), and MUC2 is known to have a tumor suppressor function in colorectal cancer (26). Hence, we examined whether PPARγ ligands increase MUC2 expression in SW480 human colorectal cancer cells. As shown in Fig. 1B, all the tested PPARγ ligands increased MUC2 expression 2- to 3-fold. On further examination of MCC-555, we found that MUC2 was increased in a dose-dependent manner (Fig. 1C). In addition, in CMT-93 mouse colorectal cancer cells, MCC-555 increased Muc2 expression in a dose-dependent manner (Fig. 1D). Because MUC2 and PPARγ ligands play an important role in lung tumorigenesis (34, 35), human
lup cancer cells NCI-H292 were treated with different PPARγ ligands, and MUC2 expression was measured. As shown in Fig. 2, MCC-555 and troglitazone also increased MUC2 expression in lung cancer cells in a dose-dependent manner.

**MUC2 Expression Is Mediated in a PPARγ-Dependent Manner**

Because PPARγ ligands can alter target gene expression in both PPARγ-dependent and PPARγ-independent manners (7, 11, 36), we examined whether PPARγ activation is responsible for MUC2 induction. As we reported previously, 5 μmol/L of the PPARγ antagonist GW9662 substantially inhibited PPARγ transactivation by MCC-555 (28). SW480 cells were treated with GW9662 and/or PPARγ ligands, and MUC2 expression was measured. As shown in Fig. 3A, pretreatment with GW9662 completely blocked induction of MUC2 expression by MCC-555 and troglitazone in SW480 cells, suggesting that MUC2 induction is probably mediated through a PPARγ-dependent pathway. Because MUC2 was increased in human and mouse cell lines in the presence of MCC-555, and PPARγ mediates its expression, we examined the transcriptional regulation of MUC2 by MCC-555. The reporter constructs containing human MUC2 promoter (phMUC2-2096/+27LUC) and mouse Muc2 promoter (pmMuc2-1001/+29LUC) were transfected into SW480, NCI-H292, and CMT-93 cells. After MCC-555 treatment for 24 h, luciferase activity was measured. As shown in Fig. 3B and C, MCC-555-treated samples showed increased luciferase activity, compared with vehicle-treated samples, indicating that MCC-555 increases MUC2 expression at the transcriptional level.

**Suppression of Intestinal Polyp Formation by MCC-555**

Because antitumorigenic effects of MCC-555 on intestinal cancer had not been determined previously, we treated Min mice with MCC-555 (30 mg/kg/d) for 4 weeks and evaluated tumor formation in the small intestine and colon. MCC-555 reduced the total numbers of small intestinal polyps to 54.8% of those in control mice (control 125.1 ± 22.6 versus MCC-555 68.6 ± 9.0). The size distribution of intestinal polyps in control and MCC-555-treated groups is shown in Fig. 4A. Significant reductions of polyp numbers by MCC-555 were observed in polyps measuring 1.0 to 1.5 and 1.5 to 2.0 mm in diameter. Tumor load analysis (polyp number × polyp size) shows that MCC-555 significantly suppressed intestinal polyp formation (Fig. 4B). We also examined polyps in the colon and found no statistical significance in polyp number (control 2.3 ± 0.6 versus MCC-555 1.4 ± 0.5). Because MCC-555 dramatically reduced the number of small intestinal polyps in Min mice and induced MUC2 expression in human and mouse cancer cells (Fig. 1), the effects of MCC-555 on intestinal Muc2 expression were examined in vivo. The histology of both small and large intestines was typical of Min adenomas, and there was no evidence of deep invasion (Fig. 4C). To examine the distribution of Muc2, immunostaining was done using small intestine and colonic tissues from Min mice containing tumors. As shown in Fig. 4C, Muc2 was positively stained in goblet cells in the small intestine and colon as reported previously (26). Interestingly, Muc2 was highly expressed in normal tissue compared with adjacent tumor tissue, supporting the previous report that MUC2 is a tumor suppressor protein.

**Increased Muc2 Expression in MCC-555-Treated Min Mice**

To investigate whether MCC-555 increases known PPARγ (Glut2, ap2, and Lpl) and/or PPARα target genes (l-Fabp and Cyp4a10; refs. 37–39), the expression of Glut2, l-Fabp, ap2, Cyp4a10, or Lpl in liver tissue was examined by reverse transcription-PCR (RT-PCR). Treatment with MCC-555 increased Glut2 and Cyp4a10 mRNA expression, whereas it did not increase other PPAR target genes, l-Fabp, Lpl, and ap2 (Fig. 5A). The RT-PCR analysis showed that the level of Muc2 expression in the normal intestine was higher than that in the corresponding tumor tissue from control mice (mouse numbers 6, 7, and 5 in Fig. 5B), consistent with the Muc2 immunohistochemical staining shown in Fig. 4C. Furthermore, Muc2 mRNA expression was significantly enhanced in MCC-555-treated mouse tumors compared with tumors from control mice (mouse numbers 11–14 in Fig. 5B). These results indicate that MUC2 expression induced by MCC-555 may play an important role in the tumors of intestinal tract.

**Figure 2.** Increased expression of MUC2 in response to PPARγ ligands in NCI-H292 human lung cancer cells. **A**, NCI-H292 cells treated with different doses of MCC-555. **B**, NCI-H292 cells treated with different doses of troglitazone. After 24 h of treatment, total RNAs were extracted, and semiquantitative RT-PCRs were done as described in Materials and Methods. GAPDH and β2-microglobulin served as the internal control. **Bottom**, fold inductions over the vehicle-treated sample.
phosphorylation status of ERK1/2 in the small intestine and colon polyps. Enhanced phosphorylation of ERK1/2 was observed in intestinal tumors of Min mice treated with MCC-555. On the other hand, expression of total ERK1/2 was not affected by MCC-555 treatment (Fig. 6A). To confirm the role of the ERK1/2 pathway in the regulation of MUC2 expression, SW480 human colorectal cancer cells were pretreated with ERK pathway inhibitors, PD98059 and U0126. As shown in Fig. 6B, U0126 completely inhibited MUC2 induction by MCC-555 in SW480 cells. Another ERK pathway inhibitor, PD98059, also markedly reduced it. Taken together with in vivo and in vitro data, these results show that MCC-555 increases ERK phosphorylation in vivo and in vitro, thereby enhancing MUC2 expression.

Discussion
Promising agents for cancer prevention have been shown to consistently suppress tumorigenesis or the rate of tumor growth in Min mice, which served as a model for familial adenomatous polyposis. Our results showed that treatment of Min mice with MCC-555 significantly suppressed polypl formation in the small intestine and slightly decreased colonic tumorigenesis (Fig. 4). Although a growing body of evidence from in vitro studies suggests that thiazolidinediones have an antiproliferative effect (41) and induce apoptosis (7, 12, 28) and differentiation (3, 5) in colorectal cancer cells, conflicting studies showing that these agents can either increase or reduce colonic tumors in mice, raising concerns about the role of PPARγ in colon cancer. These inconsistent results from in vitro studies might be explained by the dose of PPARγ ligand used and/or properties of the various PPARs. For instance, it has been reported that PPARγ ligands show either tumor-suppressing or tumor-promoting actions in breast cancer cells depending on the doses used (42). Moreover, Niino et al. showed that both PPARγ and PPARα ligands, pioglitazone and bezafibrate, respectively, suppress polyp formation in Apcmin mice (18). Indeed, pioglitazone is also a weak PPARα agonist (43). We have reported that MCC-555 showed higher PPARα transactivation activity than any other synthetic PPARγ ligand (9-fold versus troglitazone, 7-fold versus ciglitazone, and 5-fold versus rosiglitazone), although PPARα activation by MCC-555 was less than that produced by troglitazone, rosiglitazone, and ciglitazone (28). In this study, we also found that MCC-555 increased not only a PPARγ target gene but also a PPARα target gene. This evidence strongly suggests that MCC-555 is a dual agonist for PPARα and PPARγ. The dual agonist function should be considered to be involved in the antitumorigenic activity of MCC-555 and other PPARγ ligands.

Min mice were treated with MCC-555 at a dose of 30 mg/kg, a dose used in previous studies (44). This dose could increase PPARγ-responsive genes in adipose tissue of mice and also could suppress growth of prostate cancer xenografts without lowering body weight in nude mice (44). In this study, treatment with MCC-555 increased both

Figure 3. Transcriptional regulation of MUC2 by PPARγ ligands. A, SW480 cells were pretreated with PPARγ antagonist GW9662 (5 μm) for 30 min before the addition of vehicle, MCC-555 (5 μm), or troglitazone (5 μm). After 24 h, total RNAs were isolated for RT-PCR analysis. GAPDH served as the internal control. Data represent two independent experiments. B, SW480 and NCI-H292 cells transfected with pHMUC2-2096/+27LUC construct were treated with 10 μm MCC-555 for 24 h. CMT-93 cells transfected with pmMUC2-1001/+29LUC construct were treated with 10 μm MCC-555 for 24 h, and luciferase activity was measured as described in Materials and Methods. Y axis, fold increase of relative luciferase unit (RLU) compared with relative luciferase unit of vehicle-treated samples. Mean ± SD from three replicates. *, P < 0.05; **, P < 0.01, from vehicle-treated samples.
PPARγ- and PPARα-responsive genes including Glut2 and Cyp4a10, respectively, supporting a dual agonist. On the other hand, MCC-555 failed to induce PPAR-target genes l-Fabp and αP2 in liver tissue (Fig. 5A). A recent study suggested that ectopic induction of αP2 by PPAR activation is tissue specific in the mouse (39). Thus, expression of αP2 in the small intestine was investigated; however, the expression was not affected by MCC-555 treatment (data not shown). We also analyzed expression of Lpl in the liver because treatment with pioglitazone could increase its expression in Min mice (18). However, MCC-555 did not affect Lpl expression in the liver. Because MCC-555 has a weak PPARγ binding affinity and transactivation, compared with other PPARγ ligands (27, 28), the concentration of MCC-555 used may not be enough to induce all PPARγ-responsive genes.

In this study, we found that the tumor suppressor MUC2 is commonly induced by PPARγ ligands in several human cancer cells (Figs. 1 and 2). Muc2 was also found to be consistently up-regulated in intestinal tumors of Min mice following MCC-555 treatment. Because all tested PPARγ ligands increased MUC2 expression in SW480 cells, they likely work via a PPARγ-dependent pathway. Indeed, usage of GW9662, a PPARγ inhibitor, showed that MUC2 expression occurs in a PPARγ-dependent manner (Fig. 3A). Interestingly, PPARγ ligands did not induce MUC2

Figure 4. Treatment with MCC-555 suppresses tumorigenesis in Min mice. A, number of tumors in the small intestine and colon from control and MCC-555-treated mice. Small intestinal tumors were grouped at intervals of 0.5 mm according to their diameter. Mean ± SE from seven mice. *, *P < 0.05, from control mice. B, tumor load analysis shows a significant reduction of tumors in MCC-555-treated mice. Mean ± SE from seven mice. *, *P < 0.05, from control mice. C, localization of Muc2 in the small intestine and colon of Min mice. Top, H&E-stained sections of tumors arising in the small intestine and colon of Min mice; bottom, lower expressions of Muc2 in tumors compared with normal tissue in the small intestine and colon. Asterisks, areas of neoplasia; arrows, normal tissue of the small intestine and colon. Magnification, ×100.
expression in other colorectal cancer cells such as HT-29 and Caco-2 (data not shown). Recently, it was shown that MUC2 expression in HT-29 and Caco-2 cells was repressed epigenetically by DNA methylation and repressive histone code (45). This may explain why we were unable to induce MUC2 expression by PPARα ligands in these two cell types, although these cells have been shown to express active PPARα (3).

It has been suggested that alteration in mucin gene expression is likely associated with both the early steps of colon cancer development and later tumor progression. Inactivation of Muc2 causes tumor formation accompanied by reduced apoptosis and increased proliferation and migration of intestinal adenocarcinoma cells (26). Oncogenic SOX9 or tumor suppressor p53 regulates MUC2 transcriptional activity negatively and positively (46, 47). These studies on transcriptional regulation of MUC2 imply that MUC2 can be associated with tumor suppression. In agreement with its tumor-suppressing function, MUC2 expression is reduced in tumors compared with the corresponding sections of the small intestine and colon. Our results clearly showed that MCC-555 increased MUC2 expression in human cancer cells and in Min mice. Thus, MUC2 may be, in part, responsible for the antitumorigenic action of MCC-555.

To find the molecular mechanism underlying MUC2 induction by MCC-555, the ERK pathway was analyzed, because MCC-555 promotes phosphorylation of ERK1/2,

![Figure 5. Expression of PPARγ and/or PPARα target genes from control and MCC-555-treated mice. A, representative RT-PCR results of mouse liver tissue samples detecting Glut2, I-Fabp, aP2, Cyp4a10, and Lpl gene expressions. Right, normalized expression of target genes. Values obtained from control mice were defined as 1.0. Mean ± SE from three mice. Gapdh served as the internal control. *, P < 0.05; ***, P < 0.001, from control. B, increased Muc2 expression in small intestinal tumors in mice treated with MCC-555. Total RNAs were isolated from normal (N) and tumor (T) tissues of the small intestine in mice treated with vehicle and MCC-555. Muc2 mRNA expression was analyzed by RT-PCR. Bottom, ratio of intensity (tumor/normal) in adjacent pairs. Gapdh served as the internal control.](image)

![Figure 6. ERK pathway regulates MUC2 expression. A, tissue samples were prepared using polyps isolated from small intestine and colons. Western analysis was done using anti-phospho-ERK1/2 (P-ERK1/2), anti-ERK1/2, and anti-actin antibodies and actin expression served as internal control. B, SW480 cells were pretreated with ERK1/2 pathway inhibitors PD98059 (20 µmol/L) or U0126 (2 µmol/L) for 30 min before the addition of MCC-555 (10 µmol/L). After 24 h, expression of MUC2 was analyzed by RT-PCR. GAPDH served as the internal control.](image)
but not p38 mitogen-activated protein kinase and the c-Jun NH2-terminal kinase, in human colorectal cancer cells (28). It is likely that the ERK signaling pathway is a major determinant in the control of diverse cellular processes, such as cell survival, proliferation, differentiation, and motility. However, the ERK pathway contributes to apoptosis induced by some genotoxic agents, such as diallyl disulfide and l-ascorbic acid (48, 49). In fact, ERK pathway inhibition by U0126 and PD98059 reduced MUC2 induction by MCC-555, suggesting that the ERK pathway regulates MUC2 expression (Fig. 6). Recently, Li et al. reported that troglitazone-induced apoptosis is in part ERK1/2 dependent, supporting our observation (50). In addition to this study, we showed, for the first time, that MCC-555 promotes phosphorylation of ERK1/2 in vivo. These results strongly suggest that activation of the ERK signaling pathway by a synthetic PPARγ ligand is linked to tumor suppression. An understanding of the ERK pathway may be thus an important step toward using agents including PPARγ ligands for cancer prevention. Although the use of MCC-555 is not striking when compared with results of various nonsteroidal anti-inflammatory drugs or epidermal growth factor receptor inhibitors, our data provide a novel finding that the tumor suppressor MUC2 is a target of PPARγ and the possibility of combinational use of MCC-555 with nonsteroidal anti-inflammatory drugs or epidermal growth factor inhibitors in colorectal cancer prevention studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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