Curcumin, a Novel p300/CREB-binding Protein-specific Inhibitor of Acetyltransferase, Represses the Acetylation of Histone/Nonhistone Proteins and Histone Acetyltransferase-dependent Chromatin Transcription*

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Acetylation of histones and non-histone proteins is an important post-translational modification involved in the regulation of gene expression in eukaryotes and all viral DNA that integrates into the human genome (e.g. the human immunodeficiency virus). Dysfunction of histone acetyltransferases (HATs) is often associated with the manifestation of several diseases. In this respect, HATs are the new potential targets for the design of therapeutics. In this study, we report that curcumin (diferuloylmethane), a major curcuminoid in the spice turmeric, is a specific inhibitor of the p300/CREB-binding protein (CBP) HAT activity but not of p300/CBP-associated factor, in vitro and in vivo. Furthermore, curcumin could also inhibit the p300-mediated acetylation of p53 in vitro. It specifically represses the p300/CBP HAT activity-dependent transcriptional activation from chromatin but not a DNA template. It is significant that curcumin could inhibit the acetylation of HIV-Tat protein in vitro by p300 as well as proliferation of the virus, as revealed by the repression in syncytia formation upon curcumin treatment in SupT1 cells. Thus, non-toxic curcumin, which targets p300/CBP, may serve as a lead compound in combinatorial HIV therapeutics.

The eukaryotic genome is packaged into a highly complex nucleoprotein structure, chromatin. Although apparently repressive, a precise organization of chromatin is essential for replication, repair, recombination, and chromosomal segregation. Alteration in chromatin organization modulates the expression of underlying genes, which also includes the genes of integrated viral genomes (1). These dynamic changes in the chromatin structure are brought about by post-translational modifications of the amino-terminal tails of the histones and the ATP-dependent chromatin remodeling (2). Specific amino acids within the histone tail are the sites of a variety of modifications, including phosphorylation, acetylation and methylation. Among these, acetylation of histones and nonhistone proteins plays a pivotal role in the regulation of gene expression. A balance between acetylation and deacetylation states of these proteins forms the basis of the regulation of transcription. Dysfunction of histone acetyltransferases and histone deacetylases is often associated with the manifestation of several diseases, including cancer, cardiac hypertrophy, and asthma (3–5). These enzymes, therefore, are potential new targets for therapy.

Histone acetyltransferases (HATs)1 modulate gene expression by catalyzing targeted acetylation of the e-amino group of lysine residues on histones and nonhistone proteins. HATs can be classified into several families based on the number of highly conserved structural motifs. These include the GNAT family (Gcn5-related N-acetyltransferase; e.g. Gcn5, p300/CBP, the MYST ( MOZ, YBF2/SAS3, and TIP60) group, and the p300/CBP family (4, 6). The p300 and CBP are ubiquitously expressed global transcriptional coactivators that have critical roles in a wide variety of cellular phenomena including cell cycle control, differentiation, and apoptosis (7, 8). The transcriptional coactivator function of these two proteins is partially facilitated by their intrinsic HAT activity (9). Significantly, p300/CBP also acetylates several nonhistone proteins with functional consequences. The most notable example is the acetylation of p53. p300/CBP directly interacts with p53 and acetylates the tumor suppressor in vivo and in vitro to enhance its transcriptional activation ability (4, 10) and consequently DNA repair. Mutation in the HAT active site abolishes transactivation capability of p300/CBP (11). Analysis of colorectal, gastric, and epithelial cancer samples show that in several instances, there is a mis-sense mutation as well as deletion mutations in the p300 gene (12). 80% of glioblastoma cases have been associated with the loss of heterozygosity of the p300 gene (13). In acute myeloid leukemia, the gene for CBP is translocated and fused to either the monocytic leukemia zinc finger (MOZ) gene or to MLL (a homeoeator regulator, mixed lineage leukemia). In both cases, 1 The abbreviations used are: HAT, histone acetyltransferase; CBP, CREB-binding protein; CREB, cAMP-response element-binding protein; p300, p300/CBP-associated factor; HDAC, histone deacetylase; CTPB, N-(4-chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide; HIV, human immunodeficiency virus.
the HAT activity of CBP remains intact. However, the fusion proteins cause aberrant gene expression through improper targeting of the genes. Retention of partial HAT function by the fusion protein may result in the altered regulation of the target gene, whereas a loss of function of the fusion protein may result in the normal gene not being transcribed at all. In either case, this results in aberrant cell cycle regulation, leading to cancer (4). Mutations in HATs cause several other disorders apart from cancer. Rubinstein-Taybi syndrome has been found to be a result of mutations in CBP (4). A single mutation at the PHD-type zinc finger in the HAT domain of CBP, resulting in an alteration of a conserved amino acid (E1278K), causes this syndrome. It is interesting that this mutation in CBP also abolishes its HAT activity (14, 15). The degradation of p300/CBP is also found to be associated with certain neurodegenerative diseases (16). Though the role of HAT activity in cardiac hypertrophy is still speculative, overexpression of p300 is sufficient to induce hypertrophy. The HAT domain of p300 was found to be essential for the stimulation of hypertrophy (17). Hyperacetylation of histones was also observed in the lung cells under asthmatic conditions (17). Chromatin analysis of the HIV genome shows that a single nucleosome (nuc-1) located at the transcription start site is specifically disrupted during transcriptional activation. Treatment of the cell lines latently infected with HIV-1 with histone deacetylase inhibitors (e.g., trichostatin A/trapoxin/valproic acid) causes a global acetylation of the cellular histones. Therefore, this treatment also results in the transcriptional activation of the HIV promoter and a robust increase in virus production (18, 19). Furthermore, recruitment of HDAC1 near nuc-1 by host factors YY1 and LSF to the HIV-1 long terminal repeat have been shown to inhibit transcription by maintaining nuc-1 in the hypoacetylated state (20, 21). Taken together, these data establish the fact that histone acetylation of nuc-1 is at least partly essential for the multiplication of HIV-1. Acetylation of the HIV-1 transactivator Tat by p300, PCAF, and human GCN5 has also been demonstrated to be important for HIV transcriptional activity (22–24; reviewed in Ref. 25). Therefore, both HAT and HDAC modulators (activator/inhibitor) could serve as new generation anti-HIV therapeutics.

Significant progress has been made in the field of histone deacetylase inhibitors as antineoplastic drugs and also against cardiac hypertrophy. However, very few inhibitors of histone acetyltransferases are known so far. Availability of recombinant HATs made it possible to synthesize and test more target-specific inhibitors, Lys-CoA for p300, PCAF, and human GCN5 (26). Although it has been extensively employed for in vitro transcription studies, cells are not permeable to Lys-CoA (27). Recently, we have isolated the first naturally occurring HAT inhibitor, anacardic acid, from cashew nut shell liquid and garcinol from Garcinia indica, which are nonspecific inhibitors of p300/CBP and PCAF but are capable of easily permeating the cells in culture (28, 29). Different chemical modifications of

![Figure 1](https://example.com/figure1.png)
these inhibitors were attempted to identify enzyme-specific inhibitors, but it serendipitously led to the synthesis of the only known p300-specific activator, N-(4-chloro-3-trifluoromethylphenyl)-2-ethoxy-6-pentadecyl-benzamide. Herein, we describe the discovery of curcumin as the first p300/CBP-specific cell permeable HAT inhibitor. We have shown that it does not affect the HAT activity of PCAF or histone deacetylase and methyltransferase activities. However, p300 HAT activity-dependent chromatin transcription was efficiently repressed by curcumin but not transcription from DNA template. It could also inhibit the acetylation of histones in vivo. Significantly, curcumin repressed the multiplication of human immunodeficiency virus 1 (HIV1) and also inhibited the acetylation of HIV-Tat protein.

**EXPERIMENTAL PROCEDURES**

**Purification and Structural Analysis of Curcumin**—Curcumin was purified from *Curcuma longa* rhizome extract as described previously (29). The purity and identity of the compound was determined by mass spectroscopy and NMR spectroscopy. The purified compound was stored at room temperature and dissolved freshly in Me2SO for each use.

**Histone Acetyltransferase Assay**—HAT assays were performed as described previously (30). 2.4 µg of highly purified HeLa core histones were incubated in HAT assay buffer containing 50 mM Tris-HCl, pH 8.0, 10% (v/v) glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA, pH 8.0, and 10 mM sodium butyrate at 30 °C for 10 min with or without baculovirus-expressed recombinant p300/CBP or PCAF in the presence and absence of curcumin using either G9a (C) or nuclear extract (NE) (D) as the enzyme sources. The reaction products were trichloroacetic acid-precipitated, resolved on 15% SDS-PAGE, and subjected to fluorography followed by autoradiography. B and C, lane 1, histones; lane 2, histones with enzyme (nuclear extract/G9a), lane 3, histones with the enzymes and Me2SO, lanes 4–6, histones with the enzymes in the presence of 25, 50, and 100 µM concentrations of curcumin, respectively. SAM, (S)-adenosyl-methionine.

**Histone Acetyltransferase Assay**—HAT assays were performed as described previously (30). 2.4 µg of highly purified HeLa core histones were incubated in HAT assay buffer containing 50 mM Tris-HCl, pH 8.0, 10% (v/v) glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA, pH 8.0, and 10 mM sodium butyrate at 30 °C for 10 min with or without baculovirus-expressed recombinant p300/CBP or PCAF in the presence and absence of curcumin using either G9a (C) or nuclear extract (NE) (D) as the enzyme sources. The reaction products were trichloroacetic acid-precipitated, resolved on 15% SDS-PAGE, and subjected to fluorography followed by autoradiography. B and C, lane 1, histones; lane 2, histones with enzyme (nuclear extract/G9a), lane 3, histones with the enzymes and Me2SO, lanes 4–6, histones with the enzymes in the presence of 25, 50, and 100 µM concentrations of curcumin, respectively. SAM, (S)-adenosyl-methionine.
the presence or absence of curcumin for 10 min at 30 °C. After the initial incubation, 1 μl of 8.3 Ci/mmol [3H]S-adenosyl methionine was added to the reaction mixtures, and the incubation continued for 1 h (in the case of nuclear extract) or for 15 min (in the case of G9a). The reaction products were TCA-precipitated, resolved on 15% SDS-PAGE, and subjected to fluorography followed by autoradiography.

For the deacetylation assays, histones were acetylated by the recombinant p300 (20 ng) using 2.4 μg of core histones and 1 μl of 4.7 Ci/mmol [3H]acetyl CoA in HAT assay buffer without sodium butyrate for 30 min at 30 °C (29). The activity of p300 was inhibited by incubating the reaction mixture with a 5 μM concentration of the p300-specific inhibitor Iysyl-CoA (20) for 15 min at 30 °C, after which 50 ng of baculovirus-expressed recombinant HDAC1 was added in the presence or absence of curcumin and incubated further for 45 min at 30 °C. The samples were processed as described above.

**In Vitro Chromatin Assembly**—Chromatin template for in vitro transcription experiments was assembled and characterized as described elsewhere (9).

**In Vitro Transcription Assay**—Transcription assays were carried out as described elsewhere (9) with the necessary modifications. The schematic representation of the in vitro transcription protocol is given in Fig. 4A. The reconstituted chromatin template (containing 30 ng of DNA) or an equimolar amount of histone-free DNA was incubated with 50 ng of activator (Gal4-VP16) in a buffer containing 4 mM HEPES, pH 7.8, 20 mM KCl, 2 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 10 mM sodium butyrate, 0.1 mg/ml bovine serum albumin, and 2% glycerol. Baculovirus-expressed recombinant full-length p300 was preincubated with indicated amounts of curcumin at 20 °C for 20 min, after which it was added to the transcription reaction, and the acetylation reaction was carried out for 30 min at 30 °C. HeLa nuclear extract (5 μl, which contains 8 mg/ml protein) was added then to initiate the preinitiation complex formation. Transcription reaction was started by the addition of NTP-mix and [α-32P]UTP after the preinitiation complex formation and incubated for 40 min further at 30 °C. For loading control, a separate reaction was set up with ~25 ng of supercoiled ML200 DNA, and the transcription assay was carried out as described above, without the addition of the activator (Gal4-VP16), and 2 μl of this reaction was added to each of the transcription reactions. Reactions were terminated by the addition of 250 μl of stop buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl, 1% SDS, and 0.025 ng/μl RNA). Transcripts were analyzed by 5% Urea-PAGE, and quantification of transcription was done with the use of the Fujix Bioimaging Analyzer. To visualize the transcripts, the gels were exposed to x-ray film.

**Acetic Acid/Urea/Triton X-100 (AUT) Polyacrylamide Gel Electrophoresis and Western Blotting for In Vivo-Acetylated Histones**—HeLa cells (3 × 10⁶ cells per 90-mm dish) were seeded overnight, and histones were extracted from 24 h of compound treated cells as reported previously (29, 32). In brief, cells were harvested, washed in ice-cold buffer A (150 mM KCl, 20 mM HEPES, pH 7.9, 0.1 mM EDTA, and 2.5 mM MgCl₂) and lysed in buffer A containing 250 mM sucrose and 1% (v/v) Triton X-100. Nuclei were recovered by centrifugation and washed, and proteins were extracted for 1 h using 0.25 M HCl. The proteins were precipitated with 25% (w/v) trichloroacetic acid and sequentially washed with ice-cold acidified acetone (20 μl of 12 N HCl in 100 ml of acetone) and acetone, air-dried, and dissolved in the sample buffer (5.8 M urea, 0.9 M glacial acetic acid, 16% glycerol, and 4.8% 2-mercaptoethanol). The histones (equal amounts in all lanes) were resolved on AUT gel as described elsewhere (33, 34).

For Western blotting, the quantitated protein samples were run on a 12% SDS-polyacrylamide gel; after electrophoresis, proteins on the gel were electrotransferred onto an Immobilon membrane (polyvinylidene difluoride; Millipore Corp., Bedford, MA). The membranes were then blocked in 5% nonfat dry milk solution in 1× PBS containing 0.05% Tween 20 and then immunoblotted with anti-acetyl H3 (Calbiochem), anti-acetyl H4 (a kind gift from Dr. Alaine Verrault), or anti-H3. Detection was performed using goat anti-rabbit secondary antibody (Bangalore Genei), and bands were visualized with the use of the ECL detection system (Pierce).

**Apoptosis Assay**—Curcumin-induced apoptosis was monitored by the extent of nuclear fragmentation. Nuclear fragmentation was visualized by Hoechst staining of apoptotic nuclei. The apoptotic cells were collected by centrifugation, washed with PBS, and fixed in 4% paraformaldehyde for 20 min at room temperature. Thereafter, the cells were washed and resuspended in 20 μl of PBS before deposition on polysine-coated coverslips and were left to adhere on cover slips for 30 min at room temperature, after which the cover slips were washed twice with PBS. The adhered cells were then incubated with 0.1% Triton X-100 for 5 min at room temperature and rinsed three times with PBS. The coverslips were treated with Hoechst 33258 for 30 min at 37 °C, rinsed with PBS, mounted on slides with glycerol-PBS, and processed as described previously (29).

**Transient Transfection and Immunoprecipitation**—293 T cells were transfected with CMV-p53 and CMV-p300 using LipofectAMINE 2000 reagent (Invitrogen) according to the manufacturer’s instructions. The medium was replaced, and the cells were incubated for an additional 24 h with curcumin (100 μM) or vehicle (Me₂SO). The cells were harvested using radioimmunoprecipitation assay buffer (150 mM NaCl, 50 mM Tris, pH 7.4, 1% Nonidet P-40, 0.1% sodium deoxycholate, 1 mM EDTA, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, and 0.5 μg/ml pepstatin), and the p53 protein was immunoprecipitated from the lysates using mouse monoclonal anti-p53 antibody, DO1 (oncogene). The immunocomplexes were bound on protein G-Sepharose beads (Amersham Biosciences) for 12 h at 4 °C, washed three times with radioimmunoprecipitation assay buffer, and subjected to SDS-PAGE on a 10% gel followed by Western blotting using mouse monoclonal anti-p53 antibody, pAb421, or mouse monoclonal anti-acetylated lysine antibody.

**Syncytium Inhibition Assay**—The cell lines SupT1 and H9/HTLV-IIB harboring the HIV-1 LTR promoter, obtained from the NIH AIDS Research and Reference Reagent Program, were kindly provided by Dr. Paul Clapham, Harvard University. The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. Curcumin in Me₂SO was added to the cells to final concentrations of 0.1–10 μM, and the cells were incubated for 12 h at 37 °C. The cells were then washed and resuspended in fresh medium containing 10% FBS and antibiotics. The cultures were then treated with 10 μg/ml of l-phenylalanine for 24 h, and the syncytia were scored after washing with PBS and incubation for 48 h.
Me2SO, including the control wells. Formation of syncytia was visible under light microscope within 12 h. The total number of syncytia in 10 representative wells was counted at different time points (12, 24, and 48 h); data for the 12-h time point are presented. Identical results were obtained at other time points. All the assays were performed in triplicate wells, and the experiment was performed two times.

RESULTS AND DISCUSSION

Screening of plant extracts known to possess anticancer properties led us to a polyphenolic compound from Curcuma longa rhizome, which is a potent and specific inhibitor of histone acetyltransferases p300/CBP but not of PCAF. By employing mass spectroscopy and NMR spectroscopy, it was identified as curcumin. As can be seen from both the filter binding (Fig. 1A) and gel (Fig. 1, B–D) HAT assays, the acetylation of histones H3 and H4 by p300/CBP was strongly inhibited by curcumin (Fig. 1, A–C, lanes 5–7), with an IC50 of ~25 μM, whereas the PCAF HAT activity showed no change even in the presence of 100 μM curcumin (Fig. 1, A and D). These data establish curcumin as the first known p300/CBP-specific natural HAT inhibitor.

To ensure absolute enzyme specificity, we went on to check the effect of curcumin on HDAC1 and histone methyltransferase activities. Deacetylation of acetylated core histones by recombinant baculovirus-expressed histone deacetylase 1 (HDAC1) was not affected by the presence of curcumin (Fig. 2, A, lane 3 versus lanes 7 and 8, and B, lane 2 versus lanes 4 and 5). We have also investigated the effect of curcumin on histone methyltransferase activity. HeLa core histones were methylated with [3H]S-adenosyl methionine by recombinant lysine methyltransferase G9a, which specifically methylates lysine residues 9 and 27 of histone H3 (31) in the presence or absence of curcumin. As depicted in Fig. 2C, histone methylation by G9a remains the same in the presence or absence of curcumin (Fig. 2C, lane 3 versus lanes 4–6). Furthermore, to find out whether curcumin had any effect on other histone methyltransferases, we have performed HMTase assay using HeLa nuclear extract as a source of other histone methyltransferase enzymes. Similar to G9a-mediated methylation of core histones, methylation by nuclear extract showed no difference whatsoever in the presence or absence of curcumin (Fig. 2D, lane 3 versus lanes 4–6). Taken together these results suggest that curcumin is absolutely specific for the histone acetyltransferase activity of p300/CBP but not other enzymes for which histones are the substrate. It also indicates that curcumin presumably binds to the enzyme rather than the substrate histones.

We went on to characterize the nature of inhibition of curcumin on the p300 HAT activity. Enzyme kinetics was studied to understand the mechanism of curcumin-mediated inhibition of p300 HAT activity by changing both acetyl CoA (Fig. 3A) and
FIG. 5. Curcumin induces apoptosis (A) and inhibits acetylation of histones and p53 in vivo (B–D). A, Hoechst staining of untreated HeLa cells and cells treated with Me2SO and 75 and 100 μM curcumin. Arrows indicate apoptotic nuclear fragmentation. B, HeLa cells were treated as indicated for 24 h; histones were extracted and separated on an 18% AUT PAGE. The protein bands were visualized by Coomassie Brilliant blue staining. Lane 1, histones extracted from untreated cells; lane 2, Me2SO (solvent control) treated cells; lane 3, curcumin (75 μM) treated cells; lane 4, curcumin (100 μM) treated cells; lane 5, trichostatin (2 μM) and sodium butyrate (10 mM) treated cells; lane 6, trichostatin A (2 μM), sodium butyrate (10 mM), and curcumin (100 μM) treated cells are shown. Asterisk (*) indicates hyperacetylation of histones in response to HDAC inhibition by TSA and sodium butyrate. Arrow (→) indicates inhibition of TSA-induced hyperacetylation of H4 and H2B by curcumin. C, the acid-extracted histones were resolved over 12% SDS-PAGE and were analyzed by Western blot using antibodies against acetylated histone H3 and H4. Loading and transfer of equal amounts of protein were confirmed by immunodetection of histone H3. D, 293 T cells were transiently transfected with...
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histone concentrations (Fig 3B) keeping the other constant at a time. In both cases, $K_m$, $V_{max}$, and $K_{cat}$ decreased, indicating that curcumin binds not to the active sites of either histone or acetyl CoA but to some other site on the enzyme. Presumably, this binding site on p300/CBP is specific for curcumin, and binding leads to a conformational change, resulting in a decrease in the binding efficiency of both histones and acetyl CoA to p300. In this connection, curcumin behaves in a unique manner compared with another polyphenolic cell-permeable HAT inhibitor, garcinol, in which case, upon changing the concentration of acetyl CoA, the inhibitor acts as an uncompetitive type but as a competitive inhibitor for core histones (29).

To investigate the effect of curcumin on transcription, in vitro transcription experiments were performed using DNA and reconstituted chromatin template. Transcription from the DNA template was not affected by curcumin even at 300 $\mu$m (Fig. 4B, lane 3 versus lanes 4-7). Significantly, increasing concentrations of curcumin repressed p300 HAT activity-dependent chromatin transcription up to 3-fold at 100 $\mu$m and up to 8-fold at 300 $\mu$m. (Fig. 4C, lane 4 versus lanes 7 and 9). Taken together, these data suggest that curcumin is a potent and specific inhibitor of p300 HAT activity in the transcriptional context.

Curcumin permeates the cell, and it is known to have a role in cancer chemoprevention and also in tumor growth suppression (35). Exposure of tumor cells to curcumin in vitro results in the inhibition of cell proliferation and also induction of apoptosis (36). Consistent with the previous reports on other cell lines, treatment of HeLa cells with curcumin induces apoptosis (Fig. 5A, after a 24-h exposure to 75–100 $\mu$m of the compound). Because curcumin inhibits p300/CBP HAT activity in vitro, we were interested to find out the effect of curcumin on the acetylation of histones in vivo. Histones were isolated from curcumin-treated cells and subjected to AUT polycaryamide gel electrophoresis. Although it is difficult to detect a change in overall acetylation status from the histone fractions, treatment with curcumin modestly increased the unacetylated form of histone H4 and H2B (Fig. 5B, compare lane 2 with lanes 3 and 4). To visualize the effect of curcumin on in vivo histone acetylation more distinctly, histones were hyperacetylated by the treatment with deacetylase inhibitors, TSA and sodium butyrate (Fig. 5B, compare lane 1 and lane 5). When these cells were treated with 100 $\mu$m curcumin, acetylation of histones was found to be inhibited as marked by the appearance of more unacetylated H4 and H2B (Fig. 5B, lane 5 versus 6). In vitro acetylation experiments suggested that p300/CBP mediated acetylation of H3 and H4 was strongly inhibited by curcumin, although the overall analysis of total histones by AUT polycaryamide gel electrophoresis showed no significant change in the histone H3 acetylation. Therefore, we employed Western blotting analysis to check the extent of histone acetylation upon curcumin treatment. As depicted in Fig. 5C, acetylation of both H3 and H4 were significantly (6-fold for H3 and 10-fold for H4; Fig. 5C, lane 5 versus 6) inhibited in the presence of curcumin in vivo. These results convincingly establish curcumin as a potent inhibitor of p300/CBP HAT activity in vitro and in vivo. Because p300/CBP also possesses factor acetyltransferase activity and acetylates several nonhistone proteins with functional consequences, we were interested to find out the effect of curcumin on p53 acetylation by p300 in vivo. Cells (293 T) were transfected with p53 and p300 mammalian expression vectors, and p53 was immunoprecipitated by anti-p53 monoclonal anti-body. Analysis of the immunoprecipitated protein by Western blotting shows that incubation of the cells with curcumin completely inhibits the p300-mediated acetylation of p53 (Fig. 5D, compare lane 2 and lane 5). It is interesting that p53 could be acetylated by the endogenous factor acetyltransferases even without the transfection of p300 (Fig. 5D, lane 1). However, the endogenous p53 does not seem to be acetylated by the overexpressed p300 (Fig. 5D, lane 3 versus lane 1). This could be because the proteins do not localize together. Significantly, the acetylation status of the endogenous p53 is not altered in the presence of curcumin (Fig. 5D, lane 3 versus lane 6), suggesting that other factor acetyltransferases (GCN5/PCAF/TIP60) (37, 38) could acetylate p53 and that this acetylation was not inhibited by curcumin (also see Fig. 5D, compare lane 1 and lane 4). This result points out to the fact that the in vivo target of curcumin is p300/CBP and not the other factor acetyltransferases.

p53 is often referred to as the “guardian of the genome,” and its importance is emphasized by the discovery of mutations of p53 in more than 50% of all human cancers. One of the key regulators of p53 function is acetylation. The acetylation levels of p53 are significantly enhanced in response to every type of stress in vitro (10). This acetylation enhances the activation and stabilization of p53 (39). p53 acetylation is critically important for the recruitment of coactivators (which also includes the acetyltransferases) to promoter regions and the activation of p53-targeted genes in vivo. (40). Therefore, inhibition of p300-specific acetylation of p53 by curcumin should be helpful for the molecular elucidation of acetylation-dependent regulation of p53 function.

Curcumin exhibits a variety of pharmacological effects including anti-tumor, anti-inflammatory, and anti-infectious activities. It was found to be a potent inhibitor of the HIV-1 integrase (41). Furthermore, curcumin could also inhibit the HIV-1 Tat-mediated transactivation (42) and the UV induced activation of the HIV-LTR gene expression, presumably through the inhibition of nuclear factor-κB activation (43). Although these reports suggest that curcumin may act as a repressor of HIV multiplication, its effect on viral replication was not demonstrated. During the course of infection, the HIV genome is integrated into the human chromatin. A single nucleosome called nc1 is precisely positioned immediately after the transcription start site in cell lines where the HIV promoter is silent. The nc1 is disrupted during transcriptional activation by acetylation (18). It was elegantly demonstrated that histone deacetylase inhibitors, such as trichostatin A, trapoxin, valproic acid and sodium butyrate activate the transcription from HIV promoter. HIV transcriptional activation after the treatment with HDAC inhibitors is associated with nc1 remodeling (19). Moreover, it has been demonstrated that acetylation of HIV-1 transactivator Tat by p300 is important for its transcriptional activity (22). Thus, presumably curcumin would be an effective agent to stop the growth of this virus, through the inhibition of nc-1 histones and Tat acetylation. We have tested this possibility by investigating the effect of curcumin on syncytia formation upon viral infection to SupT1 cells.

Various experimental formats have been used to evaluate infection of target cells by HIV or inhibition of the viral infection in the presence of an anti-viral compound (44, 45). The standard format is to add diluted viral stock to the target cells at a known multiplicity of infection (m.o.i.) in the presence or...
absence of an antiviral compound and monitor the synthesis of
the viral structural protein p24 or the enzyme reverse tran-
scriptase (46, 47). In the natural context, the viral transfer is
more efficient through cell-to-cell contact rather than free virus
infecting a target cell. Prevention of the viral transfer between
cells is technically more difficult than neutralizing the free
virus. SupT1 cells are highly permeable for HIV-1, and these
cells make numerous and large syncytia when infected with the
virus. Taking advantage of this property, we co-cultured SupT1
cells in the presence of H9/HTLV-IIIb NIH 1983 cells that
produce a T-cell tropic virus. A dose-dependent reduction in the
number of syncytia was evident with increasing concentration
of curcumin, and no syncytia were seen at the highest concen-
tration of curcumin (Fig. 6A). To rule out the possibility of
cytotoxicity and cell death, we determined the number of viable
cells in all the wells using a trypan blue exclusion analysis. The
cells were healthy even in the presence of 100 μM curcumin at
the end of 48 h, suggesting that the drug was not cytotoxic for
these cells at the concentrations used (data not shown). These
results thus show that the p300-specific HAT inhibitor curcu-
min inhibits the multiplication of HIV, presumably through the
inhibition of acetylation of Nuc1 as well as Tat (18, 22). It is
interesting that we have found that curcumin strongly inhibits
the acetylation of Tat by p300 in vitro (Fig. 6, B and C).

Curcumin is able to inhibit different enzymatic activities
that include HIV-1 integrase (41), nuclear factor-κB activation
(43), and p300-specific HAT/factor acetyltransferase activity.
Repression of the HIV replication by curcumin could be caused
by any of the above or a combination thereof. It has been
established that histone acetylation (of HIV nuc-1) and the
factor (Tat) acetylation is essential for the HIV gene expression
as well as multiplication (18, 22, 23). Combinatorial therapeu-
tics has been the only recourse to preventing the spread of HIV
and that, too, has had only moderate success. The identification
of novel targets, which are involved in the regulation of HIV
disease progression, would help in the design of a multipronged
therapeutic response aimed at the complete eradication of the
virus from the body. In this regard, we have introduced yet
another target for the HIV therapeutics, the histone acetyl-
transferase p300/CBP. By regulating the acetylation of Tat and
nuc1, this HAT mediates the activation of HIV from its latency.
We have also introduced curcumin, a p300/CBP-specific, cell-
permeable, non-toxic (48) HAT inhibitor that has been shown
to inhibit the spread of HIV to the neighboring cells, as high-
lighted by the syncytia formation assay. These results, in conjunction with earlier studies on HAT inhibition, open up a new target with a wide array of potential therapeutic agents in HIV combinatorial therapy. Furthermore, dysfunction of histone acetyltransferases has been found to be associated with several diseases, such as cardiac hypertrophy, asthma, and cancer. In all of these diseases, it has been found that the cellular histone and nonhistone proteins are hyperacetylated (3–5, 49). Thus, the present finding that curcumin, a non-toxic dietary component, is a p300-specific inhibitor may find therapeutic applicability for a wide spectrum of diseases, apart from being used as a probe to dissect the molecular pathways in which p300 HAT activity is involved.

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