Interplay of bromodomain and histone acetylation in the regulation of p300-dependent genes

Jihong Chen, Feras M. Ghazawi, and Qiao Li

Key words: gene regulation, histone acetylation, transcription, acetyltransferase, coactivator, bromodomain

Abbreviations: HAT, histone acetyltransferase; HDAC, histone deacetylase

The bromodomain is an evolutionarily conserved motif found in many transcriptional activators including p300 which contains an intrinsic histone acetyltransferase activity and is a general coactivator for many transcription factors. One mode of bromodomain action is to serve as a binding module to recognize specific acetyl-lysine residue of histones during chromatin remodeling and transcriptional activation. The function of p300 is required for diverse sets of gene expression. However, it is not known whether the p300 bromodomain is involved in the expression of all or only subset of p300-dependent genes. In this study, we examined the impact of either a wild-type or a bromo-deficient p300 on the expression of several p300-dependent genes. The effects of histone acetylation on the expression of these genes were also assessed by targeting histone deacetylase activities with an inhibitor approach. We show that the impact of these inhibitors on the transcriptional activation of p300-dependent genes is impaired in cells containing the bromo-deficient p300, indicating that the interplay of p300 and histone acetylation in p300-dependent gene transcription requires the bromodomain. We also observed an increase in the expression of bromo-deficient p300 at the level of transcription possibly to compensate for the loss of p300 function. However, the high level of bromo-deficient p300 is not able to maintain the basal level of histone acetylation. Thus, the bromodomain is important for p300 to maintain the basal level of histone acetylation and to induce the transcriptional activation of p300-dependent genes. Nevertheless, the requirement of bromodomain and histone acetylation in p300-dependent gene transcription is determined by a gene specific manner.

Introduction

Transcriptional coactivator p300 was identified initially as an E1A-associated protein and later as a tumor suppressor. Mutations in p300 genes have been detected in various epithelial cancers and reintroduction of wild-type p300 leads to growth suppression of the cancer cells. Patients with Rubinstein-Taybi syndrome due to p300 heterozygosity have an increased predisposition to cancer. In addition, E1A, HPV E6 oncoprotein and SV40 large T antigen target p300, further underlining a critical role for p300 in events related to cellular transformation.

The p300 contains an intrinsic histone acetyltransferase (HAT) activity and multiple interaction surfaces for a variety of transcription factors and components of the basal transcription machinery. It thus acts as either a HAT enzyme to acetylate histones or a bridging factor to integrate different regulatory signals in transcriptional activation. One of the p300 domains is an evolutionarily conserved bromodomain which was characterized first for the Drosophila melanogaster Brahma protein and later found in many transcription regulators and nearly all nuclear HAT proteins. The function of bromodomain is important for p300 to recognize specific chromatin substrates and to coordinate chromatin remodeling and transcriptional activation. One mode of bromodomain action is to serve as acetyl-lysine binding module tethering the HAT activity to a defined chromosomal site to achieve highly specific histone acetylation. However, it is not clear whether the bromodomain is required for gene expression of all or only a limited set of p300-dependent genes. In this study, we examined the impact of either a wild-type or a bromo-deficient p300 on the expression of several p300-dependent genes. The effects of histone acetylation on the expression of these genes were also assessed by targeting histone deacetylase activities with an inhibitor approach. We show that the impact of these inhibitors on the transcriptional activation of p300-dependent genes is impaired in cells containing the bromo-deficient p300, indicating that the interplay of p300 and histone acetylation in p300-dependent gene transcription requires the bromodomain. We also observed an increase in the expression of bromo-deficient p300 at the level of transcription possibly to compensate for the loss of p300 function. However, the high level of bromo-deficient p300 is not able to maintain the basal level of histone acetylation. Thus, the bromodomain is important for p300 to maintain the basal level of histone acetylation and to induce the transcriptional activation of p300-dependent genes. Nevertheless, the requirement of bromodomain and histone acetylation in p300-dependent gene transcription is determined by a gene specific manner.

*Correspondence to: Qiao Li; Email: qiaoli@uottawa.ca
Submitted: 02/07/10; Accepted: 05/04/10
Previously published online: www.landesbioscience.com/journals/epigenetics/article/12224
DOI: 10.4161/epi.5.6.12224
interaction. The bromodomain of p300 and CBP are highly homologous, yet they differ in ligand selectivity, indicating that the ligand selectivity is controlled by few but very important variations in bromodomain sequences or the action mode of the bromodomain is not solely linked to the acetyl-lysine binding.

Targeting histone deacetylase (HDAC) with inhibitors affects gene expression through inhibiting histone deacetylation, which results in the accumulation of acetylated histones and the association of activator complex to activate gene transcription. Therefore, HDAC inhibitors have emerged in recent years as a new class of cancer therapeutics since they selectively induce apoptosis in tumor cells, with limited toxicity to normal cells. There are several classes of natural and synthetic HDAC inhibitors. One is the short-chain fatty acids such as butyrate and valproic acid. Butyrate is naturally produced by anaerobic bacteria fermenting undigested dietary carbohydrates and induces cell cycle arrest and selective apoptosis of tumor cells through altering histone acetylation. Valproic acid is commonly used in the treatment of epilepsy, bipolar disorder and migraines and also selectively induces transformed cells to undergo growth arrest and apoptosis. Trichostatin A (TSA), a hydroxamate compound represents another class of HDAC inhibitors.

In this study, we examined the roles of bromodomain and histone acetylation in the expression of several p300-dependant genes such as p21, Egr1 and E2F1, to determine the interplay of bromodomain and histone acetylation in the regulation of p300-dependent genes.

Results

Bromo-deficient p300 of SiHa cells. The SiHa cervical carcinoma cells contain a homozygous internal deletion of exons 15–18 in the p300 gene (p300Δex15-18) which gives rise to a p300 protein lacking the bromodomain (bromo-deficient). Most importantly, the HAT activity of the bromodomain deficient p300 is considerably impaired, only about 30% remaining compared to the wild-type p300. To probe for cellular mechanisms involved in the regulation of p300 function, we first characterized in details the bromo-deficient p300 in SiHa cells in parallel with the wild-type p300 in HeLa cervical carcinoma cells.

Western analysis demonstrated that the level of the truncated form of p300 in SiHa cells was significantly higher than the wild-type p300 in the HeLa cells, about 5-fold (Fig. 1A and B). Quantitative real-time RT-PCR analysis revealed that indeed the mRNA level of bromo-deficient p300 in the SiHa cells was significantly higher than the wild-type p300 in the HeLa cells, about 10-fold (Fig. 1C). In addition, a pulse-labeling protocol demonstrated that the rate of protein synthesis of the bromo-deficient...
p300 in the SiHa cells was significantly higher than the wild-type p300 in the HeLa cells, about 10-fold (Fig. 1D).

To examine the impact of bromodomain turnover on p300, we employed a pulse-chase protocol to compare the metabolic stability of wild-type and bromo-deficient p300. Previous studies show that the half-life of wild-type p300 is about 11–14 h in several cell lines.31 32 We determined that in the HeLa cells, the apparent half-life of wild-type p300 is about 11 h as assessed by a pulse-chase protocol (Fig. 1E). In the SiHa cells, the apparent half-life of the bromo-deficient p300 was about 10 h, which is in the similar range of the wild-type p300 in the HeLa cells (comparing Fig. 1F with E). In addition, immunofluorescence microscopy showed that the bromo-deficient p300, as the wild-type p300, localized predominantly to the nucleus (Fig. 1G). Taken together, these data suggest that the increase in the level of the truncated form of p300 protein in the SiHa cells may stem from a feed-back regulation to compensate for the loss of p300 HAT activity, through the augmentation of p300Aex15-18 gene expression, but not a decrease of the bromo-deficient p300 turnover.

**HDAC inhibitors and p300-dependent gene expression.**

Genome-wide survey of promoter structure occupied by p300 has identified a group of p300-dependent genes, such as p21, Egr1 and E2F1, which contain the characteristic of GC rich region and some common motifs for transcription factor binding.33 However, gene transcription of p21 and E2F1 in HeLa cells harboring a wild-type p300 exhibits differential sensitivity to treatment with HDAC inhibitor butyrate.34

To define the roles of p300 and histone acetylation in the regulation of p300-dependent genes, we first examined the expression profile of p21, Egr1 and E2F1 following targeting HDAC activities with HDAC inhibitors such as butyrate, valproic acid and TSA in time course studies. As shown in Figure 2A and B, following 8 h of treatments with these HDAC inhibitors, the protein levels of p21 in the HeLa cells were significantly augmented, up to 7-fold, whereas 16 h of treatments increased the levels of p21 protein further to about 17-fold. Similarly, the protein levels of Egr1 were also significantly augmented, up to 3-fold following 8 h of treatment, and up to 9-fold following 16 h of treatments (Fig. 2A and B). On the other hand, the protein levels of E2F1 were not much affected by these treatments (Fig. 2A and B), which is consistent with previous report.35 Taken together, our data suggest that the role of histone acetylation in the expression of p300-dependent genes may be determined by a gene specific manner.

**Gene expression in cells harboring the bromo-deficient p300.**

To examine the interplay of bromodomain and histone acetylation in the regulation of p300-dependent genes, we next examined the effects of butyrate, valproic acid and TSA, on the protein levels of p21, Egr1 and E2F1 in the SiHa cells containing the bromo-deficient p300. Following 16 h of treatments, an increase in the levels of p21 protein was detected by western blotting analysis (Fig. 2C and D). However, this augmentation was only about 4-fold, significantly lower than the 17-fold increase seen in the HeLa cells (comparing Fig. 2D with B). Similarly, the protein levels of Egr1 were only increased about 2-fold in response to the treatments (Fig. 2C and D), in contrast to the 9-fold increase seen in the HeLa cells (comparing Fig. 2D with B). Interestingly, the protein levels of E2F1 were not affected by the treatments, which is the same as seen in the HeLa cells (comparing Fig. 2D with B). Taken together, these data suggest that the interplay of p300 function and histone acetylation in the expression of p300-dependent genes may require the bromodomain.

**Effects of HDAC inhibitors on the transcription of p300-dependent genes.**

To determine if the effects of these HDAC inhibitors on the protein levels of p21 and Egr1 are transcription dependent, we profiled the mRNA levels of p21 and Egr1 following HDAC inhibitor treatments by using quantitative real time RT-PCR analysis. As shown in Figure 3A, treatments of the HeLa cells with butyrate, valproic acid or TSA for 4 h resulted in a significant increase in the levels of p21 mRNA, up to 3-fold, while 16 h of treatment further increased the augmentation to about 6-fold. Likewise, the levels of Egr1 mRNA were augmented significantly to about 4-fold following 4 h of treatments, and increased further to about 21-fold following 16 h of treatments (Fig. 3A). Together, these results demonstrate that HDAC inhibitors increase the expression of p300-dependent genes such as p21 and Egr1 at the level of transcription.

Next we examined the transcript levels of p21 and Egr1 in the SiHa cells in response to these treatments. As shown in Figure 3B, following 16 h of treatments, the mRNA levels of p21 were only increased up to 3-fold, significantly lower than the 6-fold of increase observed in the HeLa cells (compare Fig. 3B with A). With respect to Egr1, 16 h of treatments resulted in about 6-fold of augmentation in the levels of Egr1 mRNA, again significantly lower than the 21-fold of increase observed in the HeLa cells (Fig. 3A and B). Taken together, these data indicate that the impact of these HDAC inhibitors on transcription of p300-dependent genes is impaired in the SiHa cells which harbor a bromo-deficient p300.

**Global histone acetylation and p300-dependent gene expression.**

To determine the status of histone acetylation, we examined the effects of HDAC inhibitors on the levels of global histone acetylation in the HeLa and SiHa cells. The cells were treated with butyrate, valproic acid or TSA for 4 h and then subjected to western analysis with a specific antibody against acetylated K9 and K14 on histone H3. As shown in Figure 4A and B, treatments with these HDAC inhibitors increased the levels of acetylated H3 similarly, about 2-fold, in both the HeLa and SiHa cells (Fig. 4A and B). However, the basal level of H3 acetylation was significantly lower in the SiHa than the HeLa cells (Fig. 4). These data suggest that the bromodomain is important for p300 to exert its HAT activity in histone acetylation since the high level of bromo-deficient p300 was not able to maintain the basal level of histone acetylation in the SiHa cells (Figs. 1 and 4).

We next examined the interplay bromodomain and histone acetylation in p300-dependent gene expression of SiHa cells. The cells were first transfected with expression plasmid for the wild-type p300 and then treated with butyrate, valproic acid or TSA for 8 h. As shown with the western analysis, p21 levels were upregulated by butyrate and TSA, whereas Egr1 levels were augmented by valproic acid and TSA (Fig. 4C). Interestingly,
Histone acetylation is generally associated with active gene loci and the acetylation of histone by p300 is an important avenue to achieve transcriptional activation. The acetylation of specific lysine residues on the histone tails plays distinct roles in gene regulation by marking the histone as a flag for transactivation. In this event, p300 is often recruited to the regulatory elements by chromatin-remodeling factors and gene specific transcription factors. On the other hand, histone acetylation also serves as a binding signal for transcriptional coactivators such as p300. In such case, the acetylated lysine residues are recognized and bound by the bromodomain to initiate the assembly of transcription factors and RNA polymerase II complex.

Here, we demonstrate that although histone acetylation is generally associated with transcriptional activation, it only regulates a subset of p300-dependent genes. HDAC inhibitors...
The bromodomain is required for the interplay of p300 and histone acetylation in the transcriptional activation of these p300-dependent genes. The bromodomain is also required for maintaining p300 HAT activity. The loss of p300 bromodomain in the SiHa cells severely impairs the HAT activity. Interestingly, the basal level of histone acetylation in cells containing the bromo-deficient p300 is significantly lower than in cells containing the wild-type p300 cells (Fig. 4). One contribution of bromodomain to histone acetylation is by tethering the HATs to specific sites at the chromatin and stabilizing the HAT/chromatin remodeling complex. It has been shown that bromo-deficient p300 is able to acetylate free histones but not the nucleosome histones in native chromatin. Therefore, the bromodomain can act as an anchoring domain which tethers p300 to the chromatin template for histone acetylation, chromatin remodeling and transcriptional activation.

Feedback is an important regulatory mechanism to control the expression of genes or regulatory networks. We observed that the p300Δex15-18 gene transcription and translation in the SiHa cells are significantly higher than the wild-type p300 in the HeLa cells, which renders an abundance of bromo-deficient p300 protein in the SiHa cells (Fig. 1). This is most likely a result of feedback regulation to compensate for the loss of p300 HAT activity. Interestingly, the stability of bromo-deficient p300 is similar to the wild-type p300. Our data suggests a new mechanism for the bromodomain involved control of p300 function, through a functional feedback regulation of p300 gene expression, but not a p300 turnover.
spectrophotometer (Thermo) was used to determine the concentrations and the quality of total RNA. Equal amounts of RNA were used in reverse transcription with High Capacity cDNA Archive Kit—Multiscribe Reverse Transcriptase (Applied Biosystems). Quantitative real time PCR amplification was carried by using the Applied Biosystems 7500 Fast Real-Time PCR System. The levels of gene transcripts were quantified by using the TaqMan® reporter assay. Specific gene primers used in the amplification were pre-designed (TaqMan® primers by Applied Biosystems). 18S rRNA was used as a reference control.23 The amount of targets, normalized to the 18S endogenous reference and relative to calibrator control is calculated using the arithmetic formula $2^{-\Delta\Delta CT}$.

Pulse-chase. Cells were cultured in DMEM supplemented with 10% of FBS for overnight and the media was replaced with methionine-free media. The cells were first pulsed for 2 h with $100 \muCi/ml$ of $^{35}$S-methionine and chased for 2–24 h in regular medium.44 The cells were then harvested for the preparation of whole cell extracts. For immunoprecipitation, the whole cell extracts were diluted to adjust the concentrations of NaCl to 150 mM by dilution buffer (10% glycerol, 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA pH 8.0, 1 mM DTT, 1 mM PMSF and 1 mg/ml of BSA). The endogenous wild-type or mutant p300 protein was immunoprecipitated with a p300 specific antibody and washed three times with washing buffer (10% glycerol, 100 mM NaCl, 20 mM Tris-HCl pH 8.0, 0.5 mM EDTA pH 8.0, 1 mM DTT, 1 mM PMSF, 0.1% NP-40). The precipitates were then separated by SDS-PAGE and quantified by PhosphoImager.

Immunofluorescence microscopy. Cells were grown on cover-slips for about 16 h before treatment, fixed in paraformaldehyde, permeabilized in 0.2% NP-40 and blocked in PBS containing 0.2% tween 20 and 10% non-fat milk.45 The cover-slips were incubated with primary antibody specific for p300, then with rhodamine-conjugated secondary antibody, mounted in 90% glycerol and visualized by fluorescence microscopy.

The conclusion of this study is that the bromodomain is important for p300 to maintain the basal level of histone acetylation and to induce the transcriptional activation of p300-dependent genes. Nevertheless, the requirement of bromodomain and histone acetylation in p300-dependent gene transcription is determined in a gene specific manner.

Materials and Methods

Cell culture and reagents. HeLa and SiHa cells were obtained from American Type Culture Collection (ATCC) and maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% of Fetal Bovine Serum (FBS, Hyclone) and 1% of MEM Non-Essential amino acids (Invitrogen). The cells were cultured at 37°C in humidified air containing 5% CO$_2$, for a minimum of 16 h prior to different treatments. Sodium butyrate, valproic acid and TSA were obtained from Sigma-Aldrich. Specific antibodies for p300, p21, Egr1 and E2F1 were obtained from Santa Cruz biotechnology. Anti-Ac-H3 (K9/14) and anti-β-actin antibodies were purchased from Sigma-Aldrich.

Transfection and western analysis. Transient transfections were performed with FuGene (Promega) following the manufacturer’s recommendation. After transfection, the cells were cultured in the growth medium for 24 h and treated as indicated. Whole cell extracts were isolated by incubating the cells in WCE buffer containing 50 mM Tris-HCl (pH 7.6), 400 mM NaCl, 10% glycerol, 0.5 mM DTT, 0.5 mM PMSF and 0.1% NP-40 for 30 min at 4°C as previously described.31 The protein concentration was determined by Bradford assay (Bio-Rad) with the use of bovine serum albumin as a standard. Equal amounts of proteins were separated on SDS-PAGE and subjected to western analysis. Quantification of the western results was performed by using Scion Image computer software (Scion Corporation).

Real-time RT-PCR analysis. Total RNA was isolated by using RNeasy Mini kit (Qiagen). Multiskan Spectrum

---

Figure 4. Global histone acetylation and p300-dependent gene expression. (A) Western analysis was performed by using specific antibody against acetylated histone 3 at K9/14 residue following 4 h of treatments of the HeLa or SiHa cells with butyrate (NaB, 5 mM), valproic acid (VPA, 5 mM) or TSA (200 nM). The blots were stripped and reprobed for β-actin as a loading control. (B) Quantification of the western blots is plotted as fold difference in reference to the untreated control of the HeLa cells. Error bars represent standard deviations of three independent experiments. Statistical significance is denoted by * to indicate p < 0.05 compared with the untreated control of HeLa cells. (C) The SiHa cells were transfected with expression plasmid (3 µg) for the full length p300, treated with butyrate, valproic acid or TSA for 8 h and then subjected to western analysis of p21 and Egr1. The blots were stripped and reprobed for β-tubulin as a loading control.
Acknowledgements
We thank Ms. Blaire Langlais for excellent technical assistance on the real time RT-PCR analysis of p300-dependent genes. This work was sponsored by operating grant from Canadian Institutes of Health Research (CIHR). During the course of this study, Q.L. was supported by an Investigator Award from the CIHR. F.M.G. is a recipient of Canada Graduate Study Master’s Award from the CIHR.

References