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MINI-REVIEW

The impact of acetylation and deacetylation on the p53 pathway

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ABSTRACT

The p53 tumor suppressor is a sequence-specific transcription factor that undergoes an abundance of post-translational modifications for its regulation and activation. Acetylation of p53 is an important reversible enzymatic process that occurs in response to DNA damage and genotoxic stress and is indispensible for p53 transcriptional activity. p53 was the first non-histone protein shown to be acetylated by histone acetyl transferases, and a number of more recent *in vivo* models have underscored the importance of this type of modification for p53 activity. Here, we review the current knowledge and recent findings of p53 acetylation and deacetylation and discuss the implications of these processes for the p53 pathway.

KEYWORDS p53, Mdm2, acetylation, deacetylation, destabilization, ubiquitination, transcriptional activation and stability

INTRODUCTION

The tumor suppressor p53 is an essential and key transcription factor in cellular stress response pathways (Levine and Oren, 2009). The abundance of upstream regulators underscores the critical importance of keeping this protein under tight control. Under normal, non-stressed physiological conditions, p53 protein is maintained at low levels within a cell by its predominant negative regulator Mdm2. However, during times of genotoxic and other cellular stresses, a number of mechanisms allow for the quick accumulation and activation of p53 in order to prevent uncontrolled growth of cells harboring deleterious genetic alterations.

A number of post-translational modifications can occur in p53 that have critical effects on its stability and function, including phosphorylation, acetylation, sumoylation, neddylation, and methylation (Kruse and Gu, 2009a). While many if not all of these modifications have been described in *in vitro* and cell culture systems, more recent animal studies have been conducted to assess the true physiological function of a number of these modifications (Feng et al., 2005; Krummel et al., 2005). The picture that has emerged is a highly intricate and complex combination of events that ultimately lead to p53-dependent cell growth arrest or apoptosis, and key post-translational modifications are imperative for these events to occur.

Post-translational acetylation, or the reversible process by which acetyl groups are enzymatically placed onto the ε-amino group of lysine residues of target proteins, is a well studied event that occurs in histone tails during transcription (MacDonald and Howe, 2009). Histone acetylation is an enzymatic event that has been shown to occur both globally throughout the genome as well as at specific promoters and is an indicator of actively transcribed genes (Harbison et al., 2004; Wang et al., 2008b). The first non-histone substrate shown to be acetylated by histone acetyl transferases (HATs) was p53, which was a novel and previously unknown mechanism for protein activation (Gu and Roeder, 1997). Since this seminal characterization, other non-histone proteins have been shown to be acetylated that are involved in diverse cellular processes such as the cell cycle, gene splicing, and nuclear transport (Kim et al., 2006; Choudhary et al., 2009). Recent descriptions of the *in vivo* acetylome have not only identified lysine acetylation sites in upwards of 1700 proteins, but they have also shown that several regulators of the p53 pathway are also acetylated, including Daxx, PML, PTEN, and HAUSP (Choudhary et al., 2009). These findings suggest that acetylation plays a key role in the regulation and activity of virtually all nuclear processes and many

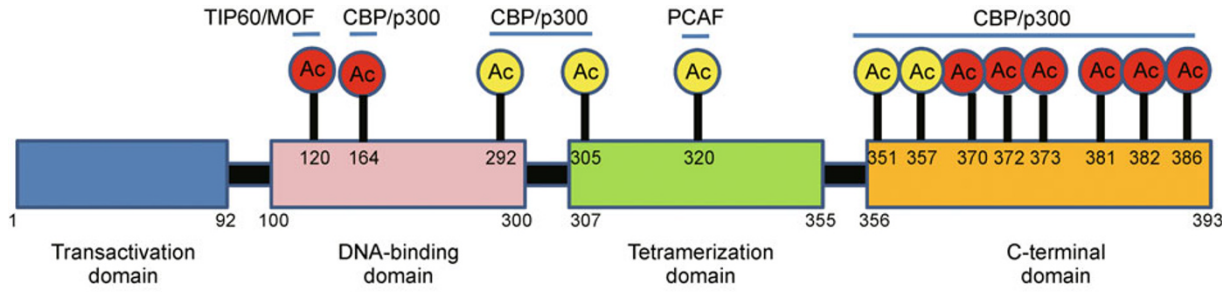

Figure 1. Schematic diagram of p53 functional domains and overview of p53 acetylation sites. The eight acetylation sites that are indispensible for p53 activation are indicated in red. The corresponding major modifying enzymes are also indicated.

cytoplasmic functions (Choudhary et al., 2009). In this review, we highlight the important effect that acetylation has on p53 function and regulation and how this type of modification may have significant implications for the signaling pathways that are induced in response to stress.

ACETYLATION OF P53

p53 was the first non-histone protein shown to be acetylated by HATs (Gu and Roeder, 1997). Enhancement of p53 acetylation levels strongly correlates with protein stabilization and activation in response to cellular stress (Luo et al., 2000; Rodriguez et al., 2000; Barlev et al., 2001; Ito et al., 2001; Knights et al., 2006; Li et al., 2007; Kim et al., 2008; Zhao et al., 2008). Moreover, acetylation of p53 was found to stimulate its sequence-specific DNA-binding (Gu and Roeder, 1997; Sakaguchi et al., 1998; Liu et al., 1999; Luo et al., 2004; Zhao et al., 2006). C-terminal lysine residues at positions 164, 305, 370, 372, 373, 381, and 382 have been shown to be acetylated by the HAT p300/CREB-binding protein (CBP) and lysine 320 by the HAT p300/CBP-associated factor (PCAF) (Fig. 1) (Gu and Roeder, 1997; Sakaguchi et al., 1998; Liu et al., 1999; Wang et al., 2003). Importantly, mutations in the p300/CBP gene are frequently found in a number of cancers (Goodman and Smolik, 2000; Iyer et al., 2004). Acetylation of these sites is also important as a docking site for the subsequent recruitment of HAT and other transcription co-factors to promoter regions, and p300/CBP can enhance histone acetylation and promote transcription in the vicinity of target genes (Goodman and Smolik, 2000).

More recently, two other acetyl transferases from the MYST family, hMOF and TIP60, were shown to acetylate p53 at position 120, which is located within the DNA binding domain (Sykes et al., 2006; Tang et al., 2006). These findings showed that p53 was acetylated at a unique lysine residue outside of the C-terminal domain. This lysine residue is also the only site of modification in p53 that is evolutionarily conserved and is a recurrent site of mutation in several human cancers, suggesting its important role in controlling p53 function. Interestingly, modification of this site has no effect on DNA binding or protein stability; however it was shown to be critical for the activation of the proapoptotic genes *PUMA* and *BAX* (Sykes et al., 2006; Tang et al., 2006). The HATs hMOF and TIP60 were also shown to be recruited to sites of transcription by p53 and were able to acetylate both p53 and histones. Importantly, K120 acetylation is induced after DNA damage and after activation of p19ARF by oncogenic stress (Tang et al., 2006; Mellert et al., 2007). Interestingly, mutation of this lysine residue to an arginine (K120R) abrogated p53-mediated apoptosis but not cell growth arrest (Tang et al., 2006). These data suggest that acetylation of lysine 120 is crucial for the differential activation of p53 target genes. The importance of this modification as well as other types of regulation for the mechanism by which p53 modulates cell cycle arrest and apoptosis are discussed below.

The dependence of the acetylation-dependent activity of p53 *in vivo* on these sites of modification seemed to be cell type specific, as the C-terminal acetylation-deficient p53-6KR knockin mouse, where the six C-terminal lysine residues subject to acetylation were substituted with arginine, had reduced p53-dependent gene expression after DNA damage in both thymocytes and embryonic stem cells (Feng et al., 2005). Significant differences were not seen in the embryonic fibroblasts from these mice. Moreover, a p53-7KR knockin mouse containing mutations at seven C-terminal acetylation sites analogous to the human p53-6KR mutant showed no defects in p53-mediated cell growth arrest and apoptosis (Krummel et al., 2005). The *in vivo* data suggested that these specific sites of acetylation may only have modest effects on overall growth arrest and apoptotic control, though some defects were observed in specific cell types. Moreover, a knockin mutation introduced at the PCAF acetylation site Lys320 also showed no defects in the expression of p53 target genes, and in fact showed an increase in the expression of proapoptotic genes *NOXA, PUMA, APAF1*, and *PIDD* in mouse embryonic fibroblasts (Chao et al., 2006). This study also demonstrated an increase in apoptosis of specific cell types from these mice after irradiation, including thymocytes, epithelial cells of the small intestine, and retinal cells (Chao et al., 2006). Though the mechanism behind these observations remains unclear, the lack of significant

defects in p53-dependent gene expression in these *in vivo* models suggests a more complex mode that may include other acetylation sites within the molecule.

Recently, a previously undiscovered acetylation site mediated by p300/CBP was described for p53 (Tang et al., 2008). The previously unfound lysine residue at position 164 was identified by a mass spectrometric analysis of all acetylation sites in p53. Interestingly, deletion of this site in combination with other known acetylation sites in p53 (p53-8KR) completely abrogated p53-dependent transactivation of p21 and the ability to induce cell growth arrest. Importantly, single acetylation defects at any of these sites individually had no effect on the transactivation ability of p53, suggesting that the acetylation sites described *in vivo* for the p53-6KR and p53-7KR knockin mice can be compensated for by acetylation at other sites. These findings not only confirm previous *in vivo* studies showing p53-dependent gene expression in p53 acetylation deficient mice, but also show that acetylation is indispensible for p53 activation. It will be interesting to see what *in vivo* effects the alterations of these sites have in animal models.

UBIQUITINATION AND ACETYLATION

The function and activity of p53 is highly regulated by acetylation, but an important step in the response to DNA damage is the rapid and efficient stabilization of p53 protein levels. The physiological levels of p53 are tightly regulated by the 26S proteosome pathway and ubiquitination, which is a mutually exclusive modification that occurs on the same lysine residues used by acetyltransferases. The predominant E3 ubiquitin ligase for p53 is the RING finger domain containing protein Mdm2, and loss of this gene causes p53-dependent embryonic lethality at day E6.5 (Jones et al., 1995; Montes de Oca Luna et al., 1995). Under normal physiological conditions, Mdm2-mediated ubiquitination targets p53 to the 26S proteosome for degradation, thereby maintaining low protein levels within the cell. However, upon a number of cellular stress conditions, p53 protein levels need to be quickly stabilized for activation, and this process occurs through several mechanisms, including post-translational modifications, physical sequestration, and degradation of the components of the p53 pathway (Brooks and Gu, 2006). For example, DNA damage-induced phosphorylation of p53 by the kinases ATM, ATR, Chk1, and Chk 2 at serine 15 and 20 leads to a disruption of the p53-Mdm2 interaction (Shieh et al., 1997; Appella and Anderson, 2001; Kruse and Gu, 2009a). Although subsequent *in vivo* knockin models of these modification sites showed no defects in stress-induced p53 stabilization, the combinatorial mutation of these sites showed a more pronounced defect in p53 stabilization (Chao et al., 2006).

Mdm2 is also a direct target of several upstream regulators for efficient stabilization of p53. The tumor suppressor ARF has been shown to indirectly stabilize p53 by interacting with Mdm2 in response to oncogenic stress (Sherr, 2001; Sharpless and DePinho, 2004). ARF can induce p53-dependent cell growth arrest or apoptosis by binding to and sequestering Mdm2 in the nucleolus, thereby stabilizing nucleoplasmic p53. ARF also directly inhibits the enzymatic activity of Mdm2 and may have effects on another p53 E3 ligase, ARF-BP1 (Chen et al., 2005; Zhong et al., 2005). Acetylation of Mdm2 within the RING domain by p300/CBP has also been shown to disrupt the p53-Mdm2 interaction and promote p53 stabilization (Wang et al., 2004). Conversely, Mdm2 can inhibit both p300/CBP and PCAF-mediated acetylation of p53 by suppressing the acetyltransferase activity and by recruiting the histone deacetylase HDAC1 to p53 (Benkirane et al., 2010). Mdm2 has also been shown to ubiquitinate Tip60 and PCAF, both of which acetylate p53. Together, these data illustrate that the abundance of mechanisms available underlying the disruption of the p53-Mdm2 interaction augments the negative regulation impact Mdm2 has on p53. Moreover, both acetylation and ubiquitination occur in the same lysine residues, showing a complex network of regulators for p53 activity, stability, and activation. Indeed, acetylation of p53 was shown to inhibit Mdm2-mediated degradation *in vitro* and in cell culture (Li et al., 2002).

The p53-dependent embryonic lethality of the Mdm2 knockout mouse reveals the importance of this protein in the p53 pathway; however more recently it has been shown that p53 is still efficiently degraded in the absence of Mdm2 in cells from Mdm2 null mice (Ringshausen et al., 2006). These results suggest that alternative mechanisms for p53 degradation exist within cells that are independent of Mdm2. Indeed, several other ubiquitin ligases have been described for p53 *in vitro* and in cell culture, including ARF-BP1/Mule, COP1, Pirh2, MSL2, p300/CBP, and E4F1 (Grossman et al., 2003; Leng et al., 2003; Dornan et al., 2004; Chen et al., 2005; Kruse and Gu, 2009b). Ubiquitination of p53 by these proteins has been shown to either cause the degradation of p53 or lead to a change in subcellular localization. Interestingly, p300/CBP possesses both acetyltransferase and ubiquitinase activity for p53. Although this protein does not possess an obvious ubiqutin ligase domain (RING or HECT), it was shown to act as an E4 ubiquitin ligase for p53 and polyubiqutinate p53 (Shi et al., 2009). In this regard, p300/CBP works in conjunction with Mdm2 to efficiently polyubiquitinate p53, which is a key signaling mechanism for recognition by the 26S proteosome. Although the p300/CBP-Mdm2 interaction has been shown to be important for Mdm2 activity on p53 *in vitro*, further *in vivo* studies will be able to dissect these interactions and their combination effects on p53.

In addition to acetyltransferase activity, PCAF was also recently shown to have intrinsic ubiquitination activity on lysine 320 of p53 (Benkirane et al., 2010). Moreover, PCAF

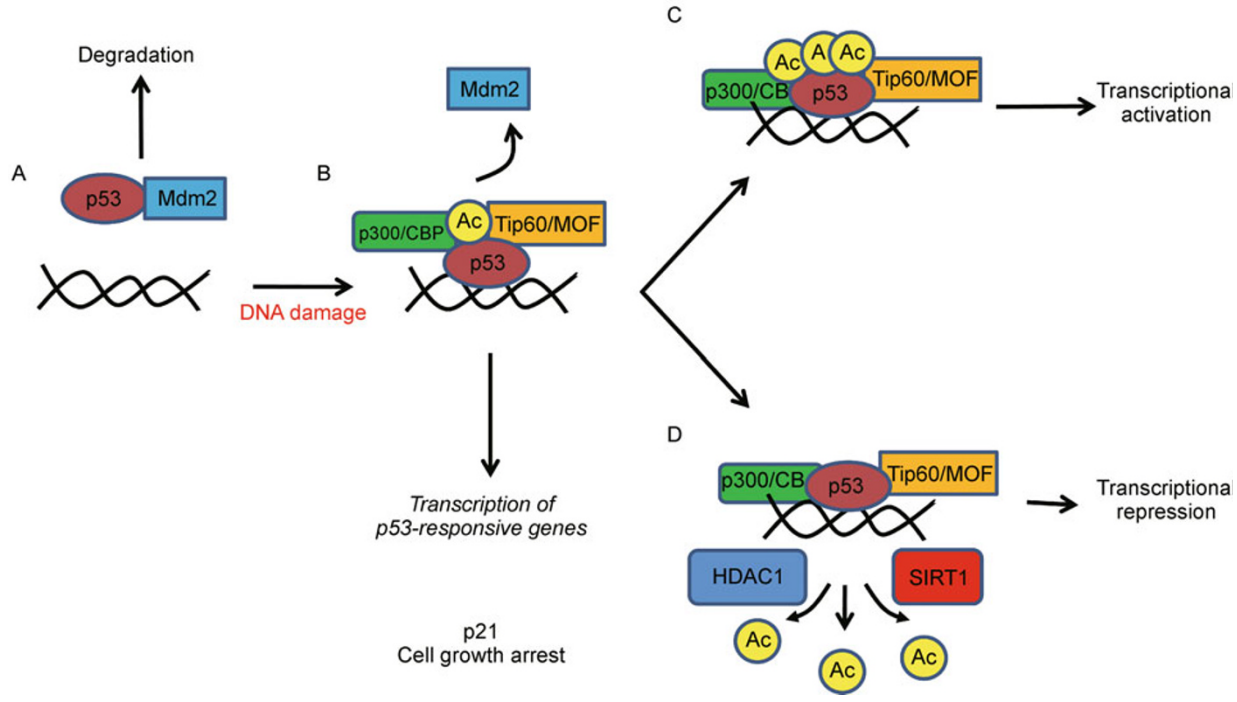

Figure 2. The role of p53 acetylation in gene regulation. (A) Unacetylated p53 is capable of activating genes that are involved in the negative regulation of p53, such as Mdm2, as a mechanism to keep p53 protein levels low during times of normal homeostasis. (B) Upon DNA damage, acetylation of p53 allows for the disruption of the Mdm2-p53 interaction and the recruitment of specific HATs to the promoters of genes involved in DNA repair and cell cycle control. (C) Full acetylation of p53 at all key acetylation sites promotes the activation of proapoptotic genes. (D) SIRT1 and HDAC1 are deacetylases that can reverse p53 acetylation and lead to transcriptional repression.

can directly ubiquitinate Mdm2, leading to PCAF-mediated degradation of the protein. Mdm2 also has auto-ubiquitinase activity and can self-ubiquitinate, and PCAF-mediated degradation of Mdm2 suggests an interesting alternative mechanism for Mdm2 degradation. Importantly, an Mdm2 RING-deficient knockin mouse model showed comparable Mdm2 protein levels with the wild-type, suggesting that alternative mechanisms exist to control Mdm2 protein levels (Itahana et al., 2007). Moreover, the newly found enzymatic activity of PCAF shows that this protein can promote p53 stability and activation by both degrading Mdm2 and activating p53.

DEACETYLATION OF P53

The critical effects of acetylation on p53 activity show that this type of modification has to be tightly regulated and reversible. Indeed, mammalian histone deacetylases (HDACs) down-regulate p53 transcriptional activity, and p53 acetylation can be antagonized by specific HDAC protein complexes (Kruse and Gu, 2009a). HDACs are categorized based on their similarities to yeast homologues and are grouped into class I, II, and III (de Ruijter et al., 2003). p53 acetylation can be reversed by a protein complex containing HDAC1 (Luo et al., 2000). Interaction with this complex reduces the steady-state levels of acetylated p53 and inhibits p53-dependent transcriptional activation, cell growth arrest, and apoptosis (Fig. 2). In addition, the class III NAD +-dependent deacetylase SIRT1 was shown to deacetylate p53 (Luo et al., 2001; Vaziri et al., 2001). SIRT1-mediated deacetylation of p53 inactivates the sequence-specific transcriptional activity of the protein and represses p53-mediated cell growth arrest and apoptosis in response to DNA damage and oxidative stress. This process also prevented p53-dependent transactivation of p21 (Luo et al., 2001).

The role of SIRT1 and deacetylation in the p53 pathway is complex. SIRT1 has roles in diverse signaling pathways from metabolism to cellular senescence and aging (Bordone and Guarente, 2005). Although SIRT1 has a direct negative effect on p53 transcriptional activity, embryonic fibroblasts from a Sir2a knockout mouse model have exhibited impaired p53 functions (Wang et al., 2008a). Despite the presence of hyperacetylated p53 in these mice, these cells had an increased capacity to proliferate in response to chronic, sub-lethal doses of oxidative stress and failed to mount an adequate DNA damage response (Cheng et al., 2003; Chua et al., 2005). Moreover, SIRT1 has been shown to relocalize to sites of DNA double stranded breaks to help promote DNA repair and can deacetylate the DNA repair protein NBS1

(Yuan et al., 2007), WRN (Li et al., 2008), and XPA (Fan and Luo, 2010). Embryonic fibroblasts from Sirt1-null mice show defects in DNA repair, despite a high steady-state level of acetylated p53. These findings, together with the seemingly opposing results of p53 regulation, suggest that SIRT1 and deacetylation may have an important role in p53-mediated cellular senescence. It is well established that SIRT1 has critical functions in longevity and aging in other organisms such as yeast (Bordone and Guarente, 2005). SIRT1-mediated deacetylation of p53 in response to low levels of DNA damage may therefore promote cellular senescence and help cellular recovery from non-lethal genotoxic events (Wang et al., 2008a). This hypothesis may be complicated by other as yet undefined regulators in this pathway or other mechanisms for balancing the ratio or levels of acetylated p53, since SIRT1 does not fit the classical model of a true tumor suppressor and has not been found mutated in human cancers (Luo et al., 2001; Huang et al., 2008). Nevertheless, p53 has a clear functional role in cellular senescence and premature aging (Tyner et al., 2002), and SIRT1-mediated deacetylation may promote this phenotype and provide a regulatory link to cellular longevity.

CONCLUSIONS

Our understanding of the intricate regulatory control of p53 function continues to evolve. It has become clear from mouse knockin studies that p53 requires a multitude of post-translational modifications as well as additional adaptations to become fully activated. Moreover, the precise combination of modifications may act as a signal to trigger specific pathways. Acetylation plays a critical role in the functional activity of p53 and is indispensible for transcriptional activity. Since several p53 coactivators, such as ASPPs and 53BP1, are known to bind to this region, the identification of acetylation sites outside the C-terminal domain raises the possibility that modification of these lysine residues may promote the activation of different p53 target genes (Joo et al., 2002; Li et al., 2007; Sullivan and Lu, 2007). Further *in vivo* investigation of these acetylation sites and the impact they have on p53 function is critical for a full understanding of these regulatory processes.

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