Cyclooxygenase-2 Regulation in Colon Cancer Cells

MODULATION OF RNA POLYMERASE II ELONGATION BY HISTONE DEACETYLASE INHIBITORS*

Received for publication, October 21, 2004, and in revised form, February 7, 2005
Published, JBC Papers in Press, February 15, 2005, DOI 10.1074/jbc.M411978200

Xin Tong‡‡, Lei Yin‡, Shree Joshi‡, Daniel W. Rosenberg‡, and Charles Giardina***

From the ‡Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269-3125, §Department of Endoerinology and Metabolism, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, and ||Department of Molecular Medicine, University of Connecticut Health Center, Farmington, Connecticut 06030

We are interested in the mechanism of cyclooxygenase-2 (Cox-2) regulation in colon cancer cells because this knowledge could provide insight into colon carcinogenesis and suggest ways to suppress Cox-2 expression in colon tumors. Studying the HT-29 colon cancer cell line as a model, we found that Cox-2 mRNA and protein levels were activated over 10-fold by the inflammatory cytokine tumor necrosis factor (TNF)-α. Moreover, we found that the histone deacetylase inhibitors butyrate and trichostatin A could block Cox-2 activation in a gene-specific manner. TNF-α and butyrate did not significantly affect Cox-2 promoter activity, mRNA stability, or negative regulation by the Cox-2 3′-untranslated RNA region. A nuclear run-on assay showed that TNF-α increased Cox-2 transcription, whereas butyrate was suppressive. Because butyrate has been reported to suppress polymerase elongation on the c-myc gene, we employed the chromatin immunoprecipitation assay to determine the influence of butyrate and trichostatin A on polymerase distribution on the Cox-2 gene. These data indicated that butyrate restricted polymerase elongation from exon 1 to 2 on both the c-myc and Cox-2 genes. We propose that histone deacetylases regulate a transcriptional block on the Cox-2 and c-myc genes and that this block may be a potential target for pharmacological intervention.

Cyclooxygenase-2 (Cox-2) overexpression has been documented in more than 80% of human colon carcinomas, where it is believed to be a major player in promoting cancer cell survival and tumor angiogenesis (1, 2). A number of cytokines, physiological stresses, oncogenes, and mitogens have been identified to be potent inducers of Cox-2 expression in various types of cells and tissues (3). Cox-2-specific inhibitors have been developed to treat inflammation and cancer (4). In addition to the pharmacological inhibition of Cox-2 activity, a number of anti-inflammatory and antineoplastic compounds, including dexamethasone, resveratrol, and flavonoids, have been shown to suppress Cox-2 gene expression (4–9). A better mechanistic understanding of Cox-2 regulation could illuminate important cellular changes that accompany inflammation and cancer and may also lead to the development of novel approaches to suppress Cox-2 expression.

Short chain fatty acids are present in the colonic lumen at millimolar concentrations (10, 11). These microbial metabolites of dietary fiber have been shown to have a profound impact on colonocyte growth, differentiation, and turnover. Among the luminal short chain fatty acids, butyrate possesses the most potent cellular effects and has been under intensive study for more than a decade for its anti-inflammatory and antineoplastic activities (12, 13). The potential benefit of butyrate for tissue health and function has prompted research on the influence of butyrate on cellular gene expression and signaling pathways. Of particular interest is the influence of butyrate on the expression of genes involved in cellular growth regulation and the inflammatory response. When applied to cells at concentrations that approximate those found in the colon, cells undergo a number of dramatic alterations such as a reduced cell proliferation rate (14), an increased expression of the cell cycle inhibitor p21 (15–17), and the acquisition of differentiated phenotype (18). Accompanying these changes in cell physiology is a dramatic alteration in the cellular response to inflammatory stimuli. Specifically, nuclear factor (NF)-κB activation by stimuli such as tumor necrosis factor (TNF)-α is dramatically down-regulated in cells that have undergone butyrate-induced growth arrest and differentiation (19–22).

Butyrate influences a number of cellular processes, but the one most closely associated with gene regulation is the ability of butyrate to inhibit histone deacetylases (HDACs) (23). Cancer cell growth arrest by butyrate has been attributed to increased expression of the p21 cell cycle inhibitor (17), which results from the inhibition of HDACs that stably associate with the p21 promoter (24). This inhibition leads to the acetylation of promoter-associated histones and the opening of the chromatin structure for pre-initiation complex formation. However, HDAC inhibitors can also trigger the down-regulation of genes that promote cancer cell proliferation. Examples of genes down-regulated by HDAC inhibitors include c-myc and c-Src (25–27). The c-Src gene has a promoter element that mediates the repression by HDAC inhibitors (26). HDAC inhibitors suppress c-myc expression by inducing an elongational arrest and/or premature termination event between exons 1 and 2 of the c-myc gene (28). Exactly how HDAC inhibitors down-regulate these genes is not well understood, but this down-regulation...
Cox-2 Suppression by HDAC Inhibitors

Reverse Transcription (RT)-PCR—Total RNA was prepared from cultured cells using the TRIzol reagent (Invitrogen). Five μg of total RNA from each sample was reverse transcribed into cDNA by Superscript II Reverse Transcriptase (Invitrogen). The resulting cDNA was treated with 1 unit of RNase H at 37 °C for 20 min. A "no RT" control reaction with all the components except reverse transcriptase was used to confirm the absence of genomic DNA contamination. One μl of each reverse transcription reaction was used for semiquantitative PCR amplification as reported previously (29).

RNAse Protection Assay (RPA)—In brief, in situ transcription was used to synthesize radiolabeled cRNA probe using T7 RNA polymerase and 1 μg of a custom-made linearized template (BD Pharmingen). After incubation at 37 °C for 1 h, the template DNA was digested with DNase I. After extraction with phenol-chloroform, the labeled RNA was ethanol-precipitated and resuspended in 10 μl of hybridization buffer. About 1 × 10^6 cpm of cRNA probe was hybridized to 10 μg of total RNA for 12 h at 56 °C. After this incubation, the reaction was digested with an RNase mixture, followed by a proteinase K digestion. The reaction was extracted with phenol-chloroform and ethanol-precipitated. The products were analyzed in a 7% polyacylamide-urea gel. The housekeeping genes L32 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used to normalize RNA loading differences.

Nuclear Run-on with Biotin Labeling—Nuclear run-on reactions were performed as described by Pitarresi et al. (5). Nuclei were prepared from the "no RT" and from sequential dilutions of nuclear extract and competed with a Cox-2 NF-κB probe competition assay. Nuclear run-on reactions were performed as described by Pitarresi et al. (5).

Cell Culture and Treatment—Human colon cancer cell line HT-29 was purchased from American Type Culture Collection (Manassas, VA). The cell line was propagated in McCoy’s 5A medium supplemented with 10% fetal bovine serum, nonessential amino acids, streptomycin (50 μg/ml), and penicillin (50 units/ml). The MHS cell line was a generous gift from Dr. David Knecht. All media components were purchased from Invitrogen (Carlsbad, CA). Myc-mRNA from a myc-myc, α-myc, and TATA promoter was purchased from BD Biosciences (San Diego, CA). All media components were purchased from Invitrogen. All media components were purchased from Invitrogen.

Electrophoretic Mobility Shift Assay—Cell Fractionation for Western Blot—Cells were washed twice with cold phosphate-buffered saline buffer and incubated in lysis buffer A (10 mM HEPES, pH 7.6, 15 mM KCl, 2 mM MgCl2, and 0.1% Nonidet P-40) at 4 °C for 20 min. The resulting supernatant was mixed with 50 μl of lysis buffer B (50 mM Tris-Cl, pH 8.3, 3 mM EDTA, and 0.1% Nonidet P-40) at 29 °C for 30 min. A TRIZol extraction, phenol-chloroform extraction, and ethanol precipitation were performed. A small aliquot was saved as “total nuclear RNA” for each treatment. Dynabeads M-280 streptavidin (Dynal Biotech, Oslo, Norway) were mixed with an equal volume of the TRIzol reagent (Invitrogen). After 1 h of incubation at 42 °C and 2 h at room temperature. With washing 15-fold formamide and 2 × SSC, the beads were resuspended in water and used in BD PowerScript RT reaction (Clontech). Six μl of this RT reaction was used for PCR cycling with primers amplifying Cox-2 (40 cycles) or actin (34 cycles). Cox-2 exon 5 primers were used for this analysis.

Transcript Transfection and Luciferase Activity Assays—Transient transfection of luciferase reporter plasmids was performed using Lipofectamine 2000 (Invitrogen) according to the protocol recommended by the manufacturer for a 24-well plate format. Luciferase assays were performed using the Promega luciferase assay system. After cell treatments, the cells were washed twice with cold phosphate-buffered saline and lysed with 75 μl of cell culture lysis reagent (Promega). Five μl of the resulting supernatant was mixed with 50 μl of luciferase assay solution, and luminescence was determined with a Turner Design Luminometer.

Immunoblotting—For immunoblotting studies, 20 μg of protein sample was denatured under reducing conditions, separated on 10% SDS polyacrylamide gels, and transferred to nitrocellulose by voltage gradient transfer. The resulting blots were blocked with 5% nonfat dry milk, and specific proteins were detected with the following primary antibodies: anti-Cox-2 (Cayman Chemical, Ann Arbor, MI) and anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA). A chemiluminescence detection system was employed for the detection of horseradish peroxidase-conjugated secondary antibodies on the immunoblots (Santa Cruz Biotechnology).

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as described in a previously reported method (19). For the DNA binding assays, double-stranded Cox-2 NF-κB binding probe GGAGGGGGATTCCTGCGCC was end-labeled with [γ-32P]ATP using T4 polynucleotide kinase. The DNA-protein complexes were resolved in a 4% native acrylamide gel and exposed to x-ray film. The protein composition was confirmed by supershift with specific antibodies against NF-κB subunits, whereas specificity was validated by cold probe competition assay.

Immunoblotting—For immunoblotting studies, 20 μg of protein sample was denatured under reducing conditions, separated on 10% SDS polyacrylamide gels, and transferred to nitrocellulose by voltage gradient transfer. The resulting blots were blocked with 5% nonfat dry milk, and specific proteins were detected with the following primary antibodies: anti-Cox-2 (Cayman Chemical, Ann Arbor, MI) and anti-actin (Santa Cruz Biotechnology).
mean ± S.E. Statistical analyses were performed using either Student’s t test for two paired data or ANOVA for three or more sets of data. p values of <0.05 were considered statistically significant.

RESULTS

Butyrate Suppresses Cox-2 Activation in HT-29 Cells—In a previous report, we showed that the HDAC inhibitors butyrate and TSA suppressed Cox-2 gene activation in the HT-29 cells (29). To determine the specificity of this inhibition, we determined the influence of butyrate on the expression of a panel of oncogenes and inflammatory response genes using an RPA. In this experiment, HT-29 cells were treated with TNF-α for 2 h in the presence or absence of butyrate or TSA. Butyrate and TSA treatments were initiated 30 min prior to TNF-α stimulation. Total RNA was prepared from the cells and analyzed by RPA as described in the Fig. 1 legend.

![FIG. 1.](image1)

**FIG. 1.** Butyrate suppresses TNF-α-induced Cox-2 activation in HT-29 cells. A, HT-29 cells were stimulated with TNF-α for 2 h, in the presence or absence of butyrate (with butyrate treatment initiated 30 min prior to TNF-α stimulation). An RPA was used to quantify the levels of a panel of RNAs (listed at left). L32 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were used as internal controls. B, HT-29 cells were stimulated with TNF-α in the presence or absence of butyrate for 4 h. Cell lysates were processed for Western blot analysis using an antibody against Cox-2. β-Actin was also probed as a protein loading control. C, the MHS macrophage cell line was treated with TNF-α or lipopolysaccharide for 4 h in the presence or absence of butyrate. Cox-2 and β-actin levels were analyzed by Western blotting.

![FIG. 2.](image2)

**FIG. 2.** The HDAC inhibitor TSA also suppressed Cox-2 activation in HT-29 cells. HT-29 cells were treated with TNF-α for 2 h, in the presence or absence of butyrate or TSA. Butyrate and TSA treatments were initiated 30 min prior to TNF-α stimulation. Total RNA was prepared from the cells and analyzed by RPA as described in the Fig. 1 legend.

![FIG. 3.](image3)

**FIG. 3.** Butyrate and TSA do not affect Cox-2 promoter activity or NF-κB activation. A, top panel. HT-29 cells were transfected with a Cox-2 promoter-luciferase reporter construct and then subjected to the indicated treatments (24 h after transfection). TNF-α treatments were performed for 4 h, with butyrate and TSA treatments initiated 30 min prior to TNF-α addition. Luciferase activity was assayed and normalized to β-galactosidase expressed by a constitutive, co-transfected lacZ reporter. An ANOVA analysis indicated that neither butyrate nor TSA significantly altered promoter activity. A, bottom panel, a schematic representation of promoter elements present on the Cox-2 promoter-luciferase construct employed in A. B, influence of butyrate on NF-κB DNA binding activity. Cells were treated with butyrate for 30 min or 18 h and then exposed to TNF-α for 30 min. Nuclear extracts were prepared, and an electrophoretic mobility shift assay was performed to determine NF-κB binding activity. The oligonucleotide used in this experiment corresponded to the primary Cox-2 κB site. Cold competition and antibody supershift assays confirmed that the indicated complex is NF-κB (data not shown).

These data indicate that Cox-2 gene activation is particularly sensitive to butyrate. Fig. 1B shows that the butyrate suppression of Cox-2 mRNA expression corresponds to a drop in Cox-2 protein levels. The effect of butyrate on Cox-2 activation does not appear to be common to all cell types. As shown in Fig. 1C, Cox-2 activation by lipopolysaccharide in a macrophage cell line (MHS) was not inhibited by butyrate. TNF-α activation of Cox-2 was not observed in MHS cells (Fig. 1C).
To determine whether another HDAC inhibitor could also suppress Cox-2 gene activation, we tested the ability of TSA to block the TNF-α-induced activation of Cox-2. Figure 2 shows that TSA also down-regulated both Cox-2 and c-myc expression. This finding supports the role of HDACs in regulating Cox-2 and c-myc expression.

Butyrate Does Not Affect Cox-2 Promoter Activity or NF-κB Activation—Cox-2 expression can be regulated at multiple steps, including transcriptional and post-transcriptional steps (31). The Cox-2 5'terminal region contains a canonical TATA box and binding sites for a number of transcriptional activators, including TCF4, SP-1, NF-κB, and NF-IL-6 (32). To determine whether butyrate affects Cox-2 promoter activity, HT-29 cells were transfected with a luciferase reporter plasmid regulated by a 1.6-kb fragment of the Cox-2 promoter. This reporter construct includes binding sites for TCF4, NF-κB, and NF-IL-6. As shown in Fig. 3A, no activation of the reporter gene was observed after TNF-α stimulation. Moreover, neither butyrate nor TSA treatment significantly affected Cox-2 promoter activity (p > 0.05).

We also considered the possibility that butyrate might be inhibiting NF-κB activation because NF-κB has been reported to contribute to Cox-2 expression (33, 34). This possibility seemed particularly appealing, given the fact that butyrate suppresses NF-κB activation in HT-29 cells (19). However, we did not observe any inhibition of NF-κB binding to the Cox-2 promoter region when the butyrate exposure was initiated just 30 min prior to TNF-α stimulation (following the protocol described above; Fig. 3B). Instead, NF-κB inhibition required an 18-h pre-incubation with butyrate, in agreement with previous reports (19, 22). These results, combined with the luciferase reporter data, indicate that Cox-2 promoter activity is not the likely target of the HDAC inhibitors.

**Fig. 4. The butyrate suppression of Cox-2 activation is independent of the Cox-2 3' UTR region.** A, HT-29 cells were transfected with cytomegalovirus promoter-driven luciferase constructs with or without the Cox-2 3' UTR region. Cells were then treated with TNF-α for 4 h, in the presence or absence of butyrate. Luciferase activity was assayed and normalized to β-galactosidase expressed by a co-transfected lacZ construct. A diagram of the constructs used is shown below the graph. The black ovals in the 3' UTR represent individual AU-rich elements. An ANOVA analysis indicated that the 3' UTR suppressed luciferase expression (*, p < 0.01), but butyrate did not significantly alter expression of the reporter construct. B, to determine whether the effect of butyrate on Cox-2 expression required an interaction between the 3' UTR and the Cox-2 promoter, Cox-2 promoter-reporter constructs with or without the Cox-2 3' UTR were analyzed. HT-29 cells were transfected with the indicated constructs and then treated with TNF-α in the presence or absence of butyrate (as indicated). The relative luciferase activities were determined as described in A. A diagram of the constructs used is shown below the graph; the minimal CR1 region of the Cox-2 UTR was present in one of the constructs. An ANOVA analysis indicated that the CR1 region suppressed luciferase expression (*, p < 0.01), but butyrate did not significantly alter expression of the reporter construct.
Butyrate Does Not Alter Cox-2 mRNA Stability in HT-29 Cells—Another layer of regulation of Cox-2 activation is achieved at the post-transcriptional level (31, 35). Cox-2 mRNA stability is primarily regulated by 23 copies of the Shaw-Kamen ARE in the 3'-H11032-UTR (36–39): in response to exogenous signals, the ARE-directed decay is temporarily suppressed to allow for rapid mRNA accumulation. The ARE is also involved in regulating Cox-2 mRNA translation (40). To test the possibility that butyrate impacts the Cox-2 ARE, cytomegalovirus promoter-driven luciferase constructs with or without the Cox-2 3'-UTR region were transfected into HT-29 cells and tested for their response to TNF-α or TNF-α + butyrate. As shown in Fig. 4A, the presence of the Cox-2 3'-UTR resulted in a significant drop in luciferase activity (p < 0.01), consistent with its destabilizing activity. Although TNF-α modestly activated the reporter genes (irrespective of the Cox-2 UTR; p < 0.05), butyrate did not reduce the reporter activity.

To determine whether the influence of butyrate required an interaction between the Cox-2 promoter and the UTR, the essential conserved region 1 (CR1) element of the Cox-2 3'-UTR was fused downstream of the luciferase coding region in a Cox-2 promoter-luciferase reporter construct (41). Again, insertion of the CR1 region caused a significant drop in reporter gene activity (p < 0.01; Fig. 4B). However, the presence of butyrate did not cause any significant decline in luciferase expression (p > 0.05). Results from Fig. 4 provide evidence that the butyrate effect is not likely to be mediated through the Cox-2 3'-UTR region.

We also performed an actinomycin D chase experiment to determine whether butyrate was influencing Cox-2 mRNA turnover. As shown in Fig. 5A, we observed that the TNF-α-induced Cox-2 mRNA levels remained relatively stable after actinomycin D halted transcription. This finding is in contrast to the rapid decay of the unstable TNF-α and c-Myc mRNAs (Fig. 5A). However, the level of Cox-2 mRNA in cells treated with butyrate was too low to be detected by an RPA. A more sensitive RT-PCR assay was employed to monitor Cox-2 mRNA degradation in the presence of actinomycin D. As shown in Fig. 5B, there was no noticeable increase of Cox-2 degradation in butyrate-treated cells. The results in Fig. 5 indicate that Cox-2 mRNA stability is not likely to be involved in the repressive activity of butyrate.

Nuclear Run-on Shows That Butyrate Suppresses Cox-2 Transcription—Because the above-mentioned experiments demonstrated that neither butyrate nor TSA alters the Cox-2 promoter activity or mRNA stability, we considered the possibility that butyrate or TSA might affect transcriptional events downstream of initiation. To determine the effect of butyrate on the Cox-2 transcription rate, we performed a nuclear run-on assay. Specifically, we determined the ability of nuclei isolated from butyrate-treated cells to incorporate biotinylated uracil into RNA during a run-on reaction. As shown in Fig. 6A, the presence of butyrate significantly reduced the incorporation of biotinylated uracil into RNA during the run-on reaction, consistent with its destabilizing activity. Although TNF-α modestly activated the reporter genes (irrespective of the Cox-2 UTR; p < 0.05), butyrate did not reduce the reporter activity.
into Cox-2 RNA. Fig. 6 shows that the level of biotinylated Cox-2 RNA generated in nuclei from TNF-α-stimulated cells was greater than that obtained from untreated cells or from cells treated with TNF-α and butyrate (a run-on reaction performed in the absence of biotinylated UTP showed no band, confirming that run-on RNA was being analyzed). Actin transcription was found to be even across the panel (except when biotinylated UTP was omitted from the run-on reaction). Fig. 6 also shows the levels of Cox-2 and actin RNA in the nuclear extract before streptavidin capture. The results of this run-on indicate that TNF-α increases Cox-2 gene transcription, whereas butyrate suppresses it.

Butyrate Suppresses Cox-2 Activation by Inhibiting Polymerase II Elongation—Based upon the nuclear run-on result, we postulated that butyrate might affect RNA polymerase II elongation and/or termination because both butyrate and TSA have been demonstrated to suppress c-myc expression by promoting pre-mature polymerase termination (42). To confirm this effect of butyrate on c-myc gene expression, HT-29 cells were treated with TNF-α for 2 h in the presence or absence of butyrate. The influence of butyrate on polymerase movement on the c-myc gene was then determined with the ChIP assay. Fig. 7A shows that c-myc gene activation by butyrate is associated with exon 1 and 2 of the c-myc gene. One-tenth of the diluted input DNA was used as loading control, and precipitates from the control IgG reaction served as a negative control. A, the ChIP protocol was performed to determine polymerase distribution on exons 1 through 5 of the Cox-2 gene. As in A, a one-tenth dilution of input chromatin served as a negative control, and precipitates from the control IgG reaction served as a negative control. Lanes marked T/TB are results from TNF-α-treated cells, and lanes marked T/TB were from cells treated with TNF-α and butyrate. B, TSA also blocks polymerase elongation on the Cox-2 gene. HT-29 cells were treated TNF-α or TNF-α + TSA. Lanes marked TNP are results from TNF-α-treated cells, and lanes marked T/TSA were from cells treated with TNF-α and TSA. A ChIP assay was then performed as described in A with exons 1 and 5 detected by PCR. Input chromatin and a control IgG precipitate are shown as controls.

**DISCUSSION**

An intriguing activity of HDAC inhibitors is their ability to alter gene expression patterns in transformed cells toward a more senescent and differentiated phenotype (43). The changes include an up-regulation of genes that promote senescence and differentiation, such as p21, alkaline phosphatase, TGFβRII, and GSK3 (44–46), and a down-regulation of genes that are aberrantly overexpressed in tumors, such as c-myc and Cox-2 (42, 47, 48). The profound influence of HDAC inhibitors on cancer cell gene expression suggests that HDACs play a fundamental role in maintaining the transformed cell phenotype. Consistent with this possibility, HDAC1 overexpression has been reported in gastric and prostate cancers (49, 50), and HDAC2 overexpression has been observed in colon cancers (51). HDAC association with transcription factors, chromatin components, and other gene-regulatory proteins may also be altered in transformed cells. For example, because HDACs associate with methylated DNA, the global changes in DNA methylation that occur during carcinogenesis may alter how HDACs are distributed and utilized in cancer cells (52, 53). A better understanding of how HDACs impact gene expression in transformed cells may ultimately improve our ability to treat and prevent cancer.

Gene activation by HDAC inhibitors results in a relatively straightforward manner: the inhibition of promoter-associated HDACs leads to histone hyperacetylation and the opening of promoter regions for the basal transcription machinery. However, results from gene array data indicate that HDAC inhibition certainly does not trigger global gene activation (54). In fact, most genes are unaffected by HDAC inhibitors. Moreover,
the number of genes that decrease in expression approximates the number of genes that are up-regulated (55). The multifaceted impact of HDACs on gene regulation surfaced after a number of reports showed that HDAC inhibitors can down-regulate steroid-inducible genes such as ovalbumin, IL-2, and mouse mammary tumor virus (56–58). HDAC inhibitors have also been shown to reduce NF-xB and STATI activation in colon cancer cells and suppress STAT5 activity in B cells (19, 22, 59–62). The mechanism for down-regulation may be different for different genes and may involve histones as well as non-histone nuclear proteins. Interestingly, down-regulation of the mouse mammary tumor virus promoter by HDAC inhibitors results from a decreased association with the Brg-1 chromatin remodeling machinery (58). In this instance, the chromatin opening effects of the HDAC inhibition were offset by a reduction in chromatin remodeling activity.

In this study, we analyzed how HDAC inhibitors caused the inhibition of Cox-2 expression. As a highly inducible enzyme, Cox-2 can be regulated at multiple steps (32). Cox-2 transactivation at the promoter region and Cox-2 mRNA stability were not greatly affected by HDAC inhibitors. Interestingly, HDAC inhibitors appear to suppress RNA polymerase II elongation on the Cox-2 gene. This same step has been reported to be the target of HDAC inhibitors on the c-myc gene (25, 42). Inhibition of polymerase II elongation may therefore be a common mechanism by which a subset of genes of cancer cells are down-regulated by HDAC inhibitors. It should be noted that the HDAC inhibitors butyrate and TSA have been reported to regulate by HDAC inhibitors. It should be noted that the inhibition in these cells involves changes in polymerase II elongation remains to be determined.

The inhibition of RNA polymerase II elongation on Cox-2 is not the result of a global decrease in polymerase processivity. Whereas Cox-2 and c-myc were suppressed upon HDAC inhibition, the activation of other genes was relatively unaffected. The possible mechanisms of this type of selective inhibition include alterations in elongation factor interaction with polymerase, alterations in RNA polymerase II C-terminal domain phosphorylation, or the activation of a termination element within the Cox-2 gene. To account for the specificity of the inhibitory effect, we postulate that the HDAC inhibitor responses are mediated by specific regulatory elements within the Cox-2 gene or promoter region. Because both c-myc and Cox-2 were similarly inhibited by butyrate or TSA at the polymerase II elongation step, it is possible that a similar sequence element resides within the c-myc gene. Understanding how HDACs are involved in Cox-2 and c-myc gene regulation could provide new insight into basic gene-regulatory mechanisms. In addition, this information could illuminate the mechanism by which these genes become overexpressed in neoplastic and inflamed tissue and suggest methods to suppress the aberrant expression of these genes.

REFERENCES

1. Chung, D. C. (2000) Gastroenterology 119, 854–865
2. Gupta, R. A., and Dubois, R. N. (2001) Nat. Rev. Cancer 1, 11–21
3. Herschman, H. R. (1996) Biochim. Biophys. Acta 1299, 125–140
4. Warner, T. D., and Mitchell, J. A. (2004) FASEB J. 18, 700–804
5. Ristimaki, A., Narko, K., and Hla, T. (1996) Biochem. J. 318, Pt 1, 325–331
6. Rosenkranz, H. S., and Thampatty, B. P. (2003) Oncogene 19, 1225–1230
7. Dixon, D. A., Kaplan, C. D., McIntyre, T. M., Zimmerman, G. A., and Prescott, S. M. (2000) J. Biol. Chem. 275, 11750–11757
8. Caput, D., Beuller, B., Hartog, K., Thayer, R., Brown-Shinm, S., and Cerami, A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1670–1674
9. Shaw, G., and Kamen, R. (1986) Cell 46, 659–667
10. Xu, N., Chen, C. Y., and Shyu, A. B. (1997) Mol. Cell. Biol. 17, 4611–4621
11. Sengupta, S., Jiang, B. C., Wu, M. T., Paik, J. H., Farnsworth, H., and Hla, T. (2003) J. Biol. Chem. 278, 25227–25233
12. Mukhopadhyay, D., Houchen, C. W., Kennedy, S., Dieckgraefe, B. K., and Anand, S. (2003) Mol. Cell 11, 115–126
13. Ou, G., Liu, C. H., Ben-Porat, L., and Hla, T. (1998) Biochem. Biophys. Res. Commun. 244, 143–148
14. Kojima, M., Morisaki, T., Inuzuka, K., Uchiyama, A., Matsunari, Y., Katano, Y., and Tanaka, M. (2002) Oncogene 19, 1225–1230
15. Dixon, D. A., Kaplan, C. D., McIntyre, T. M., Zimmerman, G. A., and Prescott, S. M. (2000) J. Biol. Chem. 275, 11750–11757
16. Caput, D., Beuller, B., Hartog, K., Thayer, R., Brown-Shinm, S., and Cerami, A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1670–1674
17. Shaw, G., and Kamen, R. (1986) Cell 46, 659–667
18. Xu, N., Chen, C. Y., and Shyu, A. B. (1997) Mol. Cell. Biol. 17, 4611–4621
19. Sengupta, S., Jiang, B. C., Wu, M. T., Paik, J. H., Farnsworth, H., and Hla, T. (2003) J. Biol. Chem. 278, 25227–25233
20. Mukhopadhyay, D., Houchen, C. W., Kennedy, S., Dieckgraefe, B. K., and Anand, S. (2003) Mol. Cell 11, 115–126
21. Ou, G., Liu, C. H., Ben-Porat, L., and Hla, T. (1998) Biochem. Biophys. Res. Commun. 244, 502–512
22. Wilson, A. J., Velech, A., Arango, D., Kurland, A. R., Shenoy, S. M., Perez, R. C., Lenkis, J. M., Singer, R. H., and Augenlicht, L. H. (2002) Cancer Res. 62, 8066–8070
23. Anuma, M., Hayashi, Y., Yoshida, H., Yanagawa, T., Tatsa, Y., Ueno, A., and Sato, M. (1996) Cancer Res. 66, 770–777
24. Ikeda, S., Blottiere, H. M., Le Foll, E., Kafer, R., Cherbut, C., and Galiche, J. P. (1997) Cell Biol. Int. 21, 281–287
25. Osada, H., Tatatematsu, Y., Masuda, A., Saito, T., Sugiyama, M., Yanagisawa, K., and Takahashi, T. (2001) Cancer Res. 61, 8351–8359
26. Chen, Z. Y., Rex, S., and Tseng, C. C. (2004) J. Natl. Cancer Inst. 96, 792–798
27. Ogawa, H., Rafei, P., Fisher, P. J., Johnson, N. A., Otterson, M. F., and Binion, D. G. (2003) Biochem. Biophys. Res. Commun. 303, 512–519
28. Kamitani, H., Geller, M., and Eling, T. (1998) J. Biol. Chem. 273, 21569–21577
29. Halkidi, K., Gaughan, L., Cook, S., Leung, H. Y., Neale, D. E., and Robson, C. N. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 177–189
30. Cowen, J. H., Kron, H. J., Yoon, B. I., Kim, J. H., Han, S. U., Joo, H. J., and Kim, D. Y. (2001) Jpn. J. Cancer Res. 92, 1300–1304
31. Zhu, P., Martin, E., Mengwasser, J., Schlag, P., Janssen, K. P., and Gottlicher, M. (2004) Cancer Cell 5, 453–465
32. Doherty, R. J., and Selker, E. U. (2001) Cell Mol. Life Sci. 58, 721–727
33. Oshiro, M. M., Watts, G. S., Wozniak, R. J., Junk, D. J., Munoz-Rodriguez, J. L., Domann, F. E., and Futscher, B. W. (2003) Oncogene 22, 3642–3643
34. Doherty, R. J., Arici, A. R., Deroo, B. J., and Archer, T. K. (2007) J. Biol. Chem. 282, 15171–15181
35. Klompfer, L., Huang, J., Sasazuki, T., Shirasawa, S., and Augenlicht, L. (2003) Mol. Cancer Res. 1, 855–862
36. Klompfer, L., Huang, J., Sasazuki, T., Shirasawa, S., and Augenlicht, L. (2003) J. Biol. Chem. 278, 30358–30368
37. Xu, M., Nie, L., Kim, S. H., and Sun, X. H. (2003) EMBO J. 22, 893–904
38. Rascle, A., Johnston, J. A., and Amati, B. (2003) Mol. Cell. Biol. 23, 4162–4173

Cox-2 Suppression by HDAC Inhibitors

15509