



Published in final edited form as:

Oncogene. 2012 September 27; 31(39): 4290–4301. doi:10.1038/onc.2011.605.

Functional interplay between p53 acetylation and H1.2 phosphorylation in p53-regulated transcription

Kyunghwan Kim^{1,2}, Kwang Won Jeong^{1,2}, Hyunjung Kim^{1,2}, Jongkyu Choi^{1,2}, Wange Lu^{1,3}, Michael R. Stallcup^{1,2}, and Woojin An^{1,2}

¹Department of Biochemistry and Molecular Biology, University of Southern California Keck School of Medicine, Los Angeles, CA 90089, USA

²Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine, Los Angeles, CA 90089, USA

³The Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, University of Southern California Keck School of Medicine, Los Angeles, CA 90089, USA

Abstract

Linker histone H1.2 has been shown to suppress p53-dependent transcription through the modulation of chromatin remodeling; however, little is known about the mechanisms governing the antagonistic effects of H1.2 in DNA damage response. Here we show that the repressive action of H1.2 on p53 function is negatively regulated via acetylation of p53 C-terminal regulatory domain and phosphorylation of H1.2 C-terminal tail. p53 acetylation by p300 impairs the interaction of p53 with H1.2 and triggers a rapid activation of p53-dependent transcription. Similarly, DNA-PK-mediated phosphorylation of H1.2 at T146 enhances p53 transcriptional activity by impeding H1.2 binding to p53 and thereby attenuating its suppressive effects on p53 transactivation. Consistent with these findings, point mutations mimicking modification states of H1.2 and p53 lead to a significant increase in p53-induced apoptosis. These data suggest that p53 acetylation-H1.2 phosphorylation cascade serves as a unique mechanism for triggering p53-dependent DNA damage response pathways.

Introduction

The p53 protein is an important tumor suppressor, and its functional inactivation is the most frequent alteration in the majority of human cancers (Junttila and Evan 2009, Toledo and Wahl 2006, Vogelstein et al 2000). In response to a variety of genotoxic stimuli, p53 is stabilized and activated to regulate downstream target genes that contain a consensus p53 response element in promoter or intronic segments (An et al 2004, Beckerman and Prives 2010, Espinosa et al 2003). Structurally and functionally, p53 can be divided into three

Users may view, print, copy, download and text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

Correspondence: Woojin An, Department of Biochemistry and Molecular Biology, University of Southern California Keck School of Medicine, Los Angeles, CA 90089. Tel.: (323) 442-4398; Fax: (323) 442-7879; woojinan@usc.edu.

Conflict of interest

The authors declare no conflict of interest.

distinct domains: the N-terminal transactivation domain, the central DNA binding domain and the C-terminal regulatory domain. The transcriptional potency of p53 is strictly regulated by posttranslational modifications, of which acetylation of the C-terminal domain has been most intensively investigated. p53 acetylation can exert its effects on transcription by altering the conformational state and DNA binding activity of p53 (Gu and Roeder 1997, Luo et al 2004). Alternatively, p53 acetylation can function by creating interaction modules for chromatin remodeling coactivators, such as p300, and thereby promoting their recruitment to cognate p53 response elements in downstream target genes (Barlev et al 2001, Mujtaba et al 2004). Stable localization of these factors at p53 target genes increases the accessibility of chromatin to the transcription machinery, leading to transcription initiation.

Linker histone H1 is one of the five main histone proteins that regulate chromatin competency through its high affinity binding to linker DNA as a structural component. There are multiple H1 isoforms that can be expressed in distinct developmental stages and localized at specific chromatin loci and tissues (Izzo et al 2008, Lennox 1984, Parseghian and Hamkalo 2001). All human H1 isoforms share a conserved structure consisting of a central globular domain flanked by a short flexible N-terminal tail and a long unstructured C-terminal tail. Numerous studies have demonstrated that the interaction of H1 with nucleosomes stabilizes the higher-order chromatin structure and interferes with DNA-dependent reactions such as transcription and replication (Brown 2003, Bustin et al 2005, Georgel and Hansen 2001, Woodcock et al 2006). In addition to having this general structural role in chromatin, H1 can exert gene specific effects by antagonizing the function of particular transcription regulators. For instance, mouse histone H1b is recruited to the MyoD promoter by Msx1 homeoprotein and enhances Msx1 activity in delaying the differentiation of progenitor cells into muscle (Lee et al 2004). The dynamic behavior of H1 has also been illustrated by the cooperative action of H1 and other factors for controlling specific gene transcription (Hale et al 2006, Ni et al 2006). In further support of specific roles for particular H1 isoforms, studies from our laboratory have shown that human H1.2 forms a stable complex with a group of proteins and represses p53-dependent, p300-mediated chromatin transcription (Kim et al 2008). More detailed analysis has revealed that the H1.2 core complex consisting of H1.2 and two repressors, YB1 and PUR α , is sufficient to recapitulate the repressive capacity of the entire H1.2 complex. H1.2-induced repression is accomplished by its direct interaction with p53 and interference with histone acetylation at promoter regions (Kim et al 2008). Although the underlying mechanisms remain elusive, the ability of H1 proteins to repress gene transcription is regulated by their posttranslational modifications (Dou et al 1999, Vaquero et al 2004). Among the modifications identified so far, the best characterized modification is phosphorylation, which is usually mapped to the N- and C-terminal tail domains of H1 (Happel and Doenecke 2009). The functional consequence of H1 phosphorylation appears to be the dissociation of H1 from nucleosomes and the increased opportunity for chromatin remodeling (Contreras et al 2003, Dou et al 1999, Horn et al 2002, Lever et al 2000, Roth and Allis 1992). Thus, given its ability to attenuate H1 effects, H1 phosphorylation is likely to reflect an initial process of activating a specific gene in chromatin.

In light of these observations, we set out to investigate whether p53 acetylation and H1.2 phosphorylation play a regulatory role in p53-mediated transcription. The data indicate that p300-mediated p53 acetylation and DNA-PK-mediated H1.2 phosphorylation impair p53-H1.2 interaction, thereby relieving the repressive effects of H1.2 on p53 transactivation. Upon DNA damage, p53 and H1.2 undergo modifications in an ordered fashion, with H1.2 T146 phosphorylation followed by p53 acetylation. Furthermore, disturbance of p53-H1.2 interaction by their modifications is critical for potentiating p53 function, because point mutations mimicking their constitutive modifications induce more efficient growth inhibition and apoptosis.

Results

p53 acetylation alleviates H1.2-mediated repression of p53 transactivation

We have recently reported that the H1.2 core complex, consisting of H1.2, YB1 and PUR α , can repress p53-dependent, p300-mediated chromatin transcription by simultaneously adding the core complex and p300 to transcription reactions (Kim et al 2008). To understand the mode of action of the H1.2 complex on chromatin transcription more precisely, we first carried out the order of addition experiments using cell free assay system (Figure 1b). For chromatin reconstitution, we employed p53ML-601 array DNA template and recombinant core histones as detailed in Supplementary Information. The successful reconstitution of periodic nucleosome arrays on the DNA template was confirmed by partial micrococcal nuclease (MNase) digestion (Supplementary Figure S1). The addition of the H1.2 core complex prior to p300 resulted in a strong repression of transcription from p53ML-601 nucleosome arrays that had been bound by p53 (Figure 1c, Transcription, lanes 1–5). Parallel results were obtained when the H1.2 core complex and p300 were added to the reactions at the same time (Transcription, lanes 11–15). Interestingly, however, the repressive effect of the H1.2 core complex was minimal if it was added to the transcription reactions after p300 (Transcription, lanes 6–10). To exclude the possibility that the transcription repression we observed by adding H1.2 prior to p300 was caused by the limited accessibility of nucleosomal DNA, we performed MNase digestion assays with nucleosome arrays incubated with p53, p300 and the H1.2 core complex. No major differences in the MNase digestion pattern were observed, when nucleosome arrays incubated with the H1.2 core complex before or after p300 addition were compared (Supplementary Figure S1b, lanes 3–5). These results are consistent with the idea that the addition of the H1.2 core complex to transcription reactions before p300 has little or no effect on the stability of the nucleosome arrays. Because p53 is acetylated by p300 at multiple lysine residues (K373, K381 and K382) within the C-terminal regulatory domain for its transcriptional activation function, a possible explanation for our transcription results is that the H1.2 core complex could interfere with p300-mediated acetylation of p53. In fact, Western blot analysis with anti-acetyl p53 (K373/K382) antibody showed that p53 C-terminal domain was acetylated by p300 to a very limited extent, when the H1.2 core complex was added to transcription reactions prior to or together with p300 (Figure 1c, Western, Acp53, lanes 1–5 and 11–15). By contrast, no change was observed in p53 acetylation following the addition of the H1.2 core complex after p300 (Western, Acp53, lanes 6–10). Similarly, individual or pairwise

addition of H1.2, YB1 and PUR α generated no detectable change in the level of p53 acetylation mediated by p300 (Supplementary Figure S2).

A direct interaction between p53 C-terminal domain and H1.2 C-terminal tail has been well-illustrated for the stable localization and action of the H1.2 complex at p53 target genes (Kim et al 2008). Thus, a plausible explanation for the transcription/modification results is that p300-mediated acetylation of p53 could perturb p53-H1.2 interaction at the promoter region. This possibility was investigated by a series of interaction studies. Recombinant H1.2 formed a stable complex with glutathione S-transferase (GST)-p53 fusion proteins pre-bound to glutathione-Sepharose beads (Figure 1d, lane 3). p53 interaction with H1.2 was dependent upon its C-terminal region, because H1.2 was not precipitated from the reaction by GST-p53 1-363, in which the C-terminal 30 residues were deleted (lane 4). The specificity of these in vitro interactions was confirmed by the failure of H1.2 to bind to GST alone (lane 2). When binding experiments were repeated with or without p300-mediated preacetylation of p53, we detected a remarkable binding preference of H1.2 for the unacetylated p53 over the acetylated p53 (Figure 1e). Moreover, in similar binding experiments using synthetic peptides derived from the p53 C-terminal domain (amino acids 364-393), the unacetylated peptides showed a distinct binding to H1.2, but the p53 peptides bearing acetylations at K373, K381 and K382 showed a markedly lower affinity to H1.2 (Figure 1f).

To further verify the above finding, we employed a transient expression approach using the p53-deficient cell line H1299 and a reporter gene construct containing p53 response elements. As summarized in Figure 1g, ectopic expression of wild type p53 reactivated the reporter gene, but co-expression of the H1.2 core complex significantly repressed p53 transcription activity (assay 2 versus assay 3). When the acetylation-mimicking mutant p53 with lysine to glutamine substitutions at K373, K381 and K382 (3KQ) was expressed, transcription activity was moderately enhanced (assay 4). Transfection of acetylation-blocking mutant (3KR), in which K373, K381 and K382 were changed to arginine showed no detectable change in the level of transcription (assay 6). In this setting, we checked whether these p53 mutants could affect the function of the H1.2 core complex as a transcriptional repressor. Remarkably, expression of the p53 mutant (3KQ) mimicking constitutive acetylation, but not the p53 mutant (3KR) blocking cellular acetylation, enhanced transcription of the reporter gene (assay 5 versus assay 7). Taken together, these assays corroborate our in vitro findings and suggest that p53 acetylation could alleviate transcriptional inhibition mediated by the H1.2 core complex.

H1.2 T146 is a substrate for DNA-PK-directed phosphorylation

The N- and C-terminal tail domains of H1 have been known as prominent substrates for various posttranslational modifications. Intrigued by our observation that DNA-PK is co-purified with H1.2 (Kim et al 2008), we explored whether DNA-PK can catalyze H1.2 phosphorylation and, if so, H1.2 phosphorylation is functionally connected to p53 acetylation in regulating p53-H1.2 interaction. As shown in Figure 2b, in vitro kinase assays demonstrated that DNA-PK can phosphorylate full length GST-H1.2, but not GST alone (lanes 1 and 2). To locate possible phosphorylation sites, we repeated the kinase assays

using three distinct domains of H1.2: the N-terminal tail domain (amino acids 1-34), the central globular domain (amino acids 35-109) and the C-terminal tail domain (amino acids 110-213) (Figure 2a). Unlike the N-terminal tail and globular domains, the C-terminal tail domain of H1.2 was efficiently phosphorylated by DNA-PK at a level similar to that of full length H1.2 (Figure 2b, lanes 3–5). In similar experiments with two subregions of the C-terminal tail domain, namely amino acids 110-138 and 139-213, we found that DNA-PK can phosphorylate amino acids 139-213, but not amino acids 110-138, of H1.2 (lanes 7 and 8). Potential phosphorylation sites in amino acids 139-213 include four threonine residues (T146, T154, T165 and T167) and three serine residues (S150, S173 and S188). To determine which of these residues are phosphorylated by DNA-PK, kinase assays were repeated with full length mutant proteins in which the seven potential sites were individually substituted with alanine. Substitution of each of T150, T154, T165, T167, S173 and S188 did not change the level of H1.2 phosphorylation (Figure 2c, compare lanes 3–8 with lane 1). Meanwhile, mutation of T146 completely abrogated H1.2 phosphorylation (lane 2), indicating that T146 is the predominant site in H1.2 C-terminal tail that is phosphorylated by DNA-PK. Western blot analysis using H1 phospho T146 antibody further confirmed that T146 serves as a major substrate for DNA-PK (Figure 2d). In agreement with these in vitro results, when human osteosarcoma U2OS cells were treated with etoposide, H1.2 phosphorylation at T146 was rapidly increased within 15 min following etoposide-induced DNA damage and decreased to the base line after 60 min of etoposide treatment (Figure 2e, DMSO, pT146). However, no such increase in H1.2 phosphorylation at the 15 min point was observed in cells pretreated with a DNA-PK specific inhibitor, NU7026 (NU7026, pT146). As a control of equivalent amounts of protein loaded in each lane, the Western blot was probed with anti-H1.2 antibody. There were equal amounts of H1.2 in all samples (H1.2). In conclusion, H1.2 is phosphorylated at T146 in response to DNA damage, and DNA-PK is mainly responsible for the observed phosphorylation.

T146 phosphorylation attenuates H1.2 repression activity

Knowing that H1.2 is specifically phosphorylated at T146 by DNA-PK, we sought to determine the effects of this modification on transrepressive activities of the H1.2 core complex. Toward this end, in vitro transcription assays were carried out by pre-phosphorylating the H1.2 core complex with DNA-PK and adding it into the reactions after p53 but before p300 (Figure 3a). When H1.2 was pre-modified by DNA-PK, the H1.2 core complex lost its ability to repress p53-dependent, p300-mediated transcription from nucleosome arrays (Figure 3b, lane 4 versus lane 5). The transcription stimulatory effect of DNA-PK, however, was largely compromised upon mutation of H1.2 T146 to alanine (lane 5 versus lane 10). These results suggest that T146 phosphorylation by DNA-PK is the major cause of the observed impairment of H1.2-induced repression. To further evaluate the relationship between H1.2 phosphorylation and p53 transcriptional activity, we performed in vitro binding experiments using GST-p53 290-393 and wild type or T146-mutated H1.2. Both wild type and mutant H1.2 showed a comparable interaction with p53 290-393, as determined by Western blot analysis of the binding reactions (Figure 3c, lanes 3 and 7). The interaction between H1.2 and p53 was markedly diminished following H1.2 phosphorylation by DNA-PK (lane 4 versus lane 3). In stark contrast, however, DNA-PK was no longer

capable of negatively regulating H1.2-p53 interaction, when H1.2 T146A mutant was used in the binding study (lane 8 versus lane 7).

We next attempted to investigate the functional importance of H1.2 phosphorylation by performing luciferase reporter gene assays in H1299 cells. Consistent with our previous results (Kim et al 2008), H1.2 alone had no effect on p53-mediated transcription of reporter gene, but cooperated with PUR α and YB1 to inhibit p53 transcriptional activity (Figure 3d, assay 4 versus assay 5). When H1.2 T146 was substituted to glutamic acid to mimic phosphorylation, the repressive effect of the H1.2 core complex was lost, and p53 transcriptional activity was fully recovered (assay 9). On the contrary, the mutant H1.2 bearing a threonine-to-alanine exchange at T146 still retained its ability to repress p53 transactivation (assay 7), thereby excluding a possible effect of mutation itself on H1.2 competency. These results, together with the results from the in vitro transcription and binding assays, strongly suggest that transrepression activity of H1.2 is strictly regulated by T146 phosphorylation. To further confirm that DNA-PK kinase activity is essential for H1.2 phosphorylation and p53 transactivation, we carried out reporter gene assays in H1299 cells treated with the DNA-PK inhibitor NU7026. As anticipated, NU7026 treatment markedly impaired DNA damage-induced p53 activation in H1299 cells expressing the wild type H1.2 core complex (Figure 3e, H1.2 WT core com). However, in repeated assays using cells transfected with H1.2 T146E mutant mimicking constitutive phosphorylation, the loss of H1.2 effects and thus no change in the level of p53 transactivation were evident (H1.2 T146E core com). When H1.2 T146A mutant blocking phosphorylation was transfected into the cells, the repressive effect of H1.2 was retained regardless of NU7026 treatment (H1.2 T146A core com). These results emphasize the essential role of DNA-PK in the control of H1.2-p53 interaction and thus H1.2 activity in p53-mediated transcription.

DNA damage induces p53 acetylation and H1.2 phosphorylation

Given the combinatorial effects of p53 acetylation and H1.2 phosphorylation on p53 transactivation, we investigated a possible alteration of these modifications in U2OS cell line after etoposide treatment for 0, 15, 30 or 60 min. As determined by Western blotting of extracts, a significant increase in p53 acetylation in response to the etoposide-induced DNA damage was observed over 60 min time period (Figure 4a, Acp53). Since etoposide treatment only modestly increased p53 protein levels (p53), p53 acetylation may be an early event in the DNA damage response. In Western blotting with H1 phospho T146 antibody, a rapid phosphorylation of H1.2 at T146 was detected within 15 min following etoposide treatment and returned to the baseline by 60 min (Figure 4b). Because the observed changes in p53 and H1.2 modifications were recapitulated with DNA damage induced by another DNA damage reagent, bleomycin, these modifications may be functional in cellular response to diverse damaging agents (Supplementary Figure 3). Likewise, immunostaining experiments showed that etoposide treatment of U2OS cells leads to a progressive increase in the level of acetylated p53 in the nucleus (Figure 4c, Acp53). In parallel experiments in which changes in H1.2 phosphorylation were analyzed over the same time period, the highest intensity of staining for H1.2 phosphorylation was scored at 15 min time point and gradually decreased thereafter (Figure 4d, pT146). These data imply that p53 and H1.2 are modified in a sequential manner upon DNA damage, with H1.2 phosphorylation followed

by p53 acetylation. In all cases, there were a rather modest increase in p53 protein level (Figure 4c, p53) and no detectable change in H1.2 protein level (Figure 4d, H1.2) in response to DNA damage.

Because p53 acetylation and H1.2 phosphorylation decrease p53-H1.2 interaction in vitro (Figures 1e and 3c), we next examined to which extent these modifications are involved in regulating H1.2 binding to p53 in U2OS cells. Cell lysates were prepared from cells treated with etoposide for the indicated times, and p53-H1.2 interaction was examined by Western blotting of immunoprecipitated materials. As shown in Figure 4e, endogenous p53 efficiently coprecipitated with H1.2 from extracts prepared before etoposide treatment (0 time point), but minimally did so from extracts prepared 15 min and 60 min after etoposide treatment. To further substantiate the role of H1.2 phosphorylation in releasing H1.2 from p53, we repeated immunoprecipitation experiments using H1299 cells transfected with expression vectors for Flag-tagged p53 290-393 and Xpress-tagged wild type H1.2 or nonphosphorylatable H1.2 harboring alanine substitution at T146. p53 stably interacted with both wild type and mutant H1.2 under normal conditions (0 time point), but there was ~90% reduction in its interaction with H1.2 60 min after etoposide treatment (Figure 4f). These results argue that despite the possible positive effects of T146A mutation on p53-H1.2 interaction, p53 acetylation is likely the primary regulator of p53-H1.2 interaction at 60 min time point. In fact, point mutations of the major p53 acetylation sites restored p53-H1.2 interaction at 60 min time point back to that at 0 time point (Figure 4g, lanes 1–4). Again, these results strongly support earlier indications that H1.2 phosphorylation plays a dominant role in regulating p53-H1.2 interaction 15–30 min after etoposide treatment, whereas p53 acetylation does so 30–60 min after the treatment. No detectable changes in p53-H1.2 interaction when p53 and H1.2 mutants simultaneously replaced their wild type counterparts further indicate that p53 acetylation and H1.2 phosphorylation are essential for their continuous dissociation (lanes 5–8).

p53 acetylation and H1.2 phosphorylation promote p53-dependent apoptotic cell death

Based on our findings that p53 acetylation and H1.2 phosphorylation are key events in p53-mediated transactivation, we explored whether these modifications coordinately regulate cell growth and apoptosis. In our clonogenic assays, H1299 cells displayed a reduced ability to form colonies after transfection of wild type p53 or acetylation mimicking mutant p53 (3KQ), as compared with empty vector-transfected cells (Figure 5a, assays 2 and 3 versus assay 1). By contrast, the observed reduction in colony formation could not be recapitulated by transfection of the H1.2 core complex (assays 4 and 5). Consistent with the transcription results indicating H1.2-induced repression of p53 activity, simultaneous expression of wild type p53 and the H1.2 core complex increased the rate of colony formation similar to that observed in the empty vector-transfected cells (assay 6). Notably, however, when wild type H1.2 in the core complex was replaced by phosphorylation mimicking mutant H1.2 (T146E), colony numbers were decreased back to those obtained by transfection of p53 alone (assay 7). Likewise, transfection of cells with acetylation mimicking mutant p53 (3KQ) decreased cell proliferation in all cases, regardless of whether wild type or mutant H1.2 is cotransfected (assays 8 and 9).

To determine whether p53 acetylation and H1.2 phosphorylation would also lead to apoptotic cell death, we assessed DNA fragmentation in cells transfected with wild type or mutant versions of p53 and H1.2. Transfection of H1299 cells with wild type p53 or acetylation mimicking mutant p53 (3KQ) resulted in a marked increase in the number of TUNEL-positive cells (Figure 5b, assays 2 and 3 versus assay 1). However, expression of the H1.2 core complex containing wild type or phosphorylation mimicking mutant (T146E) H1.2 could not induce apoptotic cell death (assays 4 and 5). Consistent with our observation in colony formation assays, cotransfection of wild type versions of p53 and the H1.2 core complex decreased the number of TUNEL-positive cells (assay 6). By contrast, simultaneous transfection of wild type p53 and the H1.2 core complex containing phosphorylation mimicking mutant H1.2 (T146E) failed to inhibit apoptotic cell death (assay 7). Additionally, transfection of cells with acetylation mimicking mutant p53 (3KQ) and wild type or mutant H1.2 core complex induced apoptotic cell death (assays 8 and 9).

To investigate whether the observed effects of p53 and H1.2 modifications are accompanied by p53 transactivation, we next measured the level of target gene transcription. H1299 cells were transfected with vectors expressing wild type or mutant forms of p53 and the H1.2 core complex, and p53-activated transcription of target genes was determined by quantitative RT-PCR. Predictably, transfection of wild type p53 activated p21 gene transcription, and the observed transactivation was evidently repressed by coexpression of wild type H1.2 core complex (Figure 6a, p21, assay 2 versus assay 6). However, the repressive action of the H1.2 core complex was impaired by substituting T146 with phosphorylation mimicking glutamic acid (T146E) (p21, assay 7). When the acetylation mimicking mutant p53 (3KQ) was coexpressed with wild type or mutant H1.2 core complex, p21 gene transcription was enhanced (p21, assays 8 and 9). Consistent with the results from p21 gene, expression of modification mimicking mutants of p53 and the H1.2 core complex greatly boosted the level of transcription of Apaf1, another p53 target gene (Apaf1, assays 7–9), indicating that p53 acetylation and H1.2 phosphorylation play a role in the activation of other p53 target genes as well. Together, these results constitute a powerful argument that H1.2 phosphorylation and p53 acetylation are cooperative for their action in p53 transcription pathway.

Discussion

A flurry of recent studies about linker histone H1 proteins has offered the new emerging aspect that this family of proteins might be linked to regulation of specific genes, in addition to their well-known roles as a structural component of the nucleosome. In further support of H1 function in gene specific transcriptional regulation, we recently showed that the binding of the H1.2 core complex consisting of H1.2, YB1 and PUR α to p53 causes an impairment of p53-mediated transcription. Although our studies uncovered a previously unrecognized role for the H1.2 complex in suppressing p53 transcriptional activity, it was not clear how H1.2 dissociates from p53 to initiate p53 transactivation in response to DNA damage. In this study, we demonstrate the functional crosstalk between p53 acetylation and H1.2 phosphorylation in triggering H1.2 release from p53 as well as in stimulating p53 transcriptional network (see Figure 6b).

A key finding from our initial study is that p300-induced acetylation of the p53 C-terminal basic region attenuates p53-H1.2 interaction, which is necessary for enhancing p53 transcriptional activity and recruiting coactivators at p53 target promoters. Supporting these biochemical data, cellular studies also demonstrate the inverse correlation between p53 acetylation and H1.2 transrepression. These observations strongly suggest that p53 acetylation is a key determinant for H1.2 dissociation from p53 and contributes to the fine tuning of p53 function in response to DNA damage. In this study, we also establish another modification pathway in which DNA-PK attenuates the repressive action of H1.2 against p53-mediated transactivation. We found that DNA-PK specifically phosphorylates H1.2 at T146, thereby disrupting p53-H1.2 interaction and leading to the enhancement of p53 acetylation and the activation of p53 target genes. Cellular studies using mutant H1.2 further demonstrate that H1.2 phosphorylation at T146 is responsible for the observed activation and consequently for timely cell growth arrest and apoptosis. Our results implicating a primary function of H1.2 T146 phosphorylation in transcription and DNA damage response seem to contrast with a recent report showing that H1.2 T146 is phosphorylated exclusively during mitosis (Zheng et al 2010). However, we speculate that mitosis-coupled phosphorylation of H1.2 does not require DNA-PK and may be mediated by cell cycle-regulated kinases, such as cyclin-dependent kinases. These results also suggest that H1.2 phosphorylation may play a broader role in cell signaling than previously expected. Additional experiments will be necessary to uncover the relative importance and regulatory mechanisms of H1.2 phosphorylation that occurs during various cellular processes.

Of particular interest in our study is the observation that etoposide treatment of cells produces H1.2 T146 phosphorylation prior to p53 acetylation. Whether these sequential modifications of H1.2 and p53 truly reflect the early programming of p53 response genes or a delayed unmasking of acetylated p53 remains unclear. However, we would suggest that DNA damage-induced phosphorylation of H1.2 may enhance the accessibility of promoter-bound p53. In this way p300 may be properly targeted to gene promoters through interaction with p53 in damaged cells. Thus, it is likely that H1.2 phosphorylation is critical in regulating the initial stage of DNA damage-facilitated, p53-mediated transactivation. Further studies will be required to determine the extent to which the preferred order of H1.2 phosphorylation and p53 acetylation described here pertains to other p53 target genes.

The underlying mechanism that p53 acetylation and H1.2 phosphorylation trigger the dissociation of H1.2 from p53 is still not clear. Considering the formation of amphipathic α -helical structure in H1.2 C-terminal tails and the tendency of hydrophobic faces of amphipathic helices to interact with other proteins (Ausio 2006, Caterino and Hayes 2011, McBryant et al 2010, Vila et al 2000), conformational change of H1.2 C-terminal domain is likely to be part of the mechanisms by which H1.2 phosphorylation regulates H1.2 binding to p53. This is somewhat similar to the case of other repressors, in which the distinct modifications in the regulatory region result in their dissociation from transcription factors and gene activation. In an analogous manner, p53 acetylation, which resides within the C-terminal regulatory domain, could also induce a non-amphipathic structure to destabilize p53 interaction with H1.2 and stimulate p53-mediated transcription. As another possible mechanism, H1.2 phosphorylation could enhance p53 transcriptional activity such that phosphorylated H1.2 remains incapable of establishing the required interactions with PUR α .

and YB1. However, in our binding experiments, H1.2 phosphorylation did not impede H1.2 interaction with PUR α and YB1 (data not shown), suggesting that H1.2 phosphorylation acts without affecting the stability of the H1.2 core complex. Structural investigations of p53-bound H1.2 core complex will provide information on the nature of p53-H1.2 interaction and how p53-H1.2 modifications cause their dissociation.

Materials and methods

Materials

H1299 and U2OS cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum in an atmosphere of 5% CO₂ at 37 °C. Antibodies specific for p53 (DO1 and FL-393) and His tag were obtained from Santa Cruz Biotechnology; antibody specific for K373/K382-acetylated p53 was from Millipore; antibodies specific for H1.2 and H1 pT146 were from Abcam; antibodies specific for actin and Flag tag were from Sigma; and antibody specific for Xpress tag was from Invitrogen.

Plasmid constructions

See the Supplementary Information for details of plasmid constructions.

Recombinant proteins and p53 peptides

See the Supplementary Information for details on preparation of recombinant proteins and p53 C-terminal peptides.

Reconstitution of nucleosome arrays and in vitro transcription and modification assays

For nucleosome array reconstitution, the p53ML601-14 plasmid was digested with EcoRI and HindIII, and the 3.4 kb p53ML-601 array DNA fragment was gel-purified. Nucleosome arrays were reconstituted by salt gradient dialysis and purified by sedimentation in a 5–30% (vol/vol) glycerol gradient (Jaskelioff et al 2000). Micrococcal nuclease (Sigma) digestion of nucleosome arrays (2 μ g) was performed as described (An et al 2004). In vitro transcription assays were carried out as described previously (Kim et al 2008) utilizing p53ML nucleosome array (100 ng), p53 (20 ng), p300 (20 ng), PUR α (30 or 60 ng), YB1 (30 or 60 ng) and/or H1.2 (30 or 60 ng). For transcription assays with phosphorylated H1.2, H1.2 was preincubated with purified DNA-PK (50 ng) (Ma and Lieber 2006) in the presence of ATP (10 mM) for 60 min and then added to transcription reactions. Following gel electrophoresis of transcription reactions, the gels were dried and subjected to autoradiography, and the bands were quantitated by Phosphorimager. In vitro acetylation assays were performed by incubating recombinant p53 (20 ng) with p300 (20 ng) in transcription buffer (40 mM HEPES, pH 7.8, 120 mM KCl, 10 mM DTT, 120 mM MgCl₂, 1 mM EDTA, and 20 mM Na-butyrate) supplemented with 10 μ M AcCoA at 30 °C for 60 min. Reactions were separated by 10% SDS-PAGE, and p53 acetylation was assessed by Western blot using anti-Acp53 antibody. For in vitro kinase assays, recombinant H1.2 proteins were incubated with DNA-PK (20 ng) in kinase buffer (50 mM Tris-HCl, pH7.5, 20 mM EGTA, 10 mM MgCl₂, 1 mM DTT, and 1 mM beta-glycerophosphate) containing 10 μ Ci of [γ -³²P]ATP and 5 mM ATP for 60 min at 30 °C. Reactions were resolved by 12% SDS-PAGE, and ³²P-labeled proteins were visualized by autoradiography.

Reporter gene assays

H1299 cells were plated in 12-well plates at 50% confluence and transfected with p53RE-luc reporter (200 ng) harboring p53 response elements and expression vectors (100 ng) for p53, H1.2, YB1 and/or PUR α for 24 h. Cells were harvested in Reporter Lysis Buffer (Promega) and assayed for luciferase activity using Odyssey System Premium (Li-Cor Bioscience). To verify the role of DNA-PK, cells were treated with the DNA-PK inhibitor NU7026 (5 μ M, Cayman).

In vitro binding experiments

In vitro binding assays to study p53-H1.2 interaction were performed by essentially following procedures of Kim et al (2008). A detailed description is provided in the Supplementary Information.

Immunoprecipitation

Immunoprecipitation assays were carried out as described previously (Kim et al 2008) with minor modifications as detailed in the Supplementary Information.

Immunofluorescence and Western blotting

All the details regarding immunofluorescence and Western blot analysis are provided in the Supplementary Information.

Colony formation and TUNEL assays

For soft agar colony formation assays, H1299 cells were transfected in 100-mm plates with a vector (12 μ g) containing the cDNA encoding p53, H1.2, PUR α or YB1 or a control vector by means of lipofectamine transfection reagent. Cells were suspended in semisolid medium (RPMI 10% FBS plus 0.3% ultra pure noble agar) at concentrations of 2×10^5 cells/ml, added over a layer of 0.6% agar in RPMI on 35-mm plate and incubated for an additional 21 days. The colonies in each well were stained with 0.005% crystal violet in 20% ethanol, counted and photographed. All assays were run in triplicate, and results presented are the average of three individual experiments. For terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assays, H1299 cells were seeded onto 18-mm glass coverslip and transfected with 2 μ g of vectors per well for 48 h. The cells were then stained by TUNEL, according to the manufacturers' instructions (GeneScript), and visualized by Zeiss microscope. The apoptotic index was defined by the percentage of TUNEL-positive cells among the total cells of each sample. More than 200 cells were examined in 10 random fields for each sample, and TUNEL-positive cells were counted.

qRT-PCR

Transcription levels of p21, Apaf1 and Gapdh were measured by qRT-PCR and normalization to β -actin mRNA level as described in the Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge Michael Lieber for DNA-PK and Daniela Rhodes for p601-7. This research was supported by NIH-R01GM84209 (W.A.), ACS RSG DMC-1005001 (W.A.) and NIH-RO1DK04393 (M.R.S)

References

- An W, Kim J, Roeder RG. Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. *Cell*. 2004; 117:735–748. [PubMed: 15186775]
- Ausio J. Histone variants--the structure behind the function. *Brief Funct Genomic Proteomic*. 2006; 5:228–243. [PubMed: 16772274]
- Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD, et al. Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. *Mol Cell*. 2001; 8:1243–1254. [PubMed: 11779500]
- Beckerman R, Prives C. Transcriptional regulation by p53. *Cold Spring Harb Perspect Biol*. 2010; 2:a000935. [PubMed: 20679336]
- Brown DT. Histone H1 and the dynamic regulation of chromatin function. *Biochem Cell Biol*. 2003; 81:221–227. [PubMed: 12897856]
- Bustin M, Catez F, Lim JH. The dynamics of histone H1 function in chromatin. *Mol Cell*. 2005; 17:617–620. [PubMed: 15749012]
- Caterino TL, Hayes JJ. Structure of the H1 C-terminal domain and function in chromatin condensation. *Biochem Cell Biol*. 2011; 89:35–44. [PubMed: 21326361]
- Contreras A, Hale TK, Stenoien DL, Rosen JM, Mancini MA, Herrera RE. The dynamic mobility of histone H1 is regulated by cyclin/CDK phosphorylation. *Mol Cell Biol*. 2003; 23:8626–8636. [PubMed: 14612406]
- Dou Y, Mizzen CA, Abrams M, Allis CD, Gorovsky MA. Phosphorylation of linker histone H1 regulates gene expression in vivo by mimicking H1 removal. *Mol Cell*. 1999; 4:641–647. [PubMed: 10549296]
- Espinosa JM, Verdun RE, Emerson BM. p53 functions through stress- and promoter-specific recruitment of transcription initiation components before and after DNA damage. *Mol Cell*. 2003; 12:1015–1027. [PubMed: 14580351]
- Georgel PT, Hansen JC. Linker histone function in chromatin: dual mechanisms of action. *Biochem Cell Biol*. 2001; 79:313–316. [PubMed: 11467744]
- Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell*. 1997; 90:595–606. [PubMed: 9288740]
- Hale TK, Contreras A, Morrison AJ, Herrera RE. Phosphorylation of the linker histone H1 by CDK regulates its binding to HP1 α . *Mol Cell*. 2006; 22:693–699. [PubMed: 16762841]
- Happel N, Doenecke D. Histone H1 and its isoforms: contribution to chromatin structure and function. *Gene*. 2009; 431:1–12. [PubMed: 19059319]
- Horn PJ, Carruthers LM, Logie C, Hill DA, Solomon MJ, Wade PA, et al. Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. *Nat Struct Biol*. 2002; 9:263–267. [PubMed: 11887184]
- Izzo A, Kamieniarz K, Schneider R. The histone H1 family: specific members, specific functions? *Biol Chem*. 2008; 389:333–343. [PubMed: 18208346]
- Jaskelioff M, Gavin IM, Peterson CL, Logie C. SWI-SNF-mediated nucleosome remodeling: role of histone octamer mobility in the persistence of the remodeled state. *Mol Cell Biol*. 2000; 20:3058–3068. [PubMed: 10757790]
- Junttila MR, Evan GI. p53--a Jack of all trades but master of none. *Nat Rev Cancer*. 2009; 9:821–829. [PubMed: 19776747]
- Kim K, Choi J, Heo K, Kim H, Levens D, Kohno K, et al. Isolation and characterization of a novel H1.2 complex that acts as a repressor of p53-mediated transcription. *J Biol Chem*. 2008; 283:9113–9126. [PubMed: 18258596]

- Lee H, Habas R, Abate-Shen C. MSX1 cooperates with histone H1b for inhibition of transcription and myogenesis. *Science*. 2004; 304:1675–1678. [PubMed: 15192231]
- Lennox, RW.; Cohen, LH. The H1 subtypes of mammals: metabolic characteristics and tissue distribution. John Wiley & Sons; New York: 1984.
- Lever MA, Th'ng JP, Sun X, Hendzel MJ. Rapid exchange of histone H1.1 on chromatin in living human cells. *Nature*. 2000; 408:873–876. [PubMed: 11130728]
- Luo J, Li M, Tang Y, Laszkowska M, Roeder RG, Gu W. Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2004; 101:2259–2264. [PubMed: 14982997]
- Ma Y, Lieber MR. In vitro nonhomologous DNA end joining system. *Methods Enzymol*. 2006; 408:502–510. [PubMed: 16793389]
- McBryant SJ, Lu X, Hansen JC. Multifunctionality of the linker histones: an emerging role for protein-protein interactions. *Cell Res*. 2010; 20:519–528. [PubMed: 20309017]
- Mujtaba S, He Y, Zeng L, Yan S, Plotnikova O, Sachchidanand, et al. Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. *Mol Cell*. 2004; 13:251–263. [PubMed: 14759370]
- Ni JQ, Liu LP, Hess D, Rietdorf J, Sun FL. Drosophila ribosomal proteins are associated with linker histone H1 and suppress gene transcription. *Genes Dev*. 2006; 20:1959–1973. [PubMed: 16816001]
- Parseghian MH, Hamkalo BA. A compendium of the histone H1 family of somatic subtypes: an elusive cast of characters and their characteristics. *Biochem Cell Biol*. 2001; 79:289–304. [PubMed: 11467742]
- Roth SY, Allis CD. Chromatin condensation: does histone H1 dephosphorylation play a role? *Trends Biochem Sci*. 1992; 17:93–98. [PubMed: 1412698]
- Toledo F, Wahl GM. Regulating the p53 pathway: in vitro hypotheses, in vivo veritas. *Nat Rev Cancer*. 2006; 6:909–923. [PubMed: 17128209]
- Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D. Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell*. 2004; 16:93–105. [PubMed: 15469825]
- Vila R, Ponte I, Jimenez MA, Rico M, Suau P. A helix-turn motif in the C-terminal domain of histone H1. *Protein Sci*. 2000; 9:627–636. [PubMed: 10794405]
- Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. *Nature*. 2000; 408:307–310. [PubMed: 11099028]
- Woodcock CL, Skoultchi AI, Fan Y. Role of linker histone in chromatin structure and function: H1 stoichiometry and nucleosome repeat length. *Chromosome Res*. 2006; 14:17–25. [PubMed: 16506093]
- Zheng Y, John S, Pesavento JJ, Schultz-Norton JR, Schiltz RL, Baek S, et al. Histone H1 phosphorylation is associated with transcription by RNA polymerases I and II. *J Cell Biol*. 2010; 189:407–415. [PubMed: 20439994]

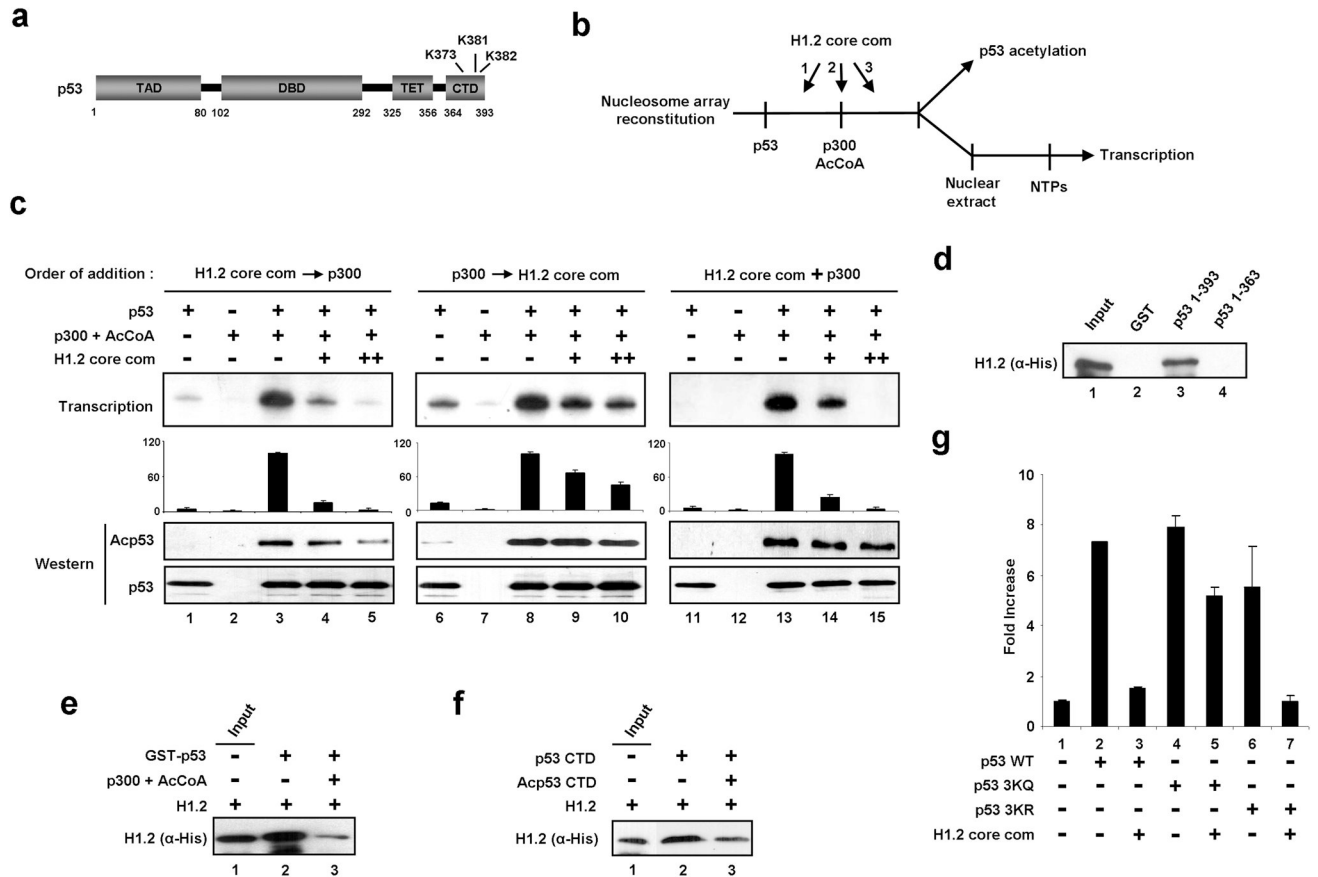


Figure 1. p53 acetylation interferes with H1.2 transrepression activity

(a) Schematic diagram of the domain structure of p53. Three major acetylation sites for p300 are indicated. TAD, transcription activation domain; DBD, DNA binding domain; TET, tetramerization domain; and CTD, C-terminal domain. Numbers denote amino acid positions.

(b) Outline of the in vitro transcription and p53 acetylation assays with nucleosome arrays. The H1.2 core complex was added in the following ways; 1, before p300 and acetyl CoA (AcCoA); 2, together with p300 and AcCoA; 3, after p300 and AcCoA. NTPs: nucleotide triphosphates.

(c) Repressive effects of the H1.2 core complex on p53-dependent transcription were determined by the order-of-addition assays, in which the H1.2 core complex was incubated with nucleosome arrays before (lanes 1–5) or after (lanes 6–10) the addition of p300 and AcCoA. After the transcription reactions, the radiolabeled RNA products were digested with RNase T1, and then analyzed by gel electrophoresis and autoradiography. In parallel transcription reactions, the H1.2 core complex was added simultaneously with p300 and AcCoA (lanes 11–15). Results are representative of three independent experiments, and the mean and standard deviation of three experiments are shown. p53 concentration and its acetylation level in transcription reactions were also determined by Western blotting using anti-p53 and anti-acetyl p53 (Acp53) antibodies (middle and lower panels).

(d) His-H1.2 was tested for binding to GST (lane 2), GST-full length p53 (lane 3) or GST-C-terminal truncated p53 (lane 4). Bound H1.2 proteins were resolved by 12% SDS-PAGE, transferred to nitrocellulose membranes and probed with anti-His antibody. Input corresponds to 10% of the protein used in the binding reactions (lane 1).

(e) GST-full length p53 immobilized on glutathione beads was acetylated by p300 and AcCoA for 60 min. After extensive washing, the interaction assays were performed with His-H1.2, as in Figure 1d.

(f) Unmodified and acetylated versions of p53 C-terminal peptides (amino acids 364-393) were synthesized and immobilized on streptavidin-agarose beads. After incubating with His-H1.2, H1.2 binding to the peptides was determined by Western blotting using anti-His antibody.

(g) H1299 cells were transfected with p53RE-luc reporter together with expression vectors for wild type p53 (p53 WT), acetylation mimicking mutant p53 (p53 3KQ), acetylation blocking mutant p53 (p53 3KR) and/or the H1.2 core complex as indicated. The luciferase activity was measured 24 h after transfection, and the relative activities of three independent experiments are presented as means \pm S.E. Values from cells transfected with the luciferase reporter alone are set to 1.

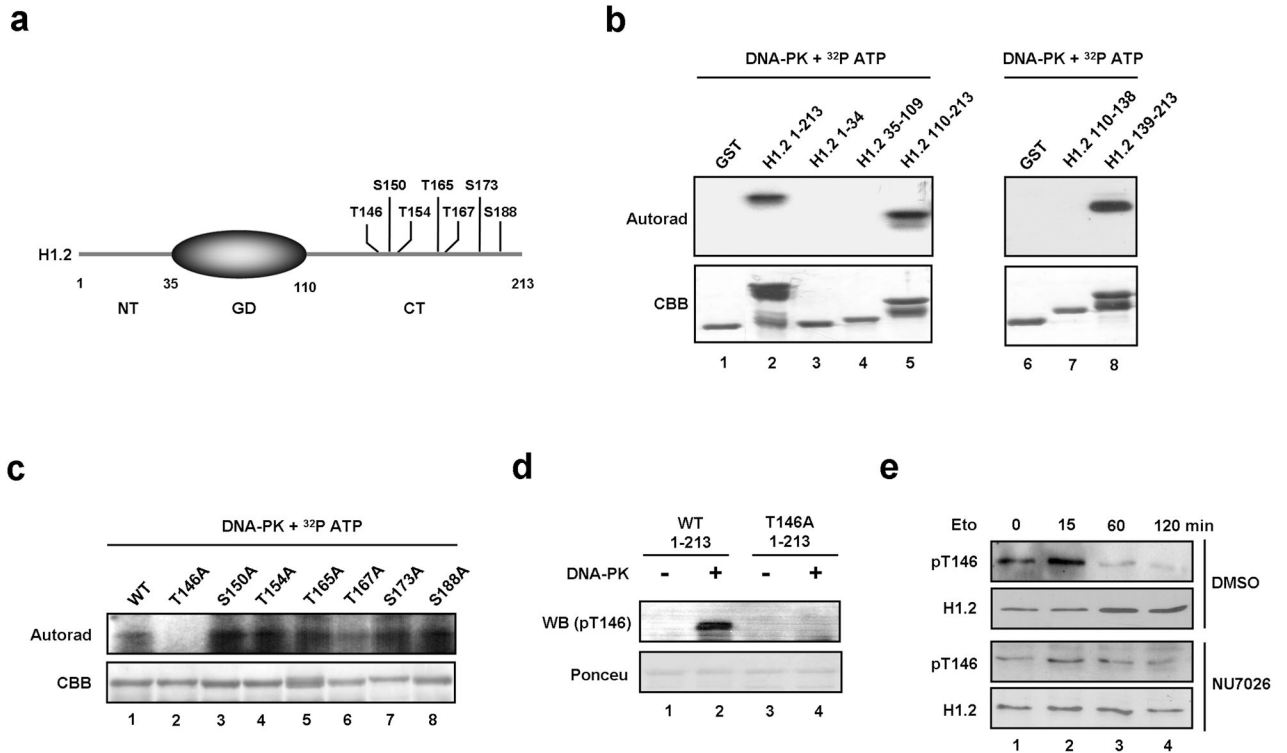


Figure 2. DNA-PK phosphorylates H1.2 at T146

(a) Schematic of the domain structure of human H1.2. Putative DNA-PK phosphorylation sites mutated in this study are indicated. NT: N-terminal tail; GD: globular domain; CT: C-terminal tail.

(b) In vitro kinase assays were performed with DNA-PK and [γ -³²P]ATP using GST-fused full length H1.2 or indicated H1.2 domains as a substrate. H1.2 phosphorylation was analyzed by 12% SDS-PAGE followed by autoradiography (upper panel). Coomassie staining of the H1.2 substrates is shown in the lower panel.

(c) Flag-tagged wild type H1.2 or mutant H1.2 lacking potential phosphorylation sites was prepared and used for in vitro kinase assays with DNA-PK and [γ -³²P]ATP. H1.2 phosphorylation was detected by autoradiography (upper panel). Coomassie staining of each substrate is also shown (lower panel).

(d) Flag-tagged wild-type and T146-mutated H1.2 proteins were subjected to in vitro kinase assays with DNA-PK and cold ATP. H1.2 phosphorylation was detected by Western blotting using anti-H1 phospho T146 antibody (upper panel). Ponceau S staining of blots shows approximately equal amounts of H1.2 proteins used in the modification assays (lower panel).

(e) U2OS cells were treated with etoposide (100 μ M) for 0, 15, 60 and 120 min in the presence or absence of the DNA-PK inhibitor NU7026 (5 μ M). Cell lysates were analyzed by Western blotting using anti-H1.2 and anti-H1 phospho T146 antibodies.

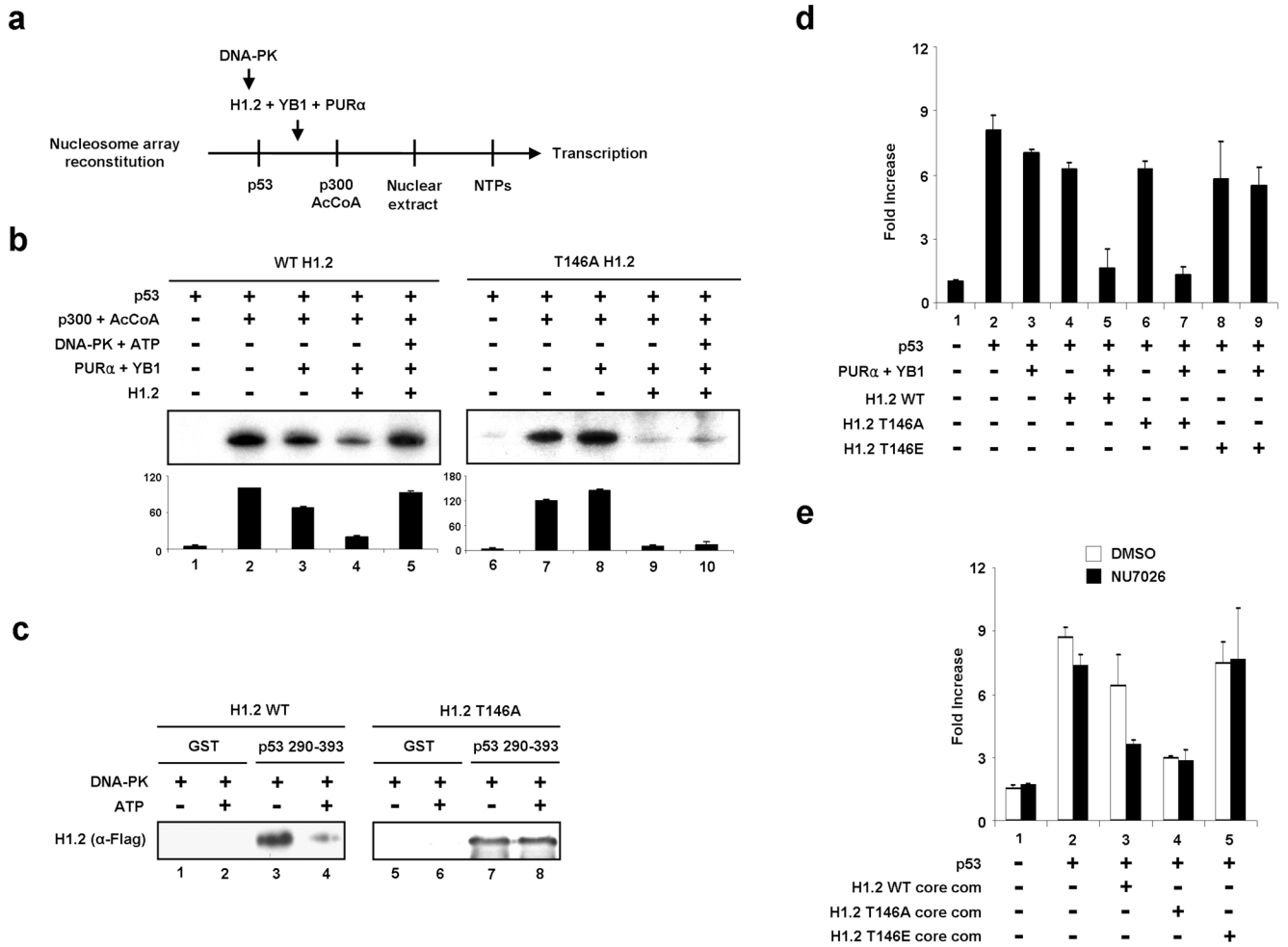


Figure 3. H1.2 phosphorylation reduces the repressive potential of H1.2

(a) Schematic diagram of H1.2 phosphorylation and transcription protocol.

(b) In vitro transcription assays were essentially as described in Figure 1c, but wild type (lanes 1–5) and T146-mutated (lanes 6–10) H1.2 proteins were premodified by DNA-PK. The H1.2 core complex was added to transcription reactions prior to p300 and AcCoA.

(c) Wild type (lanes 1–4) and T146-mutated (lanes 5–8) H1.2 proteins were unmodified (–ATP, lanes 3 and 7) or phosphorylated (+ATP, lanes 4 and 8) with DNA-PK, and incubated with GST alone (lanes 1, 2, 5 and 6) or GST-p53 290-393 (lanes 3, 4, 7 and 8) immobilized on glutathione beads. After extensive washing, H1.2 binding was assessed by Western blotting with anti-Flag antibody.

(d) H1299 cells were transfected with p53 reporter plasmid and the indicated expression vectors, and luciferase activity was measured 24 h post-transfection.

(e) Reporter gene assays were performed as in Figure 3d, but after treatment with etoposide (100 μ M) and NU7026 (5 μ M).

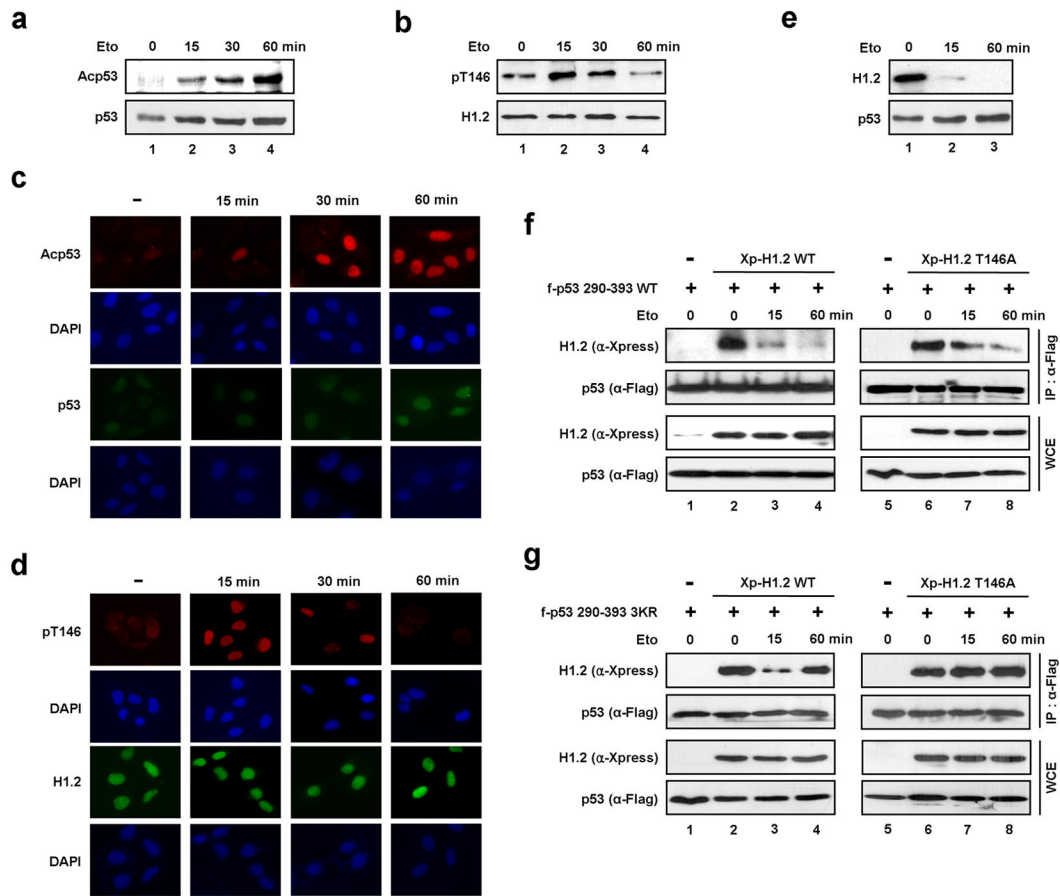


Figure 4. DNA damage induces p53 acetylation and H1.2 phosphorylation

(a) U2OS cells were treated with etoposide (100 μ M) for 0, 15, 30 and 60 min, and cell lysates were prepared. p53 and its acetylation levels were assessed by Western blotting using anti-p53 and anti-Acp53 antibodies. The blot presented is representative of three independent experiments.

(b) Cell lysates were prepared at the indicated time points after etoposide treatment, and subjected to Western blotting to analyze changes in H1.2 protein level and H1.2 phosphorylation.

(c) U2OS cells were treated with etoposide (100 μ M) for indicated times, and immunostained with antibodies against p53 and Acp53. Red and green signals represent Acp53 and total p53, respectively.

(d) U2OS cells were treated with etoposide as in Figure 4c, and immunostained with anti-H1.2 (green) and anti-H1 phospho T146 (red) antibodies.

(e) Cell extracts were prepared at the indicated time after etoposide treatment and immunoprecipitated with anti-p53 antibody. H1.2 precipitates were examined by 10% SDS-PAGE and Western blot analysis using anti-H1.2 antibody.

(f) The inhibitory effects of p53 acetylation and H1.2 phosphorylation on p53-H1.2 interaction were analyzed by transfecting expression vectors encoding Flag-tagged wild type p53 290-393 and either Xpress-tagged wild type or T146-mutated H1.2 into H1299 cells. Extracts were prepared in lysis buffer and immunoprecipitated with anti-Flag antibody.

Western blot analysis was performed with anti-Xpress antibody to detect Xpress-tagged H1.2. Similar recovery of p53 was confirmed by Western blotting using anti-Flag antibody (IP: α -Flag). Whole cell extracts were also analyzed by Western blot analysis to confirm that equivalent amounts of proteins were used for immunoprecipitation (WCE).

(g) H1299 cells were transfected with Flag-tagged p53 290-393 mutated at three acetylation sites (K373, K381 and K382) and Xpress-tagged wild type or T146-mutated H1.2. Extracts were immunoprecipitated with anti-Flag antibody and analyzed by Western blot analysis as in Figure 4f.

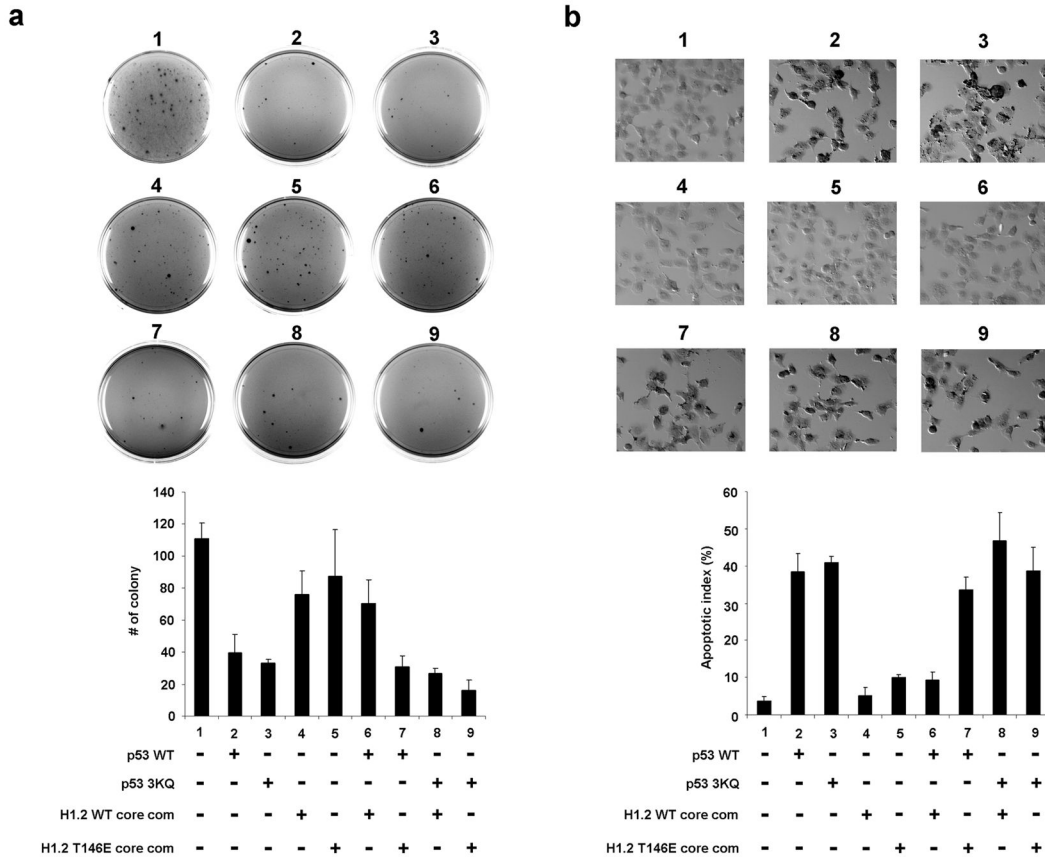
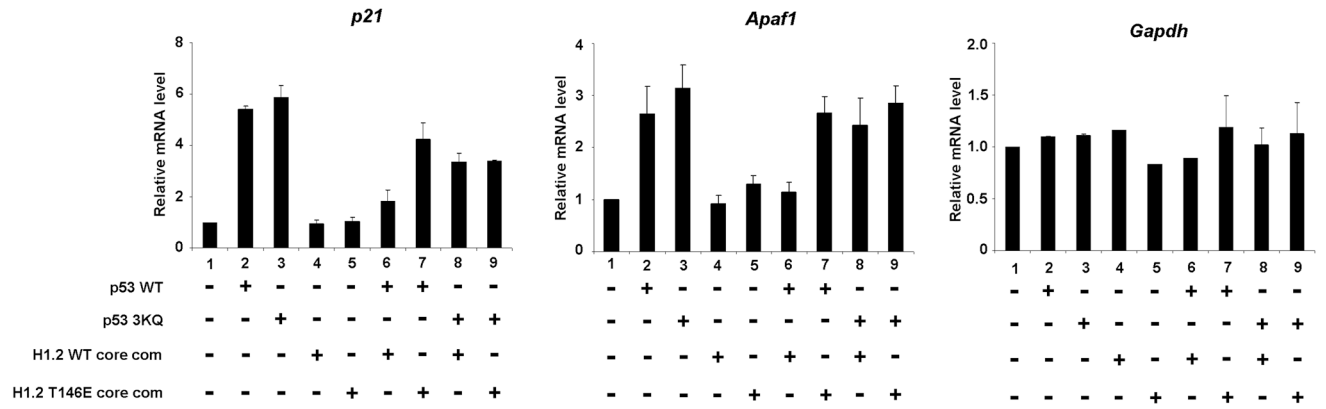


Figure 5. p53 and H1.2 mutants are defective in regulating cell growth

(a) H1299 cells were transfected with control vector or expression vectors encoding wild type or mutant forms of p53 and H1.2. Equal numbers of transfected cells (2×10^5 cells/ml) were plated into fresh media and allowed to form colonies for three weeks. The number of surviving colonies is reported as mean \pm S.D. from three independent experiments.

(b) Expression vectors encoding wild type or mutant forms of p53 and H1.2 were transfected into H1299 cells, and apoptotic cells were quantified by TUNEL staining 2 days post-transfection.

a



b

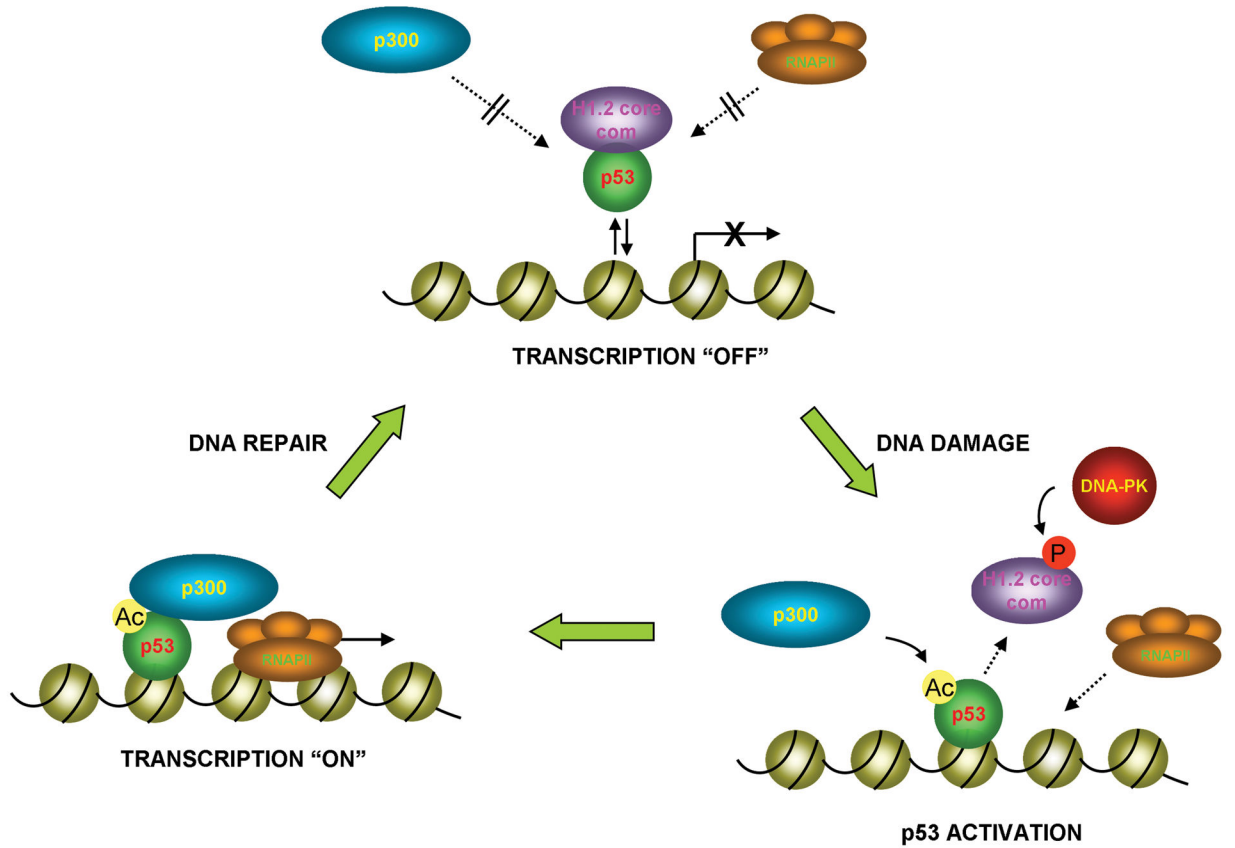


Figure 6. p53 acetylation and H1.2 phosphorylation are critical for p53 target gene transcription
(a) H1299 cells were transiently transfected with the indicated expression plasmids for 48 h. Total RNA was prepared from cells, and qRT-PCR was performed using primers specific for p21, Apaf1 and Gapdh. mRNA levels from each reaction were normalized against an

internal β -actin control. The results shown are means from three independent experiments, and values derived from mock-transfected cells are set to 1.

(b) A working model for the role of p53 acetylation and H1.2 phosphorylation in regulating p53 transactivation. In normal cells, the H1.2 core complex exists as an unphosphorylated form capable of binding p53 and maintaining p53 target genes in a quiescent state. When cells are exposed to stress environments, p53 and H1.2 are acetylated and phosphorylated, respectively. These modifications impair p53-H1.2 interaction and promote dissociation of H1.2 from p53, thereby allowing the recruitment of chromatin remodeling and transcription factors to p53 target promoters. Thus, p53 acetylation and H1.2 phosphorylation serve as signals to dictate p53-regulated transcriptional program in response to DNA damage (see discussion for more details).