Histone Deacetylase Inhibitors Suppress the Induction of c-Jun and Its Target Genes Including COX-2*

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Cyclooxygenase-2 (COX-2) is considered to be a target for anticancer therapy. Histone deacetylase (HDAC) inhibitors exhibit antitumor activity, but the mechanisms of action are incompletely understood. We investigated whether HDAC inhibitors blocked AP-1-mediated activation of COX-2 transcription. Trichostatin A and suberoylanilide hydroxamic acid, two structurally related inhibitors of HDAC activity, blocked AP-1-mediated induction of COX-2 expression and prostaglandin E2 biosynthesis. Chromatin immunoprecipitation assays indicated that HDAC inhibitors suppressed c-Jun binding to the COX-2 promoter and thereby blocked transcription. The observed reduction in binding reflected reduced levels of c-Jun. HDAC inhibitors suppressed the induction of c-jun transcription by blocking the recruitment of the preinitiation complex (RNA polymerase II and TFIIB) to the c-jun promoter. HDAC3 but not HDAC1 or HDAC2 was required for AP-1-mediated stimulation of c-jun expression. Because HDAC inhibitors suppressed the induction of c-jun gene expression, resulting in reduced COX-2 transcription, it was important to determine whether other known AP-1 target genes were also modulated. Cyclin D1 and collagenase-1 are AP-1-dependent genes that have been implicated in carcinogenesis. HDAC inhibitors suppressed the induction of both cyclin D1 and collagenase-1 transcription by inhibiting the binding of c-Jun to the respective promoters. Taken together, these results suggest that HDAC inhibitors block the induction of c-jun transcription by inhibiting the recruitment of the preinitiation complex to the c-jun promoter. This led, in turn, to reduced expression of several activator protein-1-dependent genes (COX-2, cyclin D1, collagenase-1). These findings provide new insights into the mechanisms underlying the antitumor activity of HDAC inhibitors.

The packaging of DNA into chromatin provides an important point of control for gene expression. Posttranslational modifications of histones such as acetylation, deacetylation, or phosphorylation can influence chromatin architecture and thereby gene transcription. Acetylation of histones is the best studied of these modifications and depends on net balance between histone acetyltransferase and histone deacetylase (HDAC) activities. HDAC activity is increased in cancer cells and has been linked to carcinogenesis (25). HDAC inhibitors suppress the growth of tumor cells in experimental models and exhibit antitumor activity in clinical trials (26–29). These beneficial effects can be explained by an accumulation of acetylated proteins that affect diverse cellular processes including gene transcription (30, 31). The mechanism(s) by which HDAC inhibitors modulate gene expression is incompletely understood and remains a subject of intense investigation (32).

In this study we investigated whether HDAC inhibitors suppressed the transcriptional activation of COX-2 in human carcinoma cell lines. Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), two structurally related HDAC inhibitors, inhibited AP-1-mediated induction of COX-2 transcription and PGE2 biosynthesis. This was a consequence of reduced levels of c-Jun. Based on this finding, we also evaluated whether HDAC inhibitors modulated the expression of cyclin D1 and collagenase-1, two other AP-1 target genes (33–35). Consistent with the COX-2 findings, the induction of both cyclin D1 and collagenase-1 transcription was suppressed by TSA and SAHA. Taken together, these results suggest that HDAC inhibitors block the induction of c-jun transcription, leading to decreased expression of AP-1-dependent genes. Suppression of AP-1-mediated gene expression is likely to contribute to the anticancer activity of HDAC inhibitors.
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EXPERIMENTAL PROCEDURES

Reagents—TSA and SAHA were from BIOMOL (Plymouth Meeting, PA). Antibodies to COX-2, c-Jun, phospho-c-Jun, c-Fos, cyclin D1, TBP, TFIIIB, HDAC1, –2, and –3, green fluorescent protein, acetyl lysine, and acetylated histone 3 and histone 4 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Enzyme immunoassay reagents for PGE2 assays were from Cayman Chemical (Ann Arbor, MI). Western blotting detection reagents (ECL) were from Amersham Biosciences. pSVβgal was obtained from Promega Corp. (Madison, WI). Reagents for the luciferase assay were from Analytical Luminescence (San Diego, CA). Oligonucleotides were synthesized by Sigma Genosys.

Cell Lines—KYSE450 (esophageal squamous cell carcinoma) (36), HCA7 (colon cancer) (37), 1483 (head and neck squamous cell carcinoma) (38), 184B5/HER (Neu-transformed breast cells) (39), and MSKLeuk1 oral leukoplakia cells (40) were grown as previously described. In all experiments, cells were incubated in serum-free medium for 24 h before treatment. Treatment with vehicle (0.1% Me2SO) or HDAC inhibitor was always carried out in basal medium. Cellular cytotoxicity was assessed by the release of lactate dehydrogenase and the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction assay. There was no evidence of cytotoxicity in any of the experiments described below.

Measurement of HDAC Activity—Labeled histones were isolated following the method described earlier (41). Cell lysate was incubated in a final volume of 50 µl with the labeled histone mixture (20 µl of it containing 20,000 cpm [3H]acetylated mixed histones) for 2 h at 37 °C, and the reaction was stopped by the addition of 10 µl of 0.1 M acetic acid and 700 µl of ethyl acetate. Samples were vortexed and centrifuged (17,000 × g, 5 min), and the organic layer containing released [3H]acetate was removed and counted.

PGE2 Production—Five × 104 cells/well were plated in 6-well dishes and grown to 60% confluence before treatment. The levels of PGE2 released by cells were measured by enzyme immunoassay according to the manufacturer’s instructions. Amounts of PGE2 produced were normalized to cell protein concentrations.

Western Blotting—Cell lysates were prepared by treating cells with lysis buffer (150 mM NaCl, 100 mM Tris (pH 8.0), 1% Tween 20, 50 mM diethyldithiocarbamate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 µg/ml aprotinin, 10 µg/ml trypsin inhibitor, and 10 µg/ml leupeptin). Lysates were sonicated for 20 s on ice and centrifuged at 10,000 × g for 10 min to sediment the particulate material. The protein concentration of the supernatant was measured by the method of Lowry et al. (42). SDS-polyacrylamide gel electrophoresis was performed under reducing conditions on 10% polyacrylamide gels as described by Laemmli (43). The resolved proteins were transferred into nitrocellulose sheets as detailed by Towbin et al. (44). The nitrocellulose membrane was then incubated with primary antisera. Secondary antibody to IgG conjugated to horseradish peroxidase was used. The blots were probed with the ECL Western blot detection system according to the manufacturer’s instructions.

Northern Blotting—Total cellular RNA was isolated from cell monolayers using an RNA isolation kit from Qiagen Inc. 10 µg of total cellular RNA per lane were electrophoresed in a formaldehyde-containing 1.2% agarose gel and transferred to nylon-supported membranes. After baking, membranes were prehybridized overnight in a solution containing 50% formamide, 5× sodium chloride/sodium phosphate/EDTA buffer, 5× Denhardt’s solution, 0.1% SDS, and 100 µg/ml single-stranded salmon sperm DNA and then hybridized for 12 h at 42 °C with radiolabeled cDNA probes and 18 S rRNA. After hybridization, membranes were washed twice for 20 min at room temperature in 2× sodium chloride/sodium phosphate/EDTA buffer, 0.1% SDS, twice for 20 min in the same solution at 55 °C, and twice for 20 min in 0.1× sodium chloride/ sodium phosphate/EDTA buffer, 0.1% SDS at 55 °C. Washed membranes were then subjected to autoradiography. Probes were labeled with [32P]CTP by random priming.

Plasmids—The COX-2 5′-untranslated region promoter-luciferase constructs (−1432/+59, −327/+59, −220/+59, −124/+59, −52/+59, KBM, ILM, CRM) were generous gifts of Drs. Tadashi Tanabe and Hiroyasu Inoue (National Cardiovascular Center Research Institute, Osaka, Japan) (6). c-jun promoter-luciferase constructs were provided by Dr. W. V. Vedeckis (Louisiana State University Medical Center, New Orleans, LA) (45), and c-Jun expression vectors were provided by Dr. Tom Curran (Roche Applied Science). Dr. Stephen M. Prescott (University of Utah, Salt Lake City, UT) provided the human COX-2 cDNA. siRNAs to HDAC1, -2, and -3 as well as control vector were purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). siRNA to TBP and TFIIIB were purchased from Open Biosystems (Huntsville, AL).

Transient Transfection Assays—Cells were seeded at a density of 5 × 104 cells/well in 6-well dishes and grown to 50–60% confluence. For each well, 2 µg of plasmid DNA was introduced into cells using 2 µg of Lipofectamine2000 as per the manufacturer’s instructions. After 12 h of incubation, the medium was replaced with basal medium. The activities of luciferase and β-galactosidase were measured in cellular extract as previously described (8).

Electrophoretic Mobility Shift Assay—Cells were harvested, and nuclear extracts were prepared. For binding studies, an oligonucleotide
containing the CRE of the COX-2 promoter was used: 5'-AACAGTGATTTCGTCACTGGGCTTG-3’ (sense) and 5’-CAAGCCCATGTGACGAAATGACTGTTT-3’ (antisense). The complementary oligonucleotides were annealed in 20 mM Tris (pH 7.6), 50 mM NaCl, 10 mM MgCl₂, and 1 mM dithiothreitol. The annealed oligonucleotide was phosphorylated at the 5’ end with [γ-32P]ATP and T4 polynucleotide kinase. The binding reaction was performed by incubating 5 μg of nuclear protein in 20 mM HEPES (pH 7.9), 10% glycerol, 300 μg of bovine serum albumin, and 1 μg of poly(dIdC) in a final volume of 10 μl for 10 min at 25 °C. The labeled oligonucleotide was added to the reaction mixture and allowed to incubate for an additional 20 min at 25 °C. The samples were electrophoresed on a 4% nondenaturing polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose. Immunoblots were probed with antibodies specific for COX-2 and β-actin.

**RESULTS**

HDAC Inhibitors Suppress PMA-mediated Induction of COX-2 Transcription—Initially, we investigated the effects of PMA, TSA, and SAHA on HDAC activity in KYSE450 cells. Treatment with PMA caused a severalfold increase in HDAC activity (Fig. 1). Both HDAC inhibitors caused dose-dependent suppression of PMA-mediated induction of HDAC activity (Fig. 1). Next, we determined whether TSA and SAHA could suppress PMA-mediated induction of COX-2 protein in KYSE450 cells. Both TSA and SAHA caused dose-dependent inhibition of COX-2 induction (Fig. 2). To prove that this effect was not restricted to a specific cell type, a similar analysis was carried out in several other human cell lines. Comparable inhibitory effects were observed in each of these cell lines (Fig. 2). The changes in HDAC activity correlated with differences in the amounts of COX-2, suggesting a causal relationship. To further elucidate the mechanism responsible for changes in COX-2 protein, we determined steady-state levels of COX-2 mRNA by Northern blotting. PMA-mediated induction of COX-2 mRNA was blocked by both TSA and SAHA (Fig. 3, A and B). To evaluate the functional significance of these effects, PGE₂ production was measured. Treatment with PMA led to an ∼10-fold increase in PGE₂ synthesis, an effect that was nearly completely abrogated by treatment with the HDAC inhibitors (Fig. 3C).
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 transient transfections were performed to investigate the effects of PMA on COX-2 transcription. Treatment with PMA caused a significant increase in COX-2 promoter activity with all COX-2 promoter deletion constructs except the −52/+59 construct (Fig. 4A), suggesting that the CRE site may be responsible for mediating the inductive effects of PMA. To further evaluate this possibility, transient transfections were performed using COX-2 promoter constructs in which different elements had been mutated. As shown in Fig. 4A, bottom panel, mutagenizing the CRE site caused a loss of responsiveness to PMA. Electrophoretic mobility shift assays were next performed to identify the transcription factor responsible for PMA-mediated induction of COX-2. Treatment with PMA led to increased binding of nuclear protein to the CRE site of the COX-2 promoter (Fig. 4B). The increase in binding to the COX-2 CRE was competed by incubating nuclear extract from PMA-treated cells with a 50-fold excess of unlabeled CRE probe. Supershift analysis identified c-Jun and c-Fos, components of the AP-1 transcription factor, in the binding complex (Fig. 4B). These findings are consistent with prior evidence that AP-1 binding to the CRE is important for activation of COX-2 transcription (21–24). A potential limitation of electrophoretic mobility shift assays is that DNA binding activity is examined in the absence of a cellular context and, therefore, may not reflect binding to the endogenous gene. To further examine the mechanism by which HDAC inhibitors suppressed PMA-mediated induction of COX-2, we performed a ChIP assay (Fig. 4C). Protein-DNA complexes were immunoprecipitated with an anti-phospho-c-Jun antibody, and bound DNA fragments were recovered and subjected to semiquantitative PCR with oligonucleotides specific for the COX-2 promoter. As shown in Fig. 4C, treatment of cells with PMA enhanced binding of c-Jun to the COX-2 promoter, an effect abrogated by treatment with either TSA or SAHA. Previously, CBP/p300 was found to be important for AP-1-mediated activation of COX-2 transcription (24). Given this background, we evaluated the effects of HDAC inhibitors on the interaction between c-Jun and CBP/p300. Treatment with PMA led to an increase in the interaction between c-Jun and CBP/p300 (Fig. 5), an effect that was abrogated by cotreatment with either TSA or SAHA (Fig. 5).

HDAC Inhibitors Suppress PMA-mediated Induction of c-jun Transcription—We next carried out experiments to investigate the mechanism by which HDAC inhibitors blocked PMA-mediated induction of c-Jun binding to the COX-2 promoter. One potential explanation would be altered expression of c-Jun. In fact, both TSA and SAHA inhibited PMA-mediated induction of c-Jun protein (Figs. 6, A and B). In contrast, the HDAC inhibitors augmented the induction of c-Fos by PMA (Fig. 6, A and B). Northern blotting was then performed to determine whether the HDAC inhibitors suppressed PMA-mediated induction of c-Jun by a pretranslational mechanism. As shown in Fig. 6, C and D, both TSA and SAHA caused dose-dependent suppression of PMA-mediated induction of c-jun mRNA.

Transient transfections were performed to investigate the effects of PMA on c-jun transcription. PMA caused nearly a 2-fold increase in the activity of the full-length c-jun promoter (Fig. 7B). The inductive effects of PMA were observed with all c-jun promoter deletion constructs, except the −63 construct (Fig. 7B), suggesting that the proximal (pAP-1) rather than the distal (dAP-1) AP-1 site may be responsible for mediating the inductive effect of PMA. To test this possibility, transient transfections were performed using c-jun promoter constructs in which either the dAP-1 or pAP-1 sites had been mutagenized. As shown in Fig. 7C, mutagenizing the pAP-1 but not the dAP-1 site caused a loss of responsiveness to PMA. To further define the mechanism by which PMA stimulated c-jun transcription, studies of TFIIB and TBP, components of the preinitiation complex, were conducted. siRNAs to TBP and TFIIB suppressed levels of TBP and TFIIB (Fig. 8A) and blocked PMA-mediated induction of c-jun promoter activity (Fig. 8B). siRNA to green fluorescent protein, a control treatment, did not affect PMA-mediated induction of c-jun promoter activity (data not shown). These data imply that recruitment of the preinitiation complex is required for PMA-mediated regulation of c-jun expression. Because AP-1 plays a key role in PMA-mediated stimulation of c-jun transcription (Fig. 7), experiments were next carried out to identify the role of different c-Jun domains in mediating this inductive effect. As shown in Fig. 8C, overexpressing
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C-Jun that either lacked the leucine zipper or DNA binding domain/basic region suppressed PMA-mediated activation of the c-Jun promoter.

To further examine the mechanism by which HDAC inhibitors suppressed the induction of c-Jun, ChIP assays were performed. Treatment with PMA enhanced the interaction between the c-Jun promoter and acetylated histones 3 and 4, an effect that was suppressed by either SAHA or TSA (Fig. 9). HDAC inhibitors have been reported to interfere with the initiation of transcription by blocking the recruitment of components of the preinitiation complex (49). Accordingly, protein-DNA complexes were immunoprecipitated with antibodies to RNA polymerase II and TFII B (Fig. 10), and bound DNA fragments were recovered and subjected to semiquantitative PCR with oligonucleotides specific for the c-Jun promoter. Treatment of cells with PMA for 15 min enhanced binding of RNA polymerase II and TFII B to the c-Jun promoter, an effect that was suppressed by either TSA or SAHA (Fig. 10).

Therefore, HDAC inhibitors block the induction of recruitment of the preinitiation complex and thereby block the activation of c-Jun expression. Acetylation of c-Jun has been found to suppress the expression of c-Jun-dependent genes (51). In this study treatment with HDAC inhibitors suppressed the induction of c-Jun, but increased acetylation of c-Jun was not observed (data not shown). TSA and SAHA inhibit numerous HDACs (32). It was relevant, therefore, to determine whether all HDACs are equally important for PMA-mediated activation of c-Jun transcription. siRNAs to HDACs 1–3 suppressed levels of the respective HDACs (Fig. 11A). Interestingly, silencing of HDAC3 but not HDAC1 or -2 suppressed PMA-mediated activation of the c-Jun promoter (Fig. 11B). siRNA to green fluorescent protein, a control treatment, did not

FIGURE 4. HDAC inhibitors block PMA-mediated activation of COX-2 transcription by inhibiting the binding of c-Jun to the COX-2 promoter. A, shown is a schematic of the human COX-2 promoter (top panel). In the middle panel KYSE450 cells were transfected with 1.8 μg of a series of human COX-2 promoter deletion constructs ligated to luciferase (−1432/+59, −327/+59, −220/+59, −124/+59, and −52/+59) and 0.2 μg of pRSVgal. In the bottom panel cell were transfected with 1.8 μg of a series of human COX-2 promoter-luciferase constructs (−327/+59, KBM, ILM, CRM) and 0.2 μg of pRSVgal. KBM represents the −327/+59 COX-2 promoter construct in which the NF-κB site was mutagenized; ILM represents the −327/+59 COX-2 promoter construct in which the NF-κB site was mutagenized; CRM refers to the −327/+59 COX-2 promoter construct in which the CRE site was mutagenized. After transfection, cells were treated with vehicle (open columns) or PMA (50 ng/ml, black columns). Luciferase activity represents that data have been normalized to β-galactosidase activity. Columns, means; bars, S.D.; n = 6. B, KYSE450 cells were treated with vehicle or PMA (50 ng/ml) for 2 h. In the panel on the left, 5 μg of nuclear protein was incubated with a 32P-labeled oligonucleotide containing the CRE of the COX-2 promoter (lanes 1 and 2). In lane 3, nuclear extract from PMA-treated cells was incubated with a 32P-labeled COX-2 CRE oligonucleotide and a 50-fold excess of unlabeled oligonucleotide containing the CRE of COX-2. In the panel on the right, lane 1 represents nuclear extract from PMA-treated cells; lanes 2–4 represent nuclear extract from PMA-treated cells incubated with normal IgG (lane 2) or antibodies to phospho-c-Jun (lane 3) or c-Fos (lane 4). The protein-DNA complexes that formed were separated on a 4% polyacrylamide gel. Arrows indicate shifted bands. Ab, antibody. C, KYSE450 cells were treated with vehicle (lanes 1 and 2), PMA (50 ng/ml, lanes 3 and 4), PMA plus TSA (500 nM, lanes 5 and 6), or PMA plus SAHA (25 μM, lanes 7 and 8) for 2 h. Chromatin fragments were immunoprecipitated (IP) with antibodies against phospho-c-Jun, and the COX-2 promoter region (−7 to −621) was amplified by PCR. DNA sequencing was carried out, and the PCR product was confirmed to be the COX-2 promoter. We were unable to detect the COX-2 promoter when normal IgG was used (lane 9) or antibody was omitted from immunoprecipitation step (lane 10).
affect PMA-mediated induction of c-Jun promoter activity (data not shown).

**HDAC Inhibitors Suppress the Expression of c-Jun Target Genes**—The above results indicate that HDAC inhibitors suppress the induction of c-Jun and thereby block the activation of COX-2 transcription. These results suggest that induction of other known AP-1 target genes should also be suppressed by HDAC inhibitors. To evaluate this possibility, we investigated the effects of HDAC inhibitors on the expression of cyclin D1 and collagenase-1, two AP-1-driven genes that have been implicated in carcinogenesis (33–35). As shown in Fig. 12A, PMA-mediated induction of cyclin D1 protein was suppressed in a dose-dependent manner by TSA and SAHA, respectively. Similar results were obtained by Northern blotting (data not shown). To confirm the role of c-Jun, a ChIP assay was performed (Fig. 12C). Protein-DNA complexes were immunoprecipitated with an anti-phospho-c-Jun antibody, and bound DNA fragments were recovered and subjected to semiquantitative PCR with oligonucleotides specific for the cyclin D1 promoter. Treatment of cells with PMA enhanced binding of c-Jun to the cyclin D1 promoter, an effect that was suppressed by treatment with either TSA or SAHA. Similarly, the HDAC inhibitors blocked PMA-mediated induction of collagenase-1 (Fig. 12D). The ChIP assay demonstrated that treatment with PMA enhanced binding of c-Jun to the collagenase-1 promoter, an effect that was suppressed by either TSA or SAHA (Fig. 12E).

**DISCUSSION**

In this study we showed that HDAC inhibitors suppressed phorbol ester-mediated activation of COX-2, cyclin D1, and collagenase-1 transcription. Initially, we focused on COX-2 to determine whether this was a direct or an indirect effect of HDAC inhibitors. We first defined the mechanism by which PMA stimulated COX-2 transcription. Electro-
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FIGURE 8. The preinitiation complex is important for PMA-mediated activation of c-jun transcription. A, KYSE450 cells were transfected with siRNA to TBP or TFII B. Cellular lysate protein (100 μg/lane) was prepared 36 h after transfection and loaded onto a 10% SDS-polyacrylamide gel, electrophoresed, and transferred onto nitrocellulose. Immunoblots were probed with antibodies specific for TBP, TFII B, and β-actin. B, cells were transfected with 0.9 μg of human c-jun promoter (~1780-bp construct), 0.9 μg of siRNA control vector alone, siRNA to TBP or TFII B, and 0.2 μg of pSV/βgal. C, cells were transfected with 0.9 μg of human c-jun promoter-luciferase construct (~1780-bp construct), 0.9 μg of control vector alone, wild type c-jun, c-jun without the leucine zipper (L2), or c-jun without the basic (RK) region and 0.2 μg of pSV/βgal. In B and C, after transfection, cells were treated with vehicle or PMA (50 ng/ml) for 4 h. Luciferase activity represents data that have been normalized to β-galactosidase activity. Columns, means; bars, S.D.; n = 6.

FIGURE 9. HDAC inhibitors block PMA-mediated induction of binding of acetylated histones 3 and 4 to c-jun promoter. KYSE450 cells were treated with vehicle (lanes 1), PMA (50 ng/ml, lane 2), PMA plus TSA (100 and 500 nM; lanes 3 and 4, respectively), or PMA plus SAHA (25 μM, lane 5; 15 μM, lane 6) for 15 min. B, cells were treated with vehicle (lane 1), PMA (50 ng/ml, lane 2), PMA plus TSA (250 and 500 nM; lanes 3 and 4, respectively), or PMA plus SAHA (25 μM, lane 5). Chromatin fragments were immunoprecipitated with antibodies against acetylated histone 3 (A) or acetylated histone 4 (B), and the c-jun promoter region (~60 to ~345) was amplified by PCR. DNA sequencing was carried out, and the PCR product was confirmed to be the c-jun promoter. In both A and B, we were unable to detect the c-jun promoter when normal IgG was used (lane 7 in A; lane 6 in B) or antibody was omitted from the immunoprecipitation step (lane 8 in A; lane 7 in B). Ab, antibody.

HDAC inhibitors block induction of COX-2 by suppressing the binding of c-Jun to the COX-2 promoter. Importantly, these results are consistent with previous reports that retinoic acid and phenolic antioxidants blocked the induction of COX-2 by antagonizing AP-1 (24). The suppressive effect of retinoic acid was due to squelching of CBP/p300 (24). In contrast, HDAC inhibitors blocked the induction of c-jun and thereby suppressed the interaction with CBP/p300 that is required for activation of COX-2 transcription (Fig. 5) (24, 52).

We next investigated the mechanism by which HDAC inhibitors suppressed PMA-induced binding of c-jun to the COX-2 promoter. The reduction in c-jun binding was a consequence of reduced c-jun expression. To our knowledge, this study provides the first evidence that HDAC inhibitors can block the induction of c-jun. The HDAC inhibitors blocked the activation of c-jun transcription, resulting in reduced levels of c-jun mRNA and protein. Fig. 10 shows that HDAC inhibitors blocked PMA-induced recruitment of TFII B and polymerase II to the c-jun promoter. Thus, HDAC activity is required at the c-jun promoter for the assembly of the preinitiation complex. HDAC3 appeared to be more important than HDAC1 or HDAC2 for the induction of c-jun.

These findings are consistent with previous evidence that HDAC activity is required to recruit the preinitiation complex to selected immediate
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Tumor cells are much more sensitive to the growth inhibitory effects of HDAC inhibitors than are normal cells (59). Levels of c-Jun, COX-2, cyclin D1, and collagenase-1 are commonly increased in transformed cells and in malignant tissues (10, 48, 60, 61). Increased AP-1 activity has been detected in tumor cells. c-Jun is required for Ras to transform fibroblasts and is essential for development of chemically induced early genes that are activated by the transcription factors Stat1, Stat2, and Stat5 (50, 53). Moreover, HDAC activity was recently found to be required for preinitiation complex formation at the ICER promoter, a CREB-dependent gene (49). Others have reported that HDAC inhibitors can block the induction of c-myc and COX-2 transcription by modulating RNA polymerase II elongation (54, 55). Although effects may vary depending on inducer and cell type, the current results argue that suppression of COX-2 expression is a downstream consequence of reduced levels of c-Jun. We note that changes in acetylation of c-Jun have been reported to inhibit the expression of c-Jun-dependent genes (51). In this study the primary effect of HDAC inhibitors was to suppress the induction of c-Jun rather than to alter its acetylation.

Given the significance of c-Jun in cell transformation and carcinogenesis (56–58), it was important to determine whether HDAC inhibitors suppressed the induction of other c-Jun-target genes. Hence, we evaluated the effects of HDAC inhibitors on the induction of cyclin D1 and collagenase-1. Both of these genes have been implicated in carcinogenesis and can be modulated by changes in AP-1 activity (33–35). The induction of cyclin D1 and collagenase-1 transcription was blocked by HDAC inhibitors, resulting in reduced levels of these proteins (Fig. 12). ChIP analyses indicated that binding of c-Jun to the cyclin D1 and collagenase-1 promoters was enhanced by PMA, an effect that was blocked by HDAC inhibitors.

Tumor cells are much more sensitive to the growth inhibitory effects of HDAC inhibitors than are normal cells (59). Levels of c-Jun, COX-2, cyclin D1, and collagenase-1 are commonly increased in transformed cells and in malignant tissues (10, 48, 60, 61). Increased AP-1 activity has been detected in tumor cells. c-Jun is required for Ras to transform fibroblasts and is essential for development of chemically induced
tumors in mice (61). COX-2-derived PGs stimulate cell proliferation and angiogenesis while inhibiting apoptosis and immune surveillance (10). Cyclin D1 and collagenase-1 enhance cell proliferation and increase invasiveness, respectively (48, 61). It is appealing to speculate, therefore, that the ability of HDAC inhibitors to suppress the induction of c-Jun and its target genes contributes to the antitumor activity of these agents. Moreover, these findings may help to explain why HDAC inhibitors target transformed cells in preference to normal cells. Aside from providing new insights into the mechanism of action of HDAC inhibitors, the current study highlights the potential of targeted anticancer therapies to suppress COX-2 transcription.

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