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Activation of MHC Class I, II, and CD40 Gene Expression by Histone Deacetylase Inhibitors

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Epigenetic mechanisms are involved in regulating chromatin structure and gene expression through repression. In this study, we show that histone deacetylase inhibitors (DAIs) that alter the acetylation of histones in chromatin enhance the expression of several genes on tumor cells including: MHC class I, II, and the costimulatory molecule CD40. Enhanced transcription results in a significant increase in protein expression on the tumor cell surface, and expression can be elicited on some tumors that are unresponsive to IFN-γ. The magnitude of induction of these genes cannot be explained by the effect of DAIs on the cell cycle or enhanced apoptosis. Induction of class II genes by DAIs was accompanied by activation of a repressed class II transactivator gene in a plasma cell tumor but, in several other tumor cell lines, class II was induced in the apparent absence of class II transactivator transcripts. These findings also suggest that the abnormalities observed in some tumors in the expression of genes critical to tumor immunity may result from epigenetic alterations in chromatin and gene regulation in addition to well-established mutational mechanisms. The Journal of Immunology, 2000, 165: 7017–7024.

Although the majority of normal cells express surface MHC class I Ags, most normal nonhemopoietic cells express MHC class II only after activation by IFN-γ. However, a few cell types, including mature oligodendrocytes, sensory neurons, plasma cells, and trophoblasts, cannot be induced to express class II by IFN-γ. MHC class II expression is tightly regulated primarily at the level of transcription by multiple transcriptional activators binding to the W/S, X, and Y box promoter sequences. In addition, coactivators, such as class II transactivator (CIITA), Bob1, and CBP/p300, play important roles in the transcriptional regulation of class II (1–3). Recently, CIITA has been reported to facilitate IFN-γ induction of MHC class II in addition to class II Ags (4, 5), and this, together with the similarities in the structure of the class I and II promoters (6), suggests a commonality in the regulation of MHC genes.

Many tumors are MHC class II negative and, although some can be induced to express class II by IFN-γ, a subset of tumors is noninducible by cytokines. In a few noninducible tumors, defects in IFN-γ receptors or Janus kinase-STAT signaling pathway components have been described and have been associated with tumor progression (7). In plasmacytoma cell lines, and in selected melanomas and hepatocellular carcinomas, the lack of expression of all class II isotypes appears to be due to a defect in the transcription of the CIITA gene. In these cells, transfection of CIITA results in class II expression (8, 9) and, in certain tumors, this restores their ability to present intact Ag (9). That MHC class II may play a role in tumor immunity is suggested by studies demonstrating that transfection of MHC class II-negative tumor cells with class II genes inhibits tumorigenicity and elicits immunity to subsequent challenge with wild-type tumor cells (10). However, induced expression of MHC class II does not always enhance immunity and, in the absence of costimulatory factors, may even promote tumor progression by inducing anergy (11). Thus, differences in the functional role of class II may be related to the context in which the MHC genes are expressed in different tumors, i.e., the presence of associated “escape” defects (reviewed in Refs. 12 and 13).

Considerable evidence has accumulated recently that gene expression is influenced by epigenetic mechanisms that alter chromatin structure (14). Acetylation of lysine residues on the N-terminal tails of the basic core histones in nucleosomes is generally associated with enhanced transcription, whereas deacetylation compacts chromatin and represses transcription. Thus, while targeting molecules with histone acetyltransferase activity to promoters results in gene activation, repression of certain genes is accomplished by transcriptional repressors and corepressors that bind and target histone deacetylases (HDACs) (15, 16). Several noncompetitive inhibitors of HDACs have been described, including sodium butyrate (NaBu) and the highly specific inhibitor trichostatin A (TSA), that arrest cells in both the G1 and G2-M phases of the cell cycle (17), influence growth, and induce apoptosis (18). These inhibitors have been shown to activate a number of important genes by altering histone acetylation and chromatin structure, although not all genes are activated by HDAC inhibitors (DAIs), many are unaffected and some are inhibited (19).

We show here that MHC class II mRNA and cell surface protein can be induced by DAIs in mouse and human tumor cell lines that cannot be activated to express class II by IFN-γ. Evidence is presented that, in some tumors, DAIs activate a repressed CIITA gene, whereas, in others, induction may occur via a route different.

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from the classical CIITA-mediated pathway. Importantly, DAs are also shown to enhance the constitutive expression of MHC class I, as well as the costimulatory molecule CD40, on some tumor cells that were not induced by IFN-γ. These data also suggest that failure of tumors to express certain molecules critical to immunity (tumor escape) may result from chromatin repression, which is reversed by HDAC inhibitors.

Materials and Methods

Cells and reagents

The human neuroblastoma tumor cell line SK-N-MC (MC) and mouse placasmayota J558 cell line were obtained from American Type Culture Collection (Manassas, VA). Mouse adenocarcinoma Colon 26, generously provided by Elizabeth C. Repasky (Roswell Park Cancer Institute, Buffalo, NY), was maintained in MEM growth media supplemented with 10% FCS (Life Technologies, Grand Island, NY) as a cell line derived from the original transplantable tumor. BJ2.2.5 cells were a generous gift from Cheong-Hee Chang (University of Michigan, Ann Arbor, MI). Stock and working dilutions of TSA (Wako Biochemicals, Osaka, Japan) were prepared using ethanol as a diluent. Abs to murine surface markers were obtained from the following suppliers: 1) PharMingen (San Diego, CA): PE-conjugated anti-H-2D^d (clone 34-2-12), I-A^k (AMS-32.1), CD40 (3/23); 2) Coulter (Miami, FL); PE-anti CD40 (clone 5B13), streptavidin-biotinylated anti-CD25 (74D), CD29 (HA25/2), CD119 (GR20); purified anti-CD104 (346-11A), and FITC-anti-H-2D^d (34-2-12); 3) Caltag (South San Francisco, CA): PE-conjugated anti-H-2K^k (CT1Kk), I-A^k (14V.18), CD62L (MEL-14), CD80 (R8MMP-1), CD86 (R8MMP-2); FITC- and PE-annexin-V; and 3) Southern Biotechnology Associates (Birmingham, AL): PE-conjugated anti-CD54 (KAT-1), CD154 (M41). Flow cytometry was conducted on MC cells. Primary and secondary mAbs to mouse surface markers, B, C, O were obtained from PharMingen, PE-anti HLA-DR (clone 243) from Becton Dickinson (Mountain View, CA), and PE-anti CD40 (clone mAb 89) from Immunotech/Coulter (Miami, FL).

Inhibition of HDAC activity

Toxicity at various concentrations and incubation times with TSA and NaBu were first determined using three techniques: 1) trypan-blue dye exclusion; 2) apoptosis as measured by DNA ladder on agarose gels; and 3) flow cytometric analysis with propidium iodide (PI) or annexin-V. Preliminary titration was performed on each cell line at 10, 25, 50, 100, and 250 nM TSA for 6, 12, 24, 48, and 72 h and with the MC cell line at 1 mM NaBu for 12, 24, and 48 h, and concentrations and incubation times were selected to exclude toxicity and maximize expression. IFN-γ was titrated at 10, 50, 100, and 500 U/ml for each cell line. In most experiments, 100 U/ml was employed.

RT and quantitative real-time PCR

Standard RT-PCR was performed as previously described using primers for human HLA-DRα and CIITA (20) and mouse I-Ax (21). Other primers used included human and mouse GAPDH (Clontech Laboratories, Palo Alto, CA) and 5’-ACACCTGGACCTGGACTCAC-3’ (forward) and 5’-GAAACTGGTGAGT-3’ (reverse) for mouse CD40 (22), and 5’-ACACCTGGACCTGGACTCAC-3’ (forward) and 5’-CACATTGGAGAAGAAGCC-3’ (reverse) for human CD40 (22), and 5’-GTITAAAGTCCCGTGATCGCA-3’ (forward) and 5’-CTCAAGGATCTGTCGTCGTT-3’ (reverse) for mouse CD40 (22).

The real-time fluorescent PCR technique was used to quantitate fold changes in GAPDH, CIITA, MHC class II, and CD40. The detection system and quantitative PCR have been described in detail elsewhere (24). The threshold cycle number (C^t) corresponds to the cycle number at which the fluorescent emission reaches 10 SDs above the mean fluorescent emission measured during the early “baseline” cycles of the PCR. The C^t values for triplicates were averaged and normalized to GAPDH levels (i.e., average C^t for HLA-DR on sample cDNA minus average C^t for GAPDH on same sample = normalized C^t or ΔC^t for that sample). Assuming reverse transcription efficiencies were approximately the same for each sample, this allowed us to determine relative amounts of mRNA using the comparative C^t (ΔΔC^t) method (Perkin-Elmer Taqman User Bulletin 2). Fold changes in mRNA levels were calculated as 2^{-ΔΔC^t}, where x = the difference between the GAPDH normalized C^t values of the control and experimental samples.

Validation experiments showed a linear relationship between input cDNA and C^t values over a range of serial dilutions of cDNA extending from 1 to 10^{-6}. The slopes of these lines generated for each primer/probe set indicated that PCR efficiencies for each of the primer sets were close to 100% and validated the use of the ΔΔC^t (comparative C^t) method for relative quantitation. These experiments also demonstrated the linear range of amplification and allowed determination of the maximum C^t value at which linear amplification was reliable. For most of our primer/probe sets, this value was 40; if the samples did not amplify by 40 cycles, the gene for that sample was considered not detectable (ND), because C^t values beyond this point would not meet the criteria for calculation of fold change by the comparative C^t method. In such cases, to calculate a minimum fold change for other samples in the same group, the C^t value for the not detectable sample was arbitrarily set at 40. Primer sets for real-time PCR are available at http://realtime.nwu.edu.

Flow cytometry

Flow cytometric analyses were conducted by published methods (25) with cells fixed with 1% paraformaldehyde and analyzed on a FACSscan (Becton Dickinson). Cell cycle analyses were conducted by flow cytometry with PI staining of genomic DNA. Cells were fixed and permeabilized in methanol, treated with RNaseA and PI and analyzed for DNA content by determination of FL3-area on the FACSscan. The FL3-area data were analyzed with the ModFit software for determination of the percent cells in each phase of the cell cycle. In some cases, FITC-conjugated Ab staining was conducted before PI treatment and surface staining was analyzed as a function of the cell cycle phase. Apoptosis was analyzed by staining with annexin V directly conjugated with either FITC or R-PE and by sub-G0 analysis of DNA staining. Data are representative of at least three independent experiments.

Results

Induction of MHC class II in human and mouse tumor cells

Our experiments were initially designed to explore whether the failure of certain tumor cells to express MHC class II might be a result of a repressive chromatin structure. Therefore, we tested the effect of treatment with the DAs, NaBu and TSA, on the activation of class II genes in selected tumor cell lines. As shown in Fig. 1, treatment of the human neuroblastoma cell line SK-N-MC (MC) with 100 nM TSA or 1 mM NaBu resulted in a substantial increase in the levels of mRNA for HLA-DR (DR) as measured by RT-PCR, whereas CIITA transcription remained not detectable. Levels of mRNA for the housekeeping gene, GAPDH, were not significantly changed after treatment. Low levels of mRNA for the DP and DQ isotypes of MHC class II were also induced by TSA (data not shown). Although TSA and NaBu activate transcription of MHC class II in MC tumor cells, these cells cannot be induced to express mRNA for DR by IFN-γ (Fig. 1A). This is not due to the failure of MC cells to express IFN-γ receptors or to a defect in the IFN-γ signaling pathway, because mRNA for two prototype IFN-γ-inducible genes, IFN regulatory factor-1 and guanylate binding protein, is enhanced by IFN-γ treatment (data not shown). Essentially identical results to those of MC were obtained with JAR, a human trophoblast cell line, that was refractory to IFN-γ but inducible for class II by TSA and NaBu.

Real-time fluorescent PCR, capable of detecting low levels of mRNA, was employed, and fold changes in transcript levels were calculated using C^t values standardized to GAPDH (see Materials and Methods). As shown in Fig. 1B, treatment of MC cells with 100 nM TSA for 24 h resulted in a 108-fold increase in expression of DR, but no increase in CIITA was detectable after 60 cycles of PCR. NaBu treatment (1 mM, 48 h) resulted in an 88-fold enhancement in DR and no activation of CIITA (Fig. 1B). Thus, within the limits of these quantitative measurements, the activation of DR gene expression by DAs appears to occur with no change in the level of CIITA transcripts. Although IFN-γ did not induce HLA-DR transcripts on MC cells at any concentration or time point studied, this cytokine did elicit low levels of CIITA mRNA detectable by real-time PCR. Preliminary studies showed that the sequence of transcripts stimulated by IFN-γ in MC cells are very similar to a minor alternatively spliced Raji transcript, which has a deletion in the N-terminal activation domain, and is functionally inactive (26).
To determine whether the effect of DAIs on MHC class II was manifested in other tumors and in different species, we tested the effect of TSA on two commonly used mouse tumor models: the Colon 26 adenocarcinoma and the J558 plasmacytoma. Analysis by standard RT-PCR at 35 cycles showed that IFN-γ induced expression of CIITA and class II in Colon 26 and, similar to the human MC cell line, CIITA was not detected after TSA treatment (Fig. 2A). Real-time PCR analysis of Colon 26 cells was consistent with the RT-PCR data and demonstrated that TSA induced a 74-fold increase in Ia in the absence of CIITA, whereas IFN-γ elicited both CIITA (20-fold) and Ia (58-fold) expression. The J558 cell line behaved quite differently from Colon 26 in that IFN-γ did not activate either CIITA or class II expression (Fig. 2B). Because the classical IFN-γ-inducible genes IFN regulatory factor-1 and guanylate binding protein, as well as MHC class I mRNA, were not induced by IFN-γ in J558 (data not shown), this cell line may have a signaling defect in the IFN-γ pathway. However, unlike either the MC or Colon 26 cells, TSA induced both CIITA (102-fold) and Ia (537-fold) in J558 as shown in Fig. 2. In different experiments, maximum expression at 12 h occurred at TSA concentrations

FIGURE 1. Activation of MHC class II gene expression by IFN-γ and DAIs in the SK-N-MC human neuroblastoma cell line. A, MC cells were treated with 100 U/ml of IFN-γ, 100 nM TSA, or 1 mM NaBu and mRNA for HLA-DR amplified by RT-PCR at the time of maximal expression (24 h for TSA and 48 h for NaBu). DR is induced in MC by TSA in the absence of detectable mRNA for CIITA. Raji is a constitutively positive B cell control. B, Real-time PCR experiments on the same RNA preparations demonstrate that TSA induced a 108-fold and NaBu an 88-fold increase in mRNA for DR, whereas IFN-γ failed to elicit significant increases in three separate experiments. CIITA mRNA was not detectable (ND) at 60 cycles in controls or after TSA or NaBu treatment. IFN-γ consistently induced low levels of CIITA mRNA that were observed (fold changes from 3.5 to 11) by real-time PCR in three experiments. GAPDH mRNA remained essentially unchanged in both the RT-PCR and real-time PCR experiments (data not shown).

FIGURE 2. Contrasting patterns of CIITA and class II expression in response to IFN-γ and TSA in two murine cell lines. A, Colon 26 cells treated for 48 h were examined by RT-PCR (upper left) and real-time PCR (upper right). IFN-γ induced expression of both CIITA (20-fold) and Iα (58-fold), TSA elicited mRNA for Iα (74-fold), whereas CIITA remained at control levels and was not detectable (ND) at 60 cycles. B, J558 cells treated for 24 h were not responsive to IFN-γ, but CIITA was induced (102-fold) and Iα (537-fold) by TSA. A20 is a constitutively positive B cell control.
between 50 and 100 nM and at 24 h between 25 and 50 nM. At higher concentrations or longer incubation times, decreasing expression was associated with increasing apoptosis. The contrasting patterns in regard to CIITA induction suggest the possibility that TSA may function by both CIITA-dependent (in J558) and -independent (in MC and Colon 26) mechanisms in different cell types.

To assess the ability of TSA to induce MHC class II expression in the absence of functional CIITA, we analyzed the human CIITA mutant cell line RJ2.2.5 (27) that has been thoroughly characterized as functionally deficient in CIITA. The RJ2.2.5 CIITA mutation results in deletion of the activation domain (28) of CIITA, and this cell line does not express HLA-DR in response to IFN-γ.

FIGURE 3. Activation of MHC class II gene expression by TSA in the RJ2.2.5 human B cell line deficient in CIITA activity. A, RJ2.2.5 cells were treated with 100 U/ml of IFN-γ, or 50 nM TSA and mRNA for HLA-DR amplified by RT-PCR. IFN-γ did not activate CIITA for HLA-DR. However, DR is induced in RJ2.2.5 by TSA in the absence of CIITA. Raji is a constitutively positive B cell control. B, Real-time PCR experiments on the same RNA preparations demonstrate that TSA induced a 51-fold increase in mRNA for DR, whereas IFN-γ failed to induce expression. Neither IFN-γ nor TSA induced CIITA.

FIGURE 4. Expression of the costimulatory receptor CD40 gene is enhanced by TSA. A, Neuroblastoma MC cells express low levels of CD40 mRNA in untreated control cells as detected by real-time PCR, but not by RT-PCR. CD40 expression was enhanced 5-fold by IFN-γ and 12-fold by TSA at 24 h. B, Colon 26 CD40 mRNA was not detectable by RT-PCR or real-time PCR in control cells. Treatment of Colon 26 with 100 nM TSA for 24 h, elicited maximum CD40 mRNA levels (147-fold induction). Following 100 U/ml of IFN-γ for 24 h, CD40 mRNA remained undetectable at 60 cycles. Experiments using Colon 26 cells at different time points (12 and 48 h) also showed enhanced CD40 with TSA but not with IFN-γ (data not shown). C, CD40 mRNA was detected in control samples of J558 cells and the low levels did not change significantly after incubation with IFN-γ. After treatment with TSA (25 nM for 24 h), CD40 was enhanced 161-fold. Two different experiments with each cell type yielded similar results.
TSA treatment of this cell line resulted in a 51-fold increase in mRNA for HLA-DR (Fig. 3) and in the appearance of substantial levels of cell surface DR (data not shown). These data support the existence of a CIITA-independent TSA induction of MHC class II expression.

Activation of the CD40 gene by TSA

Because of the importance of costimulatory molecules in the cellular activation mechanism involving MHC complexes, we explored whether DAIs altered the expression of B7-1, B7-2, or CD40 genes. Although no consistent changes in B7-1 and B7-2 were detected, CD40 expression was enhanced by TSA in all three cell lines (Fig. 4). MC cells constitutively expressed low levels of CD40 and IFN-γ enhanced CD40 mRNA levels 5-fold, whereas TSA elicited a 12-fold increase by real-time PCR (Fig. 4A). Colon 26 cells did not constitutively express CD40 and mRNA levels remained undetectable at 60 cycles after IFN-γ treatment, although TSA induced a 147-fold increase in CD40. J558 cells showed low constitutive levels of CD40 mRNA that did not change after treatment with IFN-γ, whereas TSA induced a 161-fold increase in CD40. The lack of induction of CD40 by IFN-γ in J558 cells is consistent with the failure of IFN-γ to activate other IFN-γ inducible genes. The failure of IFN-γ to induce CD40 mRNA in Colon 26, which has no apparent defect in the IFN-γ signaling pathway, is unexplained, but may be related to the ability of TSA to activate factors in addition to those elicited by IFN-γ that are required for CD40 gene expression.

Flow cytometry analysis of cell surface protein expression of MHC class I, II, and CD40 on tumor cell lines

To determine the effect of TSA on cell surface protein expression, the J558 cell line was subjected to FACScan analyses. Of 13 markers analyzed, only class I, II, and CD40 were enhanced at low concentrations of TSA. Levels of several nonexpressed Ags were not elicited (including CD25, β7 integrin, CD40L, H-2Kk, and I-Ak). Other markers were expressed, but were not altered (including CD44, CD54, CD80, CD86, and CD119) by TSA treatment. In each cell line tested, TSA significantly induced the expression of MHC class II protein on the cell surface with induction ranging from 3- to 21-fold in different cells, as indicated by the percent positive cells shown in Fig. 5. The highest expression (21-fold induction) was achieved on J558 cells after 24 h of treatment with 25 nM TSA. IFN-γ did not induce class II on any of the cell lines described here. MHC class I expression was also found to be enhanced by TSA in all three cell types, but, in contrast to MHC class II, TSA was less effective than IFN-γ in MC and Colon 26, whereas J558 was unresponsive to IFN-γ. However, similar to the class II data, TSA induced expression of CD40 on all three cell types and did so more effectively than IFN-γ.

Effect of TSA on the cell cycle and apoptosis

TSA has been described as a cell cycle inhibitor that induces both G1 and, especially, G2-M blocks (17). MHC class II, but not class I, has been reported (29, 30) to be regulated differentially during the cell cycle. We therefore analyzed the cell cycle of our tumor cell line and found that TSA induced a 51-fold increase in mRNA for HLA-DR and in the appearance of substantial levels of cell surface DR. These data support the existence of a CIITA-independent TSA induction of MHC class II expression.
FIGURE 6. Cell cycle regulation of MHC class II by TSA. J558 cells were treated for 24 h with 10 nM TSA or 10 μM nocodazole and stained for cell surface expression of MHC class II. The cell cycle was analyzed by DNA staining with PI. A, The DNA staining after 10 nM TSA treatment (heavy line) superimposed on the DNA staining of untreated control cells (dotted line). The TSA and control curves are essentially identical. B, The DNA staining of 10 μM nocodazole-treated J558 cells (heavy line) superimposed on the DNA staining of control cells (dotted line) showing that nearly 100% of the cells have accumulated in G2-M after nocodazole treatment. C, The MHC class II staining of the nocodazole-treated cells. D, The MHC class II staining after TSA treatment. C and D, The shaded peak represents isotype control staining, the dotted line untreated cells, and the heavy line treated cells (data not shown).

Discussion

Using cells treated with low concentrations of DAIs that produced little or no apoptosis, and maintained an essentially normal cell cycle, we demonstrated that a human neuroblastoma cell line and two mouse tumor cell lines could be induced to express MHC class II mRNA by the deacetylase inhibitor TSA. In all three tumors, IFN-γ failed to induce MHC class II Ags on the cell surface, but, following TSA treatment, each cell line elicited substantial expression detected by flow cytometry. Enhanced expression of class I and CD40 were elicited by low concentrations of TSA, whereas the expression of 10 other surface Ags remained essentially unchanged. Higher concentrations of TSA induced Fas and FasL expression and apoptosis. Thus, as expected, various genes appear to differ in their sensitivity to DAIs that likely reflects inherent differences in chromatin structure and nucleosomal positioning in the promoters regulating these genes.

The current paradigm for MHC gene expression suggests that CIITA is required for constitutive and induced class II transcription. IFN-γ enhanced MHC class I expression, and perhaps plays a nonessential role in maintaining constitutive levels of transcription of class I. This study, together with other recently reported evidence (21, 32–34), suggests that, under specific experimental
Inhibition of CIITA transcription could be mediated by a repressor, a regulator of terminal B cell differentiation that has been shown to associate with HDACs (41). The surface expression of class II on TSA-treated J558 cells is high, approaching that on B cells and IFN-γ inducible cells; compared with the relatively low levels found in the CIITA-negative MC and Colon 26 cells. This suggests that, although class II expression may occur without CIITA expression, CIITA may be required for high levels of expression. The difference in inducibility of CIITA by TSA observed in the cell lines in this study could be related to the distinct promoters (PI→PIV) and their differential usage in regulating CIITA in various cell types (42). J558 is of B cell lineage and should preferentially utilize PIII, the B cell promoter. One possibility is that, in the transition from a B cell to a plasma cell, the PII promoter is repressed by chromatin and that this is reversed by TSA, leading to the activation of CIITA and class II in plasma cells.

In summary, DAIs could function in activating MHC class II in different cell types by one of several mechanisms that are not mutually exclusive: 1) Acetylating histones at the MHC promoter, thus providing a function normally performed by the CIITA-CBP/p300 coactivation complex. 2) Activating expression of the CIITA gene that is repressed by chromatin. This would include CIITA promoters that are methylated on CpG islands and bind methyl CpG binding proteins that recruit HDACs (16). In the trophoblast cell line, Jar, the PIV CIITA promoter is methylated and, although 5-azacytidine alone does not activate CIITA and class II, such treatment does support low level expression after IFN-γ treatment (43). In view of the potential synergy of demethylation and DAIs in activating certain epigenetically silenced genes (44), future studies of the effect of combinations of DAIs and demethylating agents will be of interest. 3) Altering the levels and/or activation state of essential cofactors such as CBP/p300. 4) Direct acetylation of the CIITA protein, or other factors required in class II transactivation, by DAIs could also affect their activity or nuclear transport.

Because the class I and II promoters show significant sequence homologies (6), and both utilize CIITA as a coactivator (4, 5), DAIs may be functioning by similar mechanisms in both class I and II. Tissue specific expression of class I is determined, in significant part, by transcriptional mechanisms that depend on upstream negative regulatory elements in the class I promoter (45) and factors binding to these elements may recruit HDACs and repress transcription. The mechanism of TSA activation of CD40 is unclear and we are not aware of data on the promoter structure of CD40 that would help clarify this issue. The observation that TSA can induce surface expression of CD40 has potential biological implications. CD40 ligand binds to the CD40 receptor and promotes tumor killing by CTLs, directly induces apoptosis of certain tumor cells (46), as well as enhances the ability of normal DC to present Ag and elicit CTLs (47). Thus, up-regulating CD40 expression could potentially be useful in future attempts to treat cancers by the administration of soluble CD40L or cells genetically modified to express CD40L genes.

Epigenetic phenomena including methylation and histone acetylation are critical in regulating gene expression through repression (14), and complex patterns of chromatin activation and repression are undoubtedly involved in many fundamental immune processes (48–50). These alterations in chromatin structure would likely involve changes in the pattern of histone acetylation. Thus, agents that hyperacetylate histones may be able to substitute for natural signals that release repression during cellular differentiation and, as shown in this study, these same agents may elicit expression of repressed genes critical in the immune response against tumors. The work reported here also suggests that some of
the variation in immunogenicity and tumorigenicity of different tumors may be mediated by regulatory events involving reversible acetylation patterns, and these could potentially be altered by treatment or targeting with agents that alter acetylation.

Acknowledgments

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