Deacetylation of the DNA-binding Domain Regulates p53-mediated Apoptosis*

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Hestia S. Mellert‡, Timothy J. Stanek§, Stephen M. Sykes‡, Frank J. Rauscher III¶, David C. Schultz∥, and Steven B. McMahon§

From the ‡Biomedical Graduate Studies, University of Pennsylvania, Philadelphia, Pennsylvania 19104, the §Kimmel Cancer Center, Department of Cancer Biology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, the ¶Department of Medicine, Brigham and Women’s Hospital, Boston, Massachusetts 02115, and ∥The Wistar Institute, Philadelphia, Pennsylvania 19104

In unstressed cells, the p53 tumor suppressor is highly unstable. DNA damage and other forms of cellular stress rapidly stabilize and activate p53. This process is regulated by a complex array of post-translational modifications that are dynamically deposited onto p53. Recent studies show that these modifications orchestrate p53-mediated processes such as cell cycle arrest and apoptosis. Cancer cells carry inherent genetic damage, but avoid arrest and apoptosis by inactivating p53. Defining the enzymatic machinery that regulates the stress-induced modification of p53 at single-residue resolution is critical to our understanding of the biochemical mechanisms that control this critical tumor suppressor. Specifically, acetylation of p53 at lysine 120, a DNA-binding domain residue mutated in human cancer, is essential for triggering apoptosis. Given the oncogenic properties of deacetylases and the success of deacetylase inhibitors as anticancer agents, we investigated the regulation of Lys120 deacetylation using pharmacologic and genetic approaches. This analysis revealed that histone deacetylase 1 is predominantly responsible for the deacetylation of Lys120. Furthermore, treatment with the clinical-grade deacetylase inhibitor p300/Tip60, known to exhibit high activity against histone deacetylase 1 (HDA1), KAT8 and Tip60/KAT5 (7, 8). Acetylation of this site is critical for maximal p53-dependent apoptosis but dispensable for p53-mediated cell cycle arrest (7, 8). The Ac-Lys120 isoform of p53 specifically accumulates at proapoptotic target genes such as BAX and PUMA, whereas a nonacetylated mutant is defective for transcription of these proapoptotic targets. Furthermore, point mutations at Lys120 occur in human cancer, including one that converts lysine to arginine (K120R) (9). The K120R mutation is of specific interest because the amino acid substitution maintains charge but blocks lysine modification. In addition to loss of acetylation through mutation of the targeted lysine, both acetyltransferases that act on Lys120 are also lost in certain human cancers (10, 11). When combined, these observations suggest that the Lys120 acetylation pathway is important in p53-mediated tumor suppression.

The p53 tumor suppressor pathway is inactivated in the majority of human cancers via either genetic lesions within the tp53 gene itself or in genes encoding p53 regulators (1, 2). To protect cells against malignant transformation, p53 initiates pathways including cell cycle arrest and apoptosis. These functions depend heavily on the ability of p53 to act as a sequence-specific transcriptional regulator. Although p53 activates the transcription of the cyclin-dependent kinase inhibitor p21/waf1 (3) and other key regulators to arrest the cell cycle, p53 mediates apoptosis in large part by transcriptional activation of proapoptotic genes such as BAX, PUMA, p53AIP, PERP, and NOXA (2). These potent biological outcomes mandate p53 activity be tightly regulated. In non-stressed cells, MDMX and MDM2 form a complex that prevents p53-mediated transactivation of target genes and facilitates ubiquitin-mediated proteolysis (4–6). Under conditions of cellular stress such as DNA damage, p53 is liberated from negative regulators and accumulates in the cell. p53 is also robustly modified (e.g. phosphorylated, methylated, and acetylated), which modulates DNA-binding capacity, interaction with cofactors, stability, and other functions (2). Acetylation of Lys120 (Ac-Lys120), which lies within the p53 DNA-binding domain, is catalyzed by the acetyltransferases hMOF/KAT8 and Tip60/KAT5 (7, 8). Acetylation of this site is critical for maximal p53-dependent apoptosis but dispensable for p53-mediated cell cycle arrest (7, 8). The Ac-Lys120 isoform of p53 specifically accumulates at proapoptotic target genes such as BAX and PUMA, whereas a nonacetylated mutant is defective for transcription of these proapoptotic targets. Furthermore, point mutations at Lys120 occur in human cancer, including one that converts lysine to arginine (K120R) (9). The K120R mutation is of specific interest because the amino acid substitution maintains charge but blocks lysine modification. In addition to loss of acetylation through mutation of the targeted lysine, both acetyltransferases that act on Lys120 are also lost in certain human cancers (10, 11). When combined, these observations suggest that the Lys120 acetylation pathway is important in p53-mediated tumor suppression.

A number of enzymes that reverse lysine acetylation have been described in mammals. These deacetylases, termed HDACs (histone deacetylases) or more recently KDACs (lysine deacetylases), have been grouped into four classes based on their homology to yeast proteins (12). The class I HDACs, which include HDAC1–3 and HDAC8, are small, ubiquitously expressed enzymes that localize to the nucleus. HDAC 1 and 2, which are very similar in amino acid composition, are

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10 To whom correspondence should be addressed: 609 BLSB, 233 S. 10th St., Philadelphia, PA 19107. Tel.: 215-503-9064; Fax: 215-923-0249; E-mail: Steven.McMahon@jci.tju.edu.

2 The abbreviations used are: Ac-Lys120, acetylation of lysine 120; 7-AAD, 7-amino-actinomycin D; CPT, camptothecin; HDAC, histone deacetylase; KAP1, KRAB-associated protein 1; NuRD, nucleosome remodeling deacetylase; TSA, trichostatin A.
often found together as the catalytic core of the multisubunit complexes nucleosome remodeling deacetylase (NuRD), corepressor of RE1-silencing transcription factor (CoREST), Sin3A, and Sin3B (12). Class II HDACs have more defined, tissue-specific activities and either remain constitutively cytoplasmic or shuttle between the nucleus and cytoplasm. Class III HDACs, also known as sirtuins, are NAD-dependent enzymes that are most noteworthy for their role in regulating the longevity of organisms (13). A fourth class of deacetylases, including HDAC11 and related proteins, has recently been identified (14).

Deacetylation of histones by HDACs can control transcriptional programs important for a number of biological processes including proliferation, cytoskeletal dynamics, metabolism, longevity, and oncogenesis (15). Within the past decade, many nonhistone targets of these enzymes have also been identified (16). Lysines within the C terminus of p53 can be deacetylated by either HDAC1 or SIRT1 (18–21), which affects p53 stability (17, 18), cofactor recruitment (19), and DNA binding (20). Although Ac-Lys120 is an important regulator of p53-mediated apoptosis (7, 8), regulation of Lys120 deacetylation has not been studied. Here, we report that Ac-Lys120 levels are regulated predominantly by HDAC1 and that this deacetylation is dependent on KRAB-associated protein 1 (KAP1; also known as TIF1β or TRIM28) and other NuRD complex components. Consistent with this finding, depletion of HDAC1 enhances Lys120-dependent, p53-mediated apoptosis in cancer cells. Additionally, we find that entinostat, a second-generation inhibitor that displays enhanced specificity toward HDAC1 (21), increases Ac-Lys120 levels and apoptosis. Collectively, these data demonstrate that the ability of HDAC inhibitors to enhance apoptosis in cancer cells is partially through activation of the Lys120 acetylation/apoptosis pathway.

EXPERIMENTAL PROCEDURES

Cell Culture and Chemical Treatments—MCF-7, HCT116, 293T and H1299 cells, obtained from American Type Culture Collection, were maintained in DMEM (Mediatech) with 10% FBS (Foundation) and penicillin/streptomycin at 37 °C under 5% CO2. Tetracycline-inducible p53 and K120R lines were made in H1299 cells with use of the VirapowerTM T-RExTM lentiviral expression system (Invitrogen) and were maintained in tetracycline-free FBS. HDAC inhibitors were added to cell media for final concentrations of 0.5 μM trichostatin A (TSA; Sigma), 0.5–5 μM entinostat/MS-275 (Syndax), 20 μM MC1568 (a gift from L. Altucci), and 100 μM splitomicin (Sigma). MDM2-p53 disruption was achieved with 10 μM Nutlin-3 (Sigma). DNA damage was induced with 5 μM camptothecin (CPT; Sigma).

Plasmids and Transfection—pcDNA-FLAG-hMOF was generated as described previously (7). pRC-p53 was a gift from M. Murphy. pcDNA-FLAG-HDAC constructs were a gift from T. Yao, and pCMV-FLAG-SIRT constructs were a gift from D. Reinberg. HDAC1-H141A was generated by site-directed mutagenesis (Stratagene) of the pcDNA-FLAG-HDAC1 construct. Primers used for the mutagenesis were: forward, 5′-GGG CTG GGG GCC TGC TGC CAA AGA AGT CCG ACG-3′; reverse, 5′-CCT CGG ACT TCT TTG CAG CGT GCA GGC CCC CAG CCC-3′. Transfections were performed using Lipofectamine 2000 (Invitrogen), according to manufacturer’s guidelines.

Viral Production and RNAi—For lentiviral packaging, each plKO shRNA construct (MISSION TRC shRNA library; Sigma) was co-transfected into 293T cells with the lentiviral packaging plasmids, pCMV-ΔR8.2 and pCMV-ΔVSV-G, which were a gift from P. Chumakov (Lerner Research Institute, Cleveland, OH). 48–72 h after transfection, viral supernatants were collected, supplemented with 8 μg/ml Polybrene (Sigma), and added to target cells. Cells were selected with puromycin (2.5 μg/ml for H1299 and H1299-derived lines, 2 μg/ml for MCF-7 cells, and 0.75 μg/ml for HCT116 cells) for 48–72 h before seeding experiments.

Immunoprecipitation and Western Blotting—To generate total cell lysates, cells were washed with cold 1 × PBS and lysed in buffer containing 20 mM NaH2PO4, 150 mM NaCl, 0.5% IGEPAL (Nonidet P-40), 30 mM sodium pyrophosphate, 10% glycerol supplemented with 30 mM sodium butyrate, and protease inhibitor mixture. For the detection of histones, cell lysates were subjected to sonication using a Bioruptor (Diagenode). Total protein concentrations were determined using the BCA assay (Pierce). Immunoprecipitation was performed on 0.5–2.0 mg of lysate with protein G-Sepharose (GE Healthcare) and the following antibodies: Ac-Lys120-p53 generated in rabbit (7), Ac-K382p53 (Upstate), and phospho-S20p53 (Cell Signaling). IPs were detected by Western blotting using FL393G antibody (Santa Cruz Biotechnology). Western blots of 20–50 μg of total lysate were probed with the following antibodies: p53 (DO-1), actin (C-2), HDAC2 (H-54), SIRT1 (H-300) from Santa Cruz Biotechnology; ORC2 (BD Pharmingen); H4, acetyl-H4, HDAC1, and phospho-H2AX (Upstate); V5 (Invitrogen); and FLAG-M2 (Sigma). KAP1 antibody was generated in rabbit (22).

Apoptosis Assay—Following treatment, cells were collected in medium and then washed with 1 × PBS. Cells were resuspended in Annexin V staining buffer supplemented with Annexin V-PE and 7-AAD according to the manufacturer’s instructions (BD Pharmingen). Cells were counted by flow cytometry using a FACScalibur (Becton Dickinson) with CellQuest (Becton Dickinson) software and further analyzed with FlowJo (Treestar) software.

RESULTS

Dynamic Deacetylation of Lys120 within the p53 DNA-binding Domain—Stress-induced acetylation plays an important role in the regulation of p53 stability and function (20, 23). For example, acetylation of Lys120 within the DNA-binding domain is essential for the full apoptotic potential of p53 (7, 8). The human breast cancer cell line MCF-7, which expresses wild-type endogenous p53, was used to investigate whether active deacetylation is a biochemical mechanism regulating Ac-Lys120. MCF-7 cells were treated with CPT, a topoisomerase inhibitor that results in DNA damage and is used as a chemotherapeutic agent, followed by treatment with class-specific HDAC inhibitors. As expected, untreated cells displayed low levels of p53 (Fig. 1A). Treatment with CPT resulted in...
stabilization of total p53 and increased Ac-Lys\(^{120}\) levels as reported previously (7, 8). In parallel, class I and II HDAC enzymes were inhibited by TSA; and class III enzymes, also known as sirtuins, were inhibited by splitomicin. The addition of TSA to CPT-treated cells robustly increased the level of Ac-Lys\(^{120}\). Treatment with splitomicin had little affect on Ac-Lys\(^{120}\) levels, but did increase acetylation on Lys\(^{382}\), a site targeted by SIRT1 (24, 25). These results suggest that active deacetylation contributes to regulating Lys\(^{120}\) acetylation after DNA damage and that the deacetylating enzyme(s) is TSA-sensitive (i.e. HDAC class I or II). To determine whether Lys\(^{120}\) deacetylation is mediated by a class I or a class II HDAC, the class I-specific inhibitor entinostat (MS275/SNDX275) was used (26). MCF-7 cells were treated with a range of entinostat concentrations, the lower of which are physiologically attainable. The addition of entinostat to CPT-treated cells caused significant accumulation of Ac-Lys\(^{120}\) similar to that achieved with the class I and II inhibitor, TSA (Fig. 1B). The acetylation of histone H4, a known substrate for class I deacetylases, followed a similar dosage trend. The increase in Ac-Lys\(^{120}\) levels was not due to an indirect effect of entinostat on SIRT1 protein levels. In parallel, MCF-7 cells were treated with the class II-specific inhibitor MC1568 (27), which had little effect on Ac-Lys\(^{120}\) levels (supplemental Fig. S1). Ac-Lys\(^{120}\) is also sensitive to TSA and entinostat in HCT116 colon carcinoma cells, which also express endogenous p53 (supplemental Fig. S2), suggesting that the mechanism of Lys\(^{120}\) deacetylation is not cell type-specific.

To identify the specific enzyme(s) responsible for Lys\(^{120}\) deacetylation, we employed a candidate-based approach using the H1299 human lung cancer cell line, which lacks endogenous p53. Lys\(^{120}\) acetylation on ectopic p53 was enforced by co-expression of the hMOF acetyltransferase (Fig. 2A). Individual candidate deacetylases from the three different enzyme classes (HDAC classes I and II, and the sirtuins), were also expressed as confirmed via detection of a shared FLAG epitope tag. Of the class I deacetylases, HDAC1 reduced Ac-Lys\(^{120}\) to basal levels, whereas HDAC2 and HDAC3 had little to no affect. This high degree of selectivity is noteworthy given that HDAC1 and HDAC2 have a number of redundant targets (such as lysines on histone H4) (28). In agreement with the inhibitor studies shown in Fig. 1, the class II and III HDACs tested had minimal ability to deacetylate Lys\(^{120}\) (Fig. 2A). This was the case even in experiments where HDAC5 and SIRT1 expression was greater than that of HDAC1 (data not shown). In some settings, HDAC1 performs a scaffolding rather than an enzymatic role. To test whether the enzymatic activity of HDAC1 is required for deacetylation of Lys\(^{120}\), HDAC1 protein with an inactivating point mutation, HDAC1-H141A (18), was compared with wild-type HDAC1. Although wild-type HDAC1 was able to deacetylate Lys\(^{120}\) robustly (Fig. 2B), the catalytically inactive mutant failed to
reduce Lys<sup>120</sup> acetylation levels, indicating that the enzymatic activity of HDAC1 is required for Lys<sup>120</sup> deacetylation.

For a more physiological assessment, we investigated the role of endogenous HDAC1 on Lys<sup>120</sup> deacetylation of endogenous p53. Consistent with the data shown above, CPT treatment of MCF-7 cells led to an accumulation of Ac-Lys<sup>120</sup>, which was further enhanced by TSA. In parallel, cells were depleted of either HDAC1 or HDAC2 using shRNA (Fig. 3). Although depletion of HDAC2 did not enhance the level of Lys<sup>120</sup> acetylation in CPT-treated cells, depletion of HDAC1 allowed Ac-Lys<sup>120</sup> levels to accumulate to a considerably higher level than in control cells. Furthermore, depletion of HDAC1 increased Ac-Lys<sup>120</sup> to a level similar to that obtained with TSA treatment, consistent with HDAC1 as a critical target of TSA in the Lys<sup>120</sup> acetylation pathway (Fig. 3). HCT116 cells depleted of HDAC1 responded similarly (supplemental Fig. S3). Together these overexpression and shRNA-depletion studies support that HDAC1 is a specific Lys<sup>120</sup>-deacetylating enzyme.

**Role of Distinct HDAC1 Complexes in Lys<sup>120</sup> Deacetylation**—HDAC1 functions via interactions with a number of protein complexes. For example, MDM2 and KAP1 bridge HDAC1 and p53 to regulate acetylation of the C-terminal sites on p53 (18, 29, 30). Because many of these sites are targets for both acetylation and ubiquitylation, it has been proposed that a competitive mechanism occurs in which acetylation counteracts a coupled deacetylation-ubiquitylation reaction carried out by the tertiary MDM2-KAP1-HDAC1 complex, which would otherwise lead to the degradation of p53 (17, 18). To gain insight into which of these partners are involved in HDAC1-mediated Lys<sup>120</sup> deacetylation, we used a combination of pharmacological inhibitors and shRNA-mediated depletion to disrupt these HDAC1 regulators. We used the small molecular inhibitor Nutlin3 to disrupt the interaction of MDM2 with p53, reasoning that if MDM2 recruited HDAC1 to p53, disturbing this interaction would lead to increased Ac-Lys<sup>120</sup> levels. As expected, the addition of Nutlin3 to MCF-7 cells was able to stabilize total p53 fully, confirming that Nutlin3 treatment disrupts p53 from the E3-ubiquitinating ligase, MDM2 (Fig. 4A). However, application of Nutlin3 did not increase levels of Ac-Lys<sup>120</sup>, suggesting that MDM2 was not required to recruit HDAC1 to p53. In contrast to Nutlin3, both DNA damage induced by CPT and reduction of HDAC1 activity by inhibition (TSA) or expression (HDAC1 shRNA) did allow accumulation of Ac-Lys<sup>120</sup>. (The lentiviral infection used to introduce shRNA in this system had no biological affect on p53 and Ac-Lys<sup>120</sup> accumulation because noninfected MCF-7 cells had acetylation levels similar to infected counterparts.)

KAP1 is a co-repressor that associates with KRAB domain-containing zinc finger proteins to facilitate transcriptional silencing (31). An unbiased screen identifying binding partners for MDM2 uncovered an additional role of KAP1 in the p53-regulatory circuit (29). As mentioned above, MDM2, KAP1 and HDAC1 can form a tertiary complex that deacetylates C-terminal lysines within p53 (29, 30). To investigate components involved in the removal of acetyl groups from Lys<sup>120</sup> further, KAP1 was depleted from cells using shRNA (Fig. 4B). DNA damage induced by CPT led to a modest increase in Ac-Lys<sup>120</sup>, in cells expressing control shRNA. Depletion of KAP1 in combination with CPT led to a substantial increase in Ac-Lys<sup>120</sup>. As described previously, the acetylation of Lys<sup>186</sup> within the C terminus modestly increased upon depletion of KAP1 as well. Neither total p53 stabilization nor the levels of phosphorylation of p53 at serine 20 changed upon KAP1 depletion, implying a direct role for KAP1 in Ac-Lys<sup>120</sup> modulation, rather than an upstream effect that leads to an overall increase in p53 activation. However, we were unable to co-immunoprecipitate this complex from MCF-7 cells leaving open the possibility that this is an indirect effect on Ac-Lys<sup>120</sup>.

Transcriptional repression by KRAB-zinc finger proteins is modulated by KAP1 recruitment of the HDAC1-containing
NuRD complex (22). Depletion of Mi2α or MBD3, two NuRD components, by shRNA resulted in a moderate increase in Ac-Lys120 levels, which was similar to levels observed in cells treated with the HDAC inhibitor entinostat (Fig. 4C and supplemental Fig. S4). The above data demonstrate that the deacetylation of Lys120 depends on the presence of KAP1 and suggest that the NuRD complex, together with KAP1, recruits HDAC1 to p53. However, based on results using Nutlin3, Lys120 deacetylation does not require interactions between p53 and MDM2.

Control of p53-mediated Apoptosis by Lys120 Deacetylation—Apoptosis is central to the tumor suppressor function of p53, and acetylation on Lys120 is essential for maximizing p53-mediated apoptosis (7, 8). To investigate the role of HDAC1 in p53-mediated apoptosis via control of Ac-Lys120, we engineered the p53-null cell line H1299 to express either wild-type p53 (TO-p53) or a nonacetylatable mutant (TO-K120R) under the control of a tetracycline-responsive promoter. In this system, induction of p53 led to increased apoptosis in cells expressing wild-type p53, with cells expressing the K120R mutant displaying a marginal defect (Fig. 5). Inhibition of HDAC activity with entinostat enhanced p53-mediated apoptosis significantly more in cells expressing the wild-type allele than in cells expressing the K120R mutant (Fig. 5A). Similar to results using entinostat, shRNA-mediated depletion of HDAC1 significantly increased apoptosis in wild-type p53 cells but only marginally in K120R-mutant cells (Fig. 5B). Collectively, these data support a model in which HDAC1 limits p53-dependent apoptosis in part through the active deacetylation of Lys120.

HDAC inhibitors sensitize cells to p53-mediated apoptosis (32, 33). This effect has been ascribed to hyperacetylated chromatin and defective DNA repair pathways (34). However, neither inhibition nor depletion of HDAC1 elevated DNA damage levels (as measured by the phosphorylation of H2AX), in the cells used in our study (supplemental Fig. S5). Therefore, the increase in apoptosis observed upon disruption of HDAC1 function is unlikely to result from DNA damage accumulation, but rather from a direct role of HDAC1 in the deacetylation of Lys120, which attenuates the apoptotic response.

DISCUSSION

p53 is rapidly acetylated at Lys120 in response to cellular stress (7, 8). This site of modification is distinct from most post-translational modifications on p53 because it lies within the DNA-binding domain and is mutated in human cancer. Some of these cancer mutations convert Lys120 to arginine, thus retaining the positive charge at this site but eliminating acetylation potential, consistent with a role for Lys120 acetylation in p53 tumor-suppressive function. In previous studies, cells expressing p53 with the cancer-derived K120R mutation were shown to be impaired in their ability to promote apoptosis, without any apparent cell cycle arrest defect (7, 8). A mechanistic explanation for this came from evidence that acetylation at Lys120 is a requisite modification for p53-mediated transactivation of certain proapoptotic target genes, but not for cell cycle arrest target genes. In addition to loss of acetylation through mutation, both acetyltransferases that target Lys120, hMOF, and Tip60, are lost in human cancer (10, 11). Given that HDACs act as oncogenes and can serve as targets of anticancer therapy (35), we explored the possibility that these enzymes are an important regulatory component of the Ac-Lys120 p53-mediated apoptotic pathway.

Here, we report that the dynamic deacetylation of Lys120 on endogenous p53 plays a key role in the regulation of p53-me-
diated apoptosis. Combined gain-of-function and loss-of-function studies implicate HDAC1, but not other HDACs or sirtuins, as the enzyme that targets Lys120. Even the highly similar HDAC2 enzyme does not have activity on Ac-Lys120. This was surprising considering that HDAC1 and HDAC2 are ubiquitously expressed and that these two enzymes reside together in several multiprotein complexes including NuRD, which we found to modulate Ac-Lys120. It is interesting to note, however, that although both of these enzymes are found in the NuRD complex, they may not both be required for activity. Recent studies in mouse embryonic stem cells null for HDAC1 or HDAC2 (28) demonstrated that NuRD, as well as other canonical HDAC1- and HDAC2-containing complexes, still have activity when purified from HDAC2 knock-out cells. Lack of HDAC1, however, had a significant effect on NuRD complex activity in these assays. Interestingly, HDAC1-null cells had only marginally higher acetylation levels on most histone lysines, with one obvious exception being acetylation of H3K56 (28). To our knowledge, this histone site, along with Lys120 and Lys382 (18) on p53, are the only substrates that are distinct between the otherwise similar and redundant HDAC1 and HDAC2 enzymes. Germ line deletion of HDAC1, however, results in embryonic lethality, whereas HDAC2 mutation does not (36). The distinction between these two enzymes is intriguing and warrants a comprehensive investigation to define which unique targets are relevant for the differences in the mouse phenotypes.

HDAC1 functions via interactions with a number of protein complexes. In some settings, MDM2 and KAP1 bridge HDAC1 and p53 to regulate acetylation of the C-terminal sites on p53 (18, 29, 30). Findings reported here suggest that HDAC1 deacetylates p53 through a KAP1-dependent but MDM2-independent mechanism. However, in contrast to results from other systems, we were unable to detect a direct interaction among p53, KAP1, and HDAC1, nor a deacetylase activity associated with KAP1 (data not shown), leaving open the possibility that KAP1 is having an indirect effect on Ac-Lys120 levels. Further studies will be required to define the specific role that KAP1 plays in this pathway (29). If Ac-Lys120 is directly targeted by KAP1, it would be of interest to assess whether this activity depends on the recently described melanoma antigen protein C2 (MAGE-C2)-KAP1 complex (37). However, we did not observe a change in p53 stability upon KAP1 depletion. This is in contrast to the KAP1-MAGE-C2 complex, which has E3 ubiquitin ligase activity toward p53 and facilitates p53 degradation. Yet another intriguing possibility is that the matrix attachment region protein, SMAR1, may interact with and recruit HDAC1 to proapoptotic targets like Bax and Puma, where it modulates Lys120 acetylation and transcription (38).

While examining the dynamic deacetylation of Lys120 on p53, we found that the addition of an HDAC inhibitor could enhance acetylation levels robustly, even beyond levels seen following DNA damage. At least two distinct models are suggested by these data: First, the DNA damage pathway may have some mechanism for decreasing HDAC activity toward p53. However, this DNA damage suppression of HDAC activity is not maximal, and chemical inhibition has an additional effect on the pathway. In cancer cells that have increased expression of HDACs, the DNA damage response mechanism may no longer be sufficient to counteract the extensive deacetylation occurring at Lys120. Without the accumulation of Ac-Lys120, the apoptosis is blunted, allowing these cells to avoid death. Second, there may be no direct influence of the DNA damage checkpoint on Lys120 deacetylation, but rather this deacetylation of Lys120 (and other target lysines on p53) is constitutive. The DNA damage-induced increase in acetylation of Lys120 may instead be due to activation of acetyltransferase pathways that counter deacetylation (at least in cells with normal HDAC expression levels). This would shift the equilibrium toward increased acetylation at Lys120 and sensitize the cell to DNA damage-induced apoptosis. In support of the first model, HDACs are regulated in response to DNA damage. For example, HDAC3 can be cleaved following DNA damage, leading to its nuclear export and enhanced apoptosis (39). In contrast, HDAC4 is shuttled into the nucleus after damage, where it acts as a co-repressor during the p53-mediated suppression of genes that promote G2/M cell cycle progression (40). However, for many HDACs (including HDAC1 and HDAC2) experimental evidence linking their activity to the DNA damage response has not been reported. Therefore, the data presented here may be more consistent with the second model, i.e. that HDAC1 constitutively deacetylates Lys120 and the DNA damage cascade is able to counteract this deacetylation by activating acetyltransferase function.

As part of these studies, we found that treatment of cells with the second-generation HDAC inhibitor, entinostat, which targets class I HDACs, increases Lys120 acetylation on endogenous p53 after DNA damage. HDAC inhibitors, including entinostat, are in clinical trials, and one of these agents is approved for the treatment of cutaneous T cell lymphoma (41). Although histones themselves may be relevant targets in some cases, HDACs also modulate the acetylation status of transcription factors such as E2F1, Myc, p53, and some nuclear hormone receptors as well as cytoplasmic targets such as tubulin (16). The functional relevance of the individual acetylated substrates that these inhibitors affect remains largely unknown. What is clear is that HDAC inhibitors counteract the cytoprotective effects that result from overexpression of HDACs in some cancers cells (32, 33) and that HDAC inhibition can render cancer cells more sensitive to lethality caused by DNA-damaging agents like radiation and chemotherapeutics (42). The data presented here suggest that modulating Lys120 acetylation levels is one of the means by which HDAC inhibitors function in tumors that retain wild-type p53. Delineating the components of the Lys120 acetylation/deacetylation pathway and understanding their regulation during cellular stress may help to identify additional events involved in tumorigenesis. Clarification of this pathway could also expose novel targets for cancer therapies. Based on the tight correlation between Lys120 acetylation levels and the tumor-suppressive function of apoptosis, this mark may be useful as a biomarker for the efficacy of therapy aimed at inducing apoptosis of cancer cells.
