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Acetylation of histone deacetylase 6 by p300 attenuates its deacetylase activity

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ABSTRACT

Protein acetyltransferases and deacetylases affect the activities of each other. This is well documented by the acetylation and inhibition of HDAC1 by p300, a transcriptional co-activator with protein acetyltransferase activity. However, the relationship between HDAC6 and p300 is poorly understood. HDAC6 is a class II histone deacetylase and differs from other members of HDAC family in that it contains two HDAC domains and an ubiquitin-binding motif. HDAC6 is a microtubule-associated deacetylase. It predominantly deacetylates non-histone proteins, including α-tubulin, and regulates cell motility. Here, we report that p300 interacts with and acetylates HDAC6 resulting down-regulation of HDAC6 deacetylase activity. Furthermore, we provide evidences that acetylation of HDAC6 by p300 inhibits tubulin deacetylation and suppression of Sp1 transcriptional activity by HDAC6. Our results demonstrate that p300 can inactivate HDAC6 by acetylation, and that p300 may regulate the activity of Sp1 indirectly through HDAC6 in addition to its direct modification of Sp1.

Introduction

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate acetylation of lysine residues, and they are key components of the cellular signaling network that coordinates fundamental cellular processes. Eleven proteins, at the least, have been identified as members of the HDAC family based on their homology of the catalytic domain, and they can be divided into four classes. HDAC6 belongs to class II and distinguishes itself from other HDAC family members in that it contains two HDAC domains and an ubiquitin-binding motif, the BUZ finger, at its C terminus [1–3]. Through its HDAC domains, HDAC6 deacetylases tubulins and the best noted substrate for HDAC6 is β-tubulin. This suggests that, through reversible acetylation, HDACs can regulate other important biological processes beside chromatin remodeling and gene transcription [4,5]. Through its BUZ domain, HDAC6 binds polyubiquitinated proteins regulating their processing and interaction with chaperones [6–8]. Moreover, HDAC6 deacetylates Hsp90 (heat shock protein 90) and affects the Hsp90-mediated maturation of glucocorticoid receptor [9].

p300 and CREB-binding protein (CBP) are transcriptional co-activators that function as integrators of numerous signaling pathways, and they are utilized by a large number of DNA-binding proteins to facilitate transcriptional activation [10]. p300/CBP have been implicated in numerous disease processes, including several forms of cancer, cardiac hypertrophy, and Huntington's disease [11–16]. The relatively abundant p300 is a rate-limiting factor for co-activation and co-repression by numerous transcription factors, and p300 serves to integrate diverse signaling pathways involved in cellular differentiation and metabolism [17,18]. Regulation of these activities by p300 involves its scaffolding function to tether transcription factors to target promoters and its enzymatic activity of the histone acetyltransferase domain [19]. In addition to histones, several substrates including various transcription factors are acetylated by p300 [17,18].

A dynamic equilibrium between acetylation and deacetylation of proteins such as transcription factors would warrant the control of distinct patterns of transcription at different cellular stages. This equilibrium implies that acetyltransferases and deacetylases would serve to the same gene regulatory loci and can affect each other. It has been reported that the acetylation of purified HDAC1 inactivates its deacetylase activity, and mutations to the critical acetylation target sites abrogate HDAC1 function *in vivo* [19,20]. We previously reported that p300 can inactivate Sirt2 by acetylation and that p300 may regulate the activity of p53 indirectly through Sirt2 in addition to its direct modification of p53. In the same context, we hypothesized that a direct cross-talk between HDAC6 and p300, which have opposing enzymatic activities, could be an efficient way to regulate gene transcription, and investigated whether they affect the function of each other. Here, we provide

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evidences for a model in which the deacetylase activity of HDAC6 is negatively regulated through acetylation by p300 and this inhibits the deacetylation of tubulin and the suppression of Sp1 transcriptional activity by HDAC6.

Materials and methods

Cell culture. All culture media and antibiotics were purchased from Invitrogen. HEK293 cells were maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics–antimycotics at 37 °C, 5% CO_2.

Plasmids and antibodies. Myc-, HA- or, GFP-tagged HDAC6, HDAC6-1, −2, −3, and p300 were constructed in a CMV promoter-derived mammalian expression vector (pCS4+). Antibodies against Myc (9E10, Santa Cruz), acetyl-lysine (Cell Signaling Technology), GFP (Santa Cruz), and HA (12CA5, Roche) were used.

DNA transfection and reporter assay. Transient transfections were performed using the calcium phosphate and Lipofectamin plus methods. For luciferase assays, HEK293 cells were plated on 24-well plates the day before transfection. pCMV-β-gal plasmid was co-transfected as an internal control for the efficiency of transfection. Cells were lysed 36 h after transfection and the luciferase activity was measured using Luciferase Reporter Assay Kit (Promega). Luciferase activities were normalized using β-galactosidase activities and expressed relative to the luciferase activity of control cells.

Immunoprecipitation and immunoblotting. Twenty-four hours after transfection, HEK293 cells were lysed in an ice-cold lysis buffer (25 mM Hepes (pH 7.5), 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 1 mM EDTA, 1 mM Na_3VO_4, 250 μM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin). Cell lysates were cleared by centrifugation and the supernatants were subjected to immunoprecipitation using appropriate antibodies and protein A or protein G-Sepharose beads. Immunoprecipitated proteins were resolved by SDS–PAGE and transferred to PVDF membranes. Proteins were visualized using appropriate primary antibodies, horseradish peroxidase-coupled secondary antibodies and chemiluminescence Western blotting reagent (Amersham Bioscience).

In vitro tubulin deacetylation assay. Cell lysates of Myc-HDAC6/p300 transfected or control HEK293 cells were immunoprecipitated for Myc and the immunoprecipitates were resuspended with 100 μl of cell lysates (50 μg total protein) from non-transfected HEK293 cells containing 1 mM ATP. After 2 h at 25 °C, reactions were terminated by adding 50 μl of 6% SDS–PAGE loading buffer. Samples were then subjected to SDS–PAGE and acetylated α-tubulin was visualized by Western blotting using antisera specific for acetylated α-tubulin.

Results

HDAC6 interacts with p300

p300 interacts with HDAC1 through its C/H3 domain [20], therefore, we investigated whether p300 can also interact with HDAC6. HEK293 cells were transfected with HA-tagged p300 and/or Myc-tagged HDAC6 and their interaction was analyzed by co-immunoprecipitation. p300 was detected in HDAC6 immunoprecipitates and vice versa (Fig. 1A and B). These results indicate that HDAC6 interacts with p300.

p300 acetylates and decreases the deacetylase activity of HDAC6

HDAC6 contains several conserved regions including two catalytic domains and a zinc-finger domain. To identify the regions of HDAC6 that are involved in the interaction with p300, the interaction between HDAC6 deletion derivates (Fig. 2A) and p300 was examined. p300 interacted with all three HDAC6 deletion derivates. But the binding affinity of HDAC6-1 and HDAC6-2, each containing a catalytic domain, to p300 was higher than that of HDAC6-3 which contains a zinc-finger domain (Fig. 2B).

p300 can regulate the activity of its down-stream effectors through acetylation. Therefore, we examined whether p300 can acetylate HDAC6. HEK293 cells were transfected with p300 and HDAC6 deletion derivates, and the levels of acetylation of HDAC6 deletion derivatives were examined by anti-acetyl-lysine immunoprecipitation followed by anti-Myc immunoblotting. The levels of HDAC6-1 and HDAC6-2 acetylation were increased in the presence of p300 (Fig. 2C).

We then examined what effect does the acetylation of HDAC6 by p300 has on its enzymatic activity. It has been reported that HDAC6 deacetylates α-tubulin. Therefore, we examined the effect of HDAC6 acetylation on its ability to deacetylate α-tubulin. HEK293 cells were transfected with Myc-tagged full-length or deletion derivates of HDAC6 and cell lysates were immunoprecipitated with anti-Myc antibody. Immunoprecipitates were incubated with cell lysates from non-transfected HEK293 cells, and the levels of acetylated α-tubulin were determined by immunoblotting with antisera specific for acetylated α-tubulin. HDAC6 and HDAC6-2 deacetylated α-tubulin (Fig. 3A, top panel lanes 3 and 5, and Fig. 3C), and deacetylation of α-tubulin by HDAC6 or

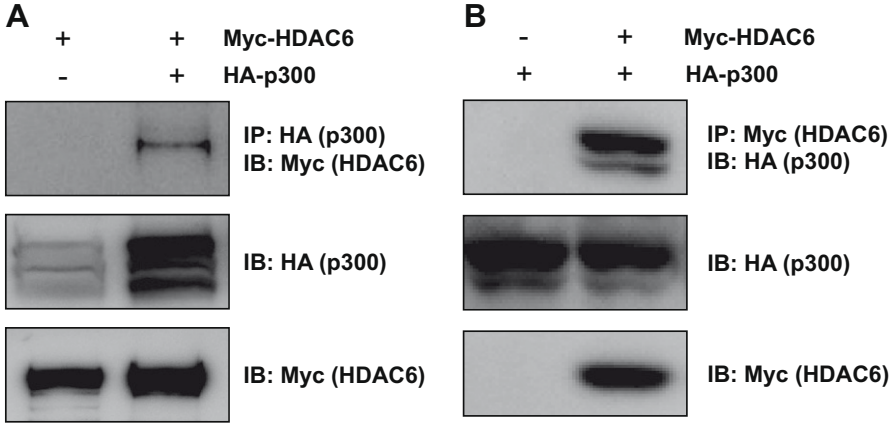

Fig. 1. HDAC6 interacts with p300. HEK293 cells were transfected with indicated combination of Myc-tagged HDAC6 and HA-tagged p300. Interaction between HDAC6 and p300 is examined (A) by immunoprecipitation using an anti-HA antibody and immunoblotting using an anti-Myc antibody [IP: HA (p300), IB: Myc (HDAC6)], or (B) by immunoprecipitation using an anti-Myc antibody and immunoblotting using an anti-HA antibody [IP: Myc (HDAC6), IB: HA (p300)]. Levels of p300 [IB: HA (p300)] and HDAC6 [IB: Myc (HDAC6)] in total lysates are also compared.

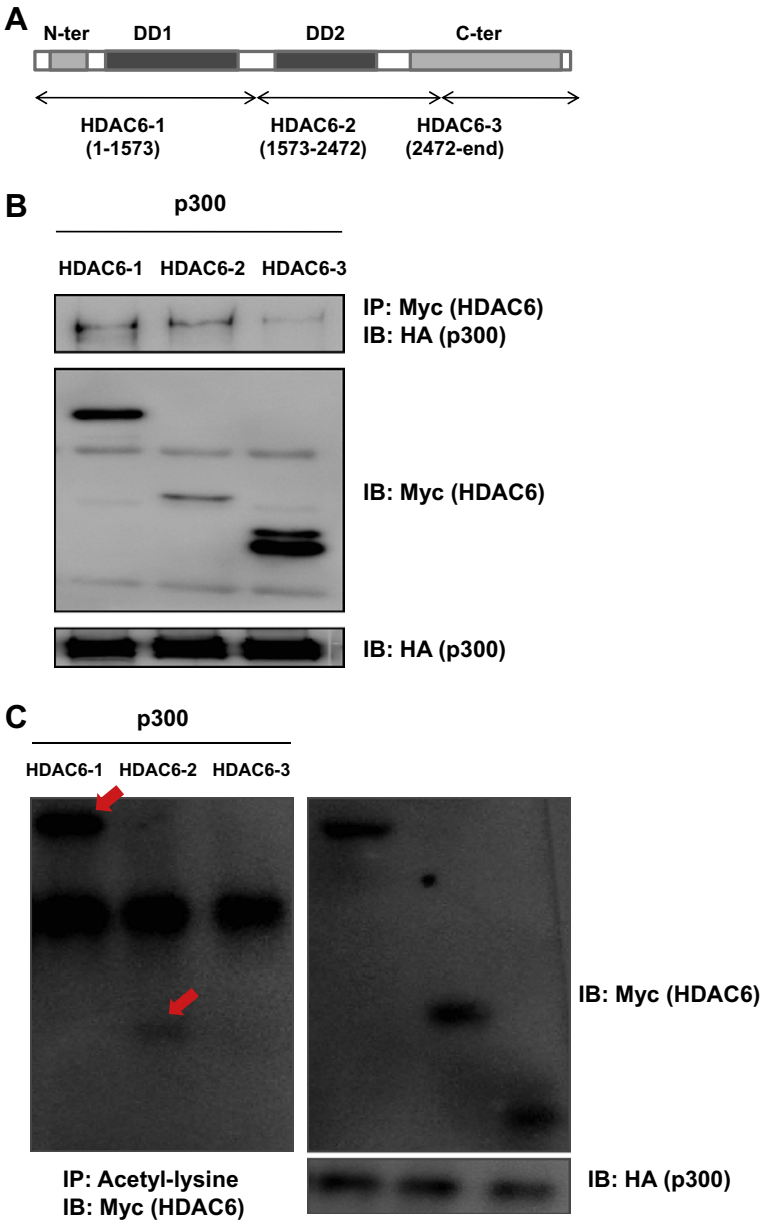

Fig. 2. Mapping of the regions of HDAC6 involved in the interaction with p300. (A) A schematic representation of HDAC6 and its deletion derivatives. The functional domains include the N-terminal domain, the deacetylase domain 1 (DD1), the deacetylase domain 2 (DD2) and the C-terminal domain of HDAC6. Deletion derivatives contain the amino acids of HDAC6 indicated in parenthesis. (B) Mapping of the regions of HDAC6 involved in the interaction with p300. HEK293 cells were transfected with indicated Myc-tagged HDAC6 deletion derivative and HA-tagged p300, and their interaction is analyzed by immunoprecipitation using an anti-Myc antibody followed by immunoblotting using an anti-HA antibody [IP: Myc (HDAC6), IB: HA (p300)]. The levels of HDAC6 deletion derivatives [IB: Myc (HDAC6)] and p300 [IB: HA (p300)] are also compared. (C) p300 acetylates HDAC6-1 and HDAC6-2. HEK293 cells were transfected with indicated Myc-tagged HDAC6 deletion derivate and HA-tagged p300. To examine the acetylation of HDAC6 deletion derivates, immunoprecipitation was performed using an anti-Acetyl-Lysine antibody followed by immunoblotting using an anti-Myc antibody [IP: Acetyl-lysine, IB: Myc (HDAC6)]. Arrows indicate acetylated HDAC6 deletion derivates. The levels of HDAC6 deletion derivatives [IB: Myc (HDAC6)] and p300 [IB: HA (p300)] in total lysates are also compared. The experiments were repeated three times and a representative result is shown.

HDAC6-2 was abolished in the presence of p300 (Fig. 3B, bottom panel lanes 3 and 5, and Fig. 3C). These results indicate that p300 can acetylate and decrease the deacetylase activity of HDAC6.

p300 relieves HDAC6-mediated down-regulation of p21 transcriptional activity

It has been previously shown that HDACs down-regulate the transcriptional activity of Sp1 [21]. To determine the biological significance of HDAC6 acetylation by p300, we examined the interaction between HDAC6 and Sp1. HEK293 cells were transfected with Flag-tagged HDAC6 and/or Myc-tagged Sp1 and their interaction was analyzed by co-immunoprecipitation. Sp1 was detected in HDAC6 immunoprecipitates (Fig. 4A). We then examined the effect of HDAC6 and p300 on the transcriptional activity of Sp1 using a Sp1-responsive luciferase reporter, p21(-143)-luc. HDAC6 suppresses the Sp1-induced activation of p21 promoter (Fig. 4B, lines 2 and 3). But the inhibition by HDAC6 was attenuated in the presence of p300 (Fig. 4B, line 5). These results suggest that p300 can relieve the inhibition of Sp1 transcriptional activity by HDAC6.

Discussion

In general, the transcriptional co-activator p300 enhances transcription by acetylating histones in chromatin to induce transcrip-

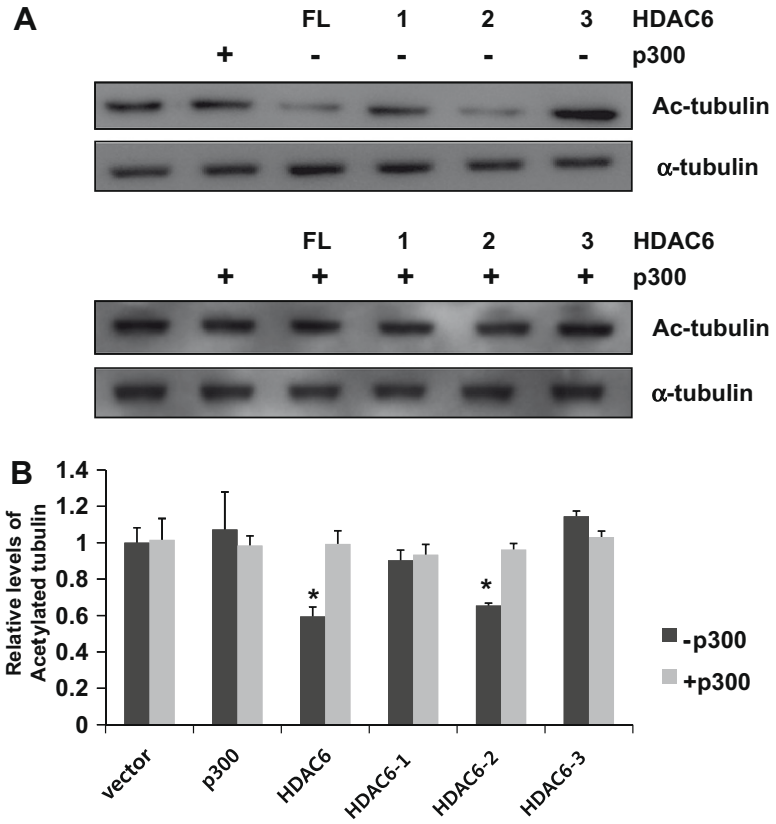

Fig. 3. Deacetylase activity of HDAC6 is attenuated by p300. (A) HEK293 cells were transfected with indicated combination of HA-tagged p300 with full-length (FL) or deletion derivatives (1, 2, or 3) of HDAC6. Cell lysates were immunoprecipitated for HDAC6 using an anti-Myc antibody and the immunoprecipitates were incubated with lysates of non-transfected HEK293 cells. Samples were subjected to SDS–PAGE and the levels of acetylated α-tubulin are examined by immunoblotting using antisera specific to acetylated tubulin (Ac-α-tubulin). The levels of α-tubulin are also compared. (B) Experiment was performed in triplicates and repeated three times. The band intensities of a representative result were measured by densitometry and the means and standard deviations are shown. $^{*}P < 0.01$, significantly different from control.

tionally active conformation or by directly modifying transcriptional factors such as p53, E2F, and GATA-1 to up-regulates their transcriptional activity [22–24]. In contrast to the well-characterized positive regulatory effects of p300 on target proteins by acetylation, our finding suggests that acetylation by p300 can also exert negative regulatory effects on target proteins. Similar effect has been previously reported that, when HDAC1 is acetylated by p300, the acetylated form of HDAC1 is no longer able to maintain the deacetylated state of chromatins near the target promoters and to repress the target promoters [19,20].

Although the precise mechanisms for the acetylation and the down-regulation of HDAC6 deacetylase activity by p300 need to

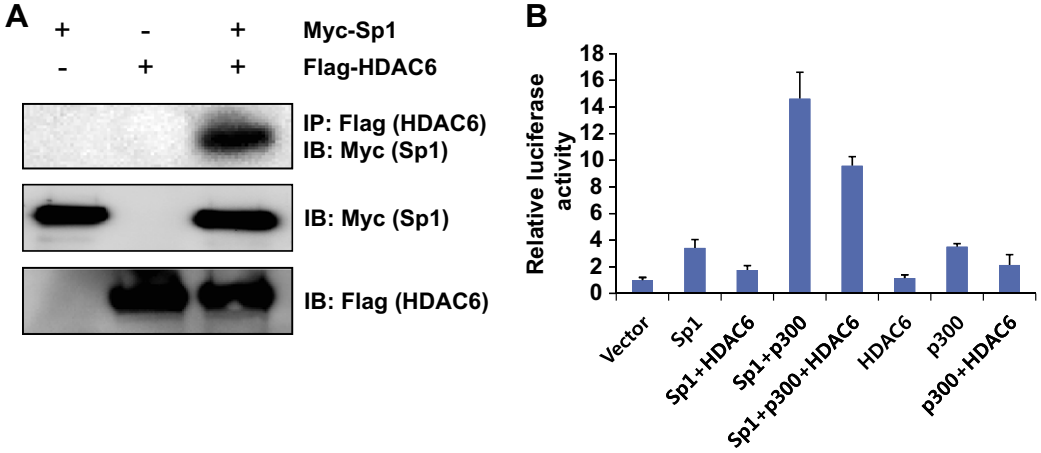

Fig. 4. p300 attenuates the down-regulation of Sp1 transcriptional activity by HDAC6. (A) HEK293 cells were transfected with indicated combinations of Myc-tagged SP1 and Flag-tagged HDAC6. Interaction between HDAC6 and Sp1 is examined by immunoprecipitation using an anti-Flag antibody and immunoblotting using an anti-Myc antibody [IP: Flag (HDAC6), IB: Myc (Sp1)]. Levels of Sp1 [IB: Myc (Sp1)] and HDAC6 [IB: Flag (HDAC6)] in total lysates are also compared. (B) HEK293 cells were transfected with p21(-143)-luc, pCMV-β-gal and indicated combinations of Sp1, p300, and HDAC6. The experiment was performed in triplicates and repeated three times. Relative luciferase activities with SD of a representative experiment are shown.

be elucidated, our results support the hypothesis that antagonism between the opposing enzymatic activities of HDACs and p300 controls the activity of common targets by regulating the acetylation/deacetylation of the same lysine residues [25]. Furthermore, HDACs and p300 may control the function of target proteins by regulating the activities of each other as demonstrated by the present study and a previous study which reported that Sirt1, a class III HDAC, deacetylates and represses the function of p300 [26]. In addition, we also demonstrated previously that acetylation of Sirt2 by p300 attenuates α-tubulin deacetylation by Sirt2 [27].

Acetylation of HDAC6 by p300 can have additional consequences in addition to down-regulation of the ability of HDAC6 to regulate the function of target proteins by deacetylation. It has been previously shown that HDAC6 interacts with Hsp90 and regulates the chaperone-depend activation of glucocorticoid receptor [9]. It is possible that the acetylation of HDAC6 by p300 may affect its ability to interact with other signaling modulators such as Hsp90 which will, in turn, affect the regulatory ability of HDAC6.

We have shown that HDAC6 acetylation by 300 attenuates its repression on Sp1 transcription activity. It is plausible to speculate that p300 modulates the activity of other HDAC6 target proteins and, therefore, other signaling pathways by acetylation and down-regulation of the deacetylase activity of HDAC6. This will have important consequences and various effects on transcriptional regulation.

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