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Regulation of p53 function by lysine methylation

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Abstract

The reversible and dynamic methylation of proteins on lysine residues can greatly increase the signaling potential of the modified factor. In addition to histones, several other nuclear factors such as the tumor suppressor and transcription factor p53 undergo lysine methylation, suggesting that this modification may be a common mechanism for modulating protein–protein interactions and key cellular signaling pathways. This article focuses on how lysine methylation events on the C-terminal tail of p53 are generated, sensed and transduced to modulate p53 functions.

Keywords

lysine methylation; p53; PKMT; protein lysine methyltransferase

The human p53 tumor suppressor is mutated in approximately 50% of human cancers. Not surprisingly, the so-called ‘guardian of the genome’ is one of the most widely studied genes in all of molecular biology [1]. Besides its central role in cancer, p53 is also involved in numerous physiologic and pathologic processes ranging from aging, differentiation and fertility, to neurodegenerative disorders, diabetes and myocardial infarction [2–15]. It is an impressive responsibility for one protein to have, a point superbly captured in a humorous anecdote about what p53 might say to a psychologist:

“Every little thing I do is watched, every decision I make is monitored...I have regulations, modifications, physiological partners that rely on me!”

– Tsvetkov and Dekel [101].

The diverse pathways that are regulated by p53 raise important questions: how is one protein with so many essential functions directed to the right places at the right time? What regulatory mechanism(s) manage the staggering complexity of this tumor suppressor protein?

In the absence of genotoxic stress, p53 is tightly regulated at the protein level by ubiquitin ligases that target it for proteolysis [16]. Various cellular stresses, including DNA damage, trigger an increase in p53 protein levels and lead to p53-dependent programs that regulate DNA repair, cell cycle arrest and apoptosis [17,18]. Another key component of p53 regulation is post-translation modification (PTM). p53 is decorated with numerous PTMs, including phosphorylation, acetylation, methylation and ubiquitination. Similar to histones, these PTMs on p53 play a critical role in regulating protein dynamics. In this article we focus on lysine methylation.

A number of studies have characterized methylation events mediated by different protein lysine methyl transferases (PKMTs) on four distinct lysines in the C-terminal regulatory region of p53 (Figure 1). Fine-tuned regulation through PTM of p53 may explain how p53 can participate in multiple cellular functions. Through lysine methylation, PKMTs dynamically generate distinct populations of p53, each methylated at a specific C-terminal lysine residue. These modified subsets of p53 can then be connected to different downstream applications by effector proteins that only recognize a specific p53 species. Thus, p53 can be linked to different tasks in response to diverse stimuli. This article discusses the PKMTs that modify p53, the effector proteins identified to date that interact with methylated p53 species, and the mechanisms by which lysine methylation events on p53 have been shown to direct downstream p53 activity.

Lysine methylation signaling: regulation of histones & p53

Lysine methylation has been best characterized on histone proteins. Lysine residues can accept up to three methyl groups forming mono-, di-, and tri-methylated derivatives (referred to here as me1, me2 and me3, respectively), with a unique activity frequently being coupled to the specific extent of methylation on the lysine residue. To date, the majority of enzymes that catalyze this covalent reaction (named PKMTs) contain a conserved catalytic motif called the SET domain. Lysine methyl marks are reversed by protein lysine demethylases (PKDMs). Switching between methylation states represents a dynamic process that can regulate fundamental nuclear processes, such as gene expression. On histones, the majority of the canonical methylation events occur on the unstructured N-terminal tails of histone H3 and histone H4 [19,20]. Similar to these histone N-terminal tails, the p53 C-terminal tail is an unstructured region containing a number of lysines available for modification (Figure 1)[21,22]. Lysine methylation is not believed to change the conformation nor overall charge of substrate proteins; rather it is believed to modulate the surface architecture of a substrate leaving a mark that can either promote or inhibit modular binding by effector protein(s) [19,20,23,24].

In chromatin biology, lysine methylation is appreciated for the complex combinatorial potential it imparts upon histone substrates. Different methyl states at a single residue can lead to divergent functional outcomes. For instance, studies have shown that H4K20me1 is frequently associated with condensed chromatin, for example, interaction with chromatin compaction factor L3MBTL1 results in gene repression [25-27]. Dimethylated H4K20 binds the tandem Tudor domains of DNA damage response mediator 53BP1 and is linked to DNA damage signaling [28,29]. Finally, H4K20me3 is involved in the maintenance and structure of constitutive heterochromatin [30-32].

A single methyl state can also diversify functional outputs through interaction with multiple chromatin 'readers' – regulatory factors that specifically recognize distinct histone modifications. For example, H3K4me3, most frequently found at the promoters of actively transcribed genes, binds dozens of readers leading to outcomes as diverse as gene activation, gene repression, and V(D)J recombination [33]. As described below, the enzymes and readers that generate, sense and transduce methyl marks on histone proteins are also now believed to regulate nonhistone targets such as the transcription factor p53. Certainly, in some cases it may be reasonable to expect that methylation activity on both a histone and nonhistone target may lead to integrated outcomes; for example, chromatin condensation and gene silencing, or chromatin relaxation and gene activation.

SET7/9 monomethylation at lysine 372 stabilizes p53

SET7/9 is an enzyme that was first identified for its ability to monomethylate H3K4, a mark associated with euchromatin [34,35]. SET7/9 has also been shown to methylate numerous

nonhistone proteins [36-39]. In general, SET7/9 appears to be associated with promoting the activity and stability of its substrates. Indeed, these themes are echoed in SET7/9 activity on p53, as described below.

A major advance in the field of lysine methylation signaling was the identification of p53 as a substrate of SET7/9 (referred to here as SET9) [40]. In this paper, the authors demonstrated that upon DNA damage, SET9 mono-methylation of p53 at lysine 372 (p53K372me1; see Figure 1) in the nucleus results in stabilization of chromatin bound p53. They also observed that induction of p53K372me1 correlated with increased p53 promoter occupancy and transcriptional activation of target genes. SET9-associated regulation of p53 has been linked to the downstream promotion of acetylation at nearby C-terminal lysine residues of p53 [41]. A dynamic sequential relationship exists between p53K372 methylation and p53 acetylation, with DNA damage increasing SET9-mediated p53K372me1 levels and this in turn promoting acetylation. One possible p53 acetyltransferase is p300, which prefers lysine-methylated, DNA-bound, tetrameric p53 versus unmodified, unbound p53 as a substrate. In addition to modifying p53, local enrichment of p300 at p53-target genes also leads to p300-mediated acetylation of histone H4 [41]. Thus, SET9 coordinates gene activation through concerted action on chromatin-bound p53 and indirectly via increasing the levels of acetylated H4. Interestingly, DNA damage was found to increase methylation of p53, but not H3K4, illustrating how environmental conditions can specify SET9 activity [41]. One important question for future research will be to understand at a molecular level how DNA damage specifically potentiates SET9 activity on p53, but not on H3K4 or other SET9 targets.

The generation of a SET9-null mouse has provided further evidence that SET9 positively regulates p53. Mouse embryonic fibroblasts derived from the SET9-knockout mice have diminished induction of p53 downstream targets (*p21* and *PUMA*) following DNA damage, and these cells are more susceptible to transformation upon addition of oncogenes (*H-rasV12* or *E1a*) compared with wild-type cells [42].

At the molecular level, how does monomethylation of p53 at K372 activate p53? The increase in acetylation could compete with ubiquitination at the same residues in the C-terminus and thereby explain how p53K372me1 promotes p53 stabilization. However, a recent report suggested that a p53K372R mutant is nonetheless still activated by SET9, suggesting alternative pathways connecting this enzyme to p53 activation that are independent of K372 [43]. In this study, the authors propose that SET9 inhibits p53 deacetylation by SIRT1. It is also possible that in the absence of its preferred substrate, SET9, a relatively promiscuous enzyme, methylates an adjacent lysine. In addition, as lysine methylation most commonly affects downstream functions via methyllysine-binding partners, it is likely that an as-yet unidentified p53K372me1-recognizing effector protein exists and can provide a molecular explanation for SET9 regulation of p53. In this context, another methylation event mediated by the PKMT Smyd2 at the adjacent p53 C-terminal lysine 370 suggests that crosstalk between modifications may also contribute to SET9-mediated stabilization of p53 through p53K372me1 inhibition of a repressive methylation event at lysine 370.

Smyd2 monomethylation at lysine 370 represses p53 activity

In contrast to the activating effect of SET9 methylation at K372, the next methylation event documented on p53, Smyd2-mediated monomethylation at lysine 370 (p53K370me1), was shown to have a repressive effect on p53 transactivation activity [44]. Smyd2 methylation of p53 reduced p53 occupancy at two target gene promoters: *p21* and *mdm2*, and depletion of Smyd2 ramped up p53-mediated apoptosis in response to various types of DNA damage.

The proximity of lysines 370 and 372 indicated a potential for crosstalk taking place between the two neighboring methyl marks. Indeed, both *in vitro* and *in vivo* data suggest that SET9-mediated methylation of K372 inhibits Smyd2-mediated methylation of K370. Cotransfection of SET9 together with flag-p53 and Smyd2 in H1299 cells decreases the p53 and Smyd2 interaction observed by flag immunoprecipitation, suggesting that SET9 physically blocks Smyd2 binding to p53 [44]. Thus, at least part of the positive effect of SET9 methylation on K372 may be explained through its inhibitory role in preventing the Smyd2 and p53 interaction.

Lysine demethylase LSD1 regulates p53

p53K370 is also dimethylated at K370 by an as-yet unidentified lysine methyltransferase. In contrast to p53K370me1, Huang *et al.* demonstrated that dimethylation at this same residue (p53K370me2) positively regulates p53 transcriptional activity: the p53K370me2 species was found to be recognized by the DNA damage response protein 53BP1 via its Tudor domain and to act as a coactivator of p53 target genes [28]. As discussed later, the Tudor domain of 53BP1 binds with little sequence specificity to dimethylated lysines, including p53 dimethylated at lysine 382 (p53K382me2) and H4K20me2 [29,45]. In the case of p53K382me2 and H4K20me2, 53BP1 binding is associated directly with DNA damage signaling and not transcription. Thus, it will be interesting to understand the molecular circumstances that dictate different 53BP1 recognition events and how they link to different functional outcomes.

Huang *et al.* also demonstrated that the lysine demethylase LSD1 removes one methyl moiety from p53K370me2 to generate p53K370me1 [28]. This finding represents the first identification of a demethylation event occurring on a nonhistone protein. Cells depleted of LSD1 and subject to DNA damage were found to have increased abundance of p53K370me2, suggesting that LSD1 primarily removes the dimethyl mark *in vivo* [28]. Consistent with its role in maintaining a repressive chromatin environment through histone demethylation activity [46,47], LSD1 represses p53 function by limiting the accumulation of p53K370me2 through dynamic demethylation, thus converting the active dimethyl species to the inactive monomethyl species. At the molecular level, Huang *et al.* proposed that p53K370me1 is no longer recognized by 53BP1 and the loss of this 53BP1 coactivator function leads to reduced capacity of p53 to associate with target gene promoters. Under conditions requiring gene activation, a yet to be identified methyltransferase increases the population of p53K370me2, thereby supporting a stabilizing interaction with 53BP1 and leading to transcriptional activation of target genes [28].

SET8 monomethylation at lysine 382 represses p53 activity

In 2007, a third physiologically relevant methylation event on p53 was described: monomethylation of lysine 382 by SET8 [48]. SET8 (PR-SET7/KMT5a) is the PKMT for H4K20me1, a mark that is, with few exceptions [49,50], generally understood to promote gene silencing and condensed chromatin states in mammals [25-27,51]. SET8 plays a role in diverse cellular processes such as S-phase progression [52,53], mitosis [54,55] and DNA-damage checkpoint signaling [56,57]. Interestingly the themes of transcriptional repression and DNA-damage checkpoint signaling are shared in SET8 regulation of p53.

The SET8-mediated monomethylation of p53 at lysine 382 represents the second example of a repressive mark that affects p53-mediated transcriptional regulation. SET8 methylation on p53 was predicted, in part, by consensus in the amino acid sequence at the substrate site of SET8 in H4 (K20) and the residues surrounding lysine 382 in the p53 C-terminus. Decreasing SET8 levels increases p53 occupancy and transcriptional activity at p53 target genes *p21* and *PUMA* [48]. Since SET8 is present under normal conditions but repressed

upon DNA damage it thus serves as a modulator of p53 activity, generating an inert population, monomethylated at K382, that suppresses p53 transactivation activity at highly responsive targets in the absence of DNA damage. Therefore, SET8 promotes survival of healthy cells through methylation-mediated repression of p53. Importantly, these phenotypes were shown to be a consequence of SET8 methylation of p53, and not an indirect effect of H4K20 methylation by SET8 since H4K20me1 levels were not changed in the experimental system upon manipulation of SET8 levels, and expression effects on specific target genes were abolished upon p53 knockdown [48].

SET8 monomethylation at lysine 382 represents another example of how lysine methylation prepares a substrate for interaction with an effector protein. While it was observed that SET8 negatively regulates acetylation at the K382 residue – SET8 overexpression in U2OS cells reduced the amount of acetylation detected by p53K382Ac antibody upon neocarzinostatin treatment – this was not thought to be the chief mechanism by which methylation represses p53 transactivational activity, since a p53K382R mutant activates target genes with an efficacy nearly comparable to the wild-type protein [48]. Interestingly, sequence similarity between the H4 tail at K20 and p53 at K382 was predictive not only of the PKMT found to methylate these residues, but also of two interacting effector proteins subsequently identified: L3MBTL1 and 53BP1.

The malignant brain tumor protein L3MBTL1 was recently identified as an interaction partner for p53K382me1 [58]. L3MBTL1 binds H4K20me1 (and other monomethylated histones) via its middle MBT repeat and compacts chromatin in a histone lysine methylation-dependent manner [25,59-61]. Recent work shows novel methylation-dependent interactions between L3MBTL1 and nonhistone targets p53 and RB by virtue of the same MBT2-mediated binding modality observed in interactions with methylated histones [58,62]. In fact, p53 joins a growing list of nonhistone interaction partners for L3MBTL1 including TEL and RB, each of which utilizes L3MBTL1 for its transcriptional repressing capability at respective target promoters [25,63].

The p53–L3MBTL1 interaction is driven *in vivo* by SET8-mediated methylation of p53 at lysine 382 [58]. Under normal conditions, p53K382me1 is thought to stabilize L3MBTL1 occupancy at the *p21* promoter. In some cases, L3MBTL1-mediated repression of target genes appears not to need transcription factor guidance to chromatin. For example, SET8-mediated monomethylation of H4K20me1 in the cyclin E1 and *RUNX1* promoters is sufficient for L3MBTL1-mediated transcriptional repression of these genes [26,27]. However, in the case of the highly responsive targets *p21* and *PUMA*, L3MBTL1 occupies the target gene promoters in p53-positive HCT116 cells, but is absent in the p53-null HCT116 line. As mentioned above, DNA damage induced by neocarzinostatin treatment leads to repression of SET8 levels and concomitant reduction of p53K382me1. This results in dissociation of p53 from L3MBTL1 and reduced L3MBTL1 occupancy at the *p21* promoter [58]. p53K382me1 interaction with L3MBTL1 thus enables quiescent p53 occupation at promoters of highly responsive target genes where it sits poised for immediate activation upon DNA damage.

53BP1 stabilizes p53K382me2 upon DNA damage

Kashirskaja *et al.* found that the tandem Tudor domains of 53BP1 recognize and bind to a second dimethylated p53 residue, p53K382me2 [29]. Incidentally, 53BP1 is the second effector protein (along with L3MBTL1) shared between K382 of p53 and K20 of histone H4. The tandem Tudor domains of 53BP1 bind to histone target H4K20me2, allowing it to accumulate at double-strand breaks [45]. p53K382me2, a species that increases with DNA damage, is similarly recognized by the tandem Tudor domains of 53BP1. This interaction

stabilizes p53 by positively regulating the accumulation of p53 protein, which accompanies double-strand break lesion formation [29]. The enzyme responsible for p53K382 dimethylation is not yet known. In light of the small collection of interacting proteins shared between these substrates, one could speculate that the enzymes responsible for H4K20me₂, Suv4-20h1/h2 [64], might also include p53 as a substrate. However, a screen of more than 30 lysine methyltransferases for dimethylation activity on p53K382 did not identify Suv4-20/h1/h2 or any other PKMT candidates as writers of this modification [29]. The identities of PKMTs that mediate K370me₂ (discussed above) and dimethylation of the novel species of unidentified function, K386me₂, remain open questions for future research.

G9a & Glp dimethylation of lysine 373 inactivates p53

The related lysine methyltransferases G9a and Glp form a stoichiometric heteromeric complex that mediates H3K9 dimethylation *in vivo* with each capable of independent H3K9 methylation *in vitro* [65,66]. G9a/Glp-mediated mono- and di-methylation of H3K9 mediate transcriptional repression in part through recruitment of HP1 protein to chromatin. The most recent work on lysine methylation of p53 has identified G9a and Glp as mediators of dimethylation of p53 lysine 373 [67]. In contrast to the activating effect of dimethylation at lysines 370 and 382, mediated through interaction with 53BP1 as described above, p53K373me₂ represents an inactivating dimethyl mark on p53. This was concluded based on the observation that, first, p53K373me₂ levels do not increase in response to at least one type of DNA damage treatment (adriamycin), and second, transient knockdown of either G9a or Glp by siRNA resulted in p53 activation as measured by an increase in the proportion of cells undergoing apoptosis [67]. The molecular mechanism of repression for K373me₂ has not yet been characterized.

Conclusion

The above examples illustrate the diverse molecular mechanisms through which non-histone protein lysine methylation can regulate the multifunctional tumor suppressor p53. It is increasingly apparent that the mechanisms first characterized on histones, such as effects on stability, crosstalk between modifications or interaction with methyl readers, also take place on p53 and other transcription factors [38,39,62,68-73]. Furthermore, a PKMT may often coordinate concurrent action on histone and nonhistone substrates leading to an integrated downstream outcome such as chromatin condensation and gene silencing, or chromatin relaxation and gene activation. SET8 represents one such example of synchronized methylation activity: under normal conditions, SET8 is expressed during S-phase and tracks with the replication fork placing H4K20 monomethylation marks essential to genome replication and stability [56,57]. SET8 contemporaneously monomethylates p53 to increase concentrations of the inactive p53K382me₁ species, thus maintaining p53 quiescent (although poised) at a time when its tumor-suppressing activities would derail healthy cell proliferation [48]. In this way, p53 regulation by lysine methylation represents a logical extension of the PTM signaling already characterized on histones, in a fashion that frequently supports coordinated response to various intracellular circumstances.

Ultimately, no lysine methylation event is essential to the overall tumor-suppressing activity of p53. This point was demonstrated in mouse genetic studies showing that p53^{K6R} and p53^{K7R} mutants (all C-terminal domain lysines mutated to arginine) are functionally equivalent to wild-type p53 with respect to stability, transactivation and induction of apoptosis [74,75]. It is important to note, however, that it is difficult to interpret the specific role of a methylation event with such mutational studies since K to R mutants abolish not only methylation but also acetylation, ubiquitination and any other modification that may occur on the lysine in question. Knockout of individual PKMTs could potentially provide

insight into the importance of p53 methylation, but this approach would be complicated by the fact that the PKMTs are likely to have multiple substrates.

Thus, lysine methylation delineates the finer points of p53 sensitization in response to diverse stimuli by generating specific populations that intercommunicate and transition through crosstalk to either augment or inhibit p53 activity via interaction with diverse methyl readers. These events contribute to the overall sensitivity and robustness of a p53 'metastable' equilibrium that promotes homeostasis under normal conditions but achieves high-level specificity in a gene-specific, promoter-specific fashion upon genotoxic stress [76].

Apart from p53, numerous nonhistone proteins are methylated on lysine residues. For the most part the mechanisms and biological implications of these events are not wholly understood [19,20,23]. However, several mechanistic themes identified in PTM regulation of p53 have recently been extended to other transcription factors such as RB and RelA [62,68-70]. These studies suggest that regulation by lysine methylation represents a broader paradigm for fine-tuned modulation of nonhistone protein activity.

Future perspective

Lysine methylation of transcription factors such as p53 is virtually certain to be a common mechanism for coordinating diverse protein–protein interactions and signaling pathways. In the next few years, both candidate and unbiased approaches will result in a dramatic increase in the number of new lysine methylation events discovered on both nuclear and cytoplasmic proteins. The development of next-generation proteomic technology and reagents will allow for the discovery of new reader domains of these methylated proteins. Together, the elucidation of new methylation signaling networks will uncover unexpected molecular modes of action for protein modifications at chromatin and other cellular regions to regulate key physiologic and pathologic processes.

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■ Website

101. Gene therapy: From the LabLit short story series. www.lablit.com/article/621

Executive summary

SET9 monomethylation at lysine 372 stabilizes p53

- SET9 monomethylates p53 at lysine 372 in the nucleus upon DNA damage.
- This methylation results in the stabilization of chromatin-bound p53 and transcriptional activation of target genes.
- One mechanism by which SET9 monomethylation at lysine 372 stabilizes p53 may be through promotion of p53 acetylation.

Smyd2 monomethylation at lysine 370 represses p53 activity

- Smyd2-mediated monomethylation at lysine 370 has a repressive effect on p53 transactivation activity.
- Smyd2 methylation of lysine 370 is inhibited by SET9 methylation at the neighboring lysine 372.

Lysine demethylase LSD1 regulates p53

- p53 dimethylation at lysine 370 by an unknown methyltransferase positively regulates p53 transcriptional activity.
- p53K370me2 is recognized by the tandem Tudor domains of DNA damage response protein 53BP1, which acts as a coactivator of p53 target genes.
- LSD1 removes one methyl moiety from p53K370me2 to generate p53K370me1, thus converting the active dimethyl species to an inactive monomethyl species.

SET8 monomethylation at lysine 382 represses p53 activity

- SET8-mediated monomethylation at lysine 382 has a repressive effect on p53 transactivation of highly responsive target genes.
- p53K382me1 is recognized by the malignant brain tumor protein L3MBTL1.
- p53K382me1 helps stabilize L3MBTL1 at target gene promoters to maintain gene repression in the absence of DNA damage.

53BP1 stabilizes p53K382me2 upon DNA damage

- p53 is dimethylated at lysine 382 by an unknown methyltransferase upon DNA damage.
- p53K382me2 is recognized by the tandem Tudor domains of 53BP1.
- 53BP1–p53K382me2 interaction is involved in transducing DNA damage signal to activate p53.

G9a & Glp dimethylation of lysine 373 inactivates p53

- The related methyltransferases G9a and Glp dimethylate p53 at lysine 373.
- p53K372me2 represents an inactivating dimethyl mark on p53.

Protein lysine methyl transferases methylate p53 at different C-terminal lysine residues to increase concentrations of methylated p53 species. Square brackets denote increased concentration of specific p53-methylated species. Protein lysine methyl transferases and methylation events are shown in blue. Readers and modifying enzymes are shown in yellow. AD: Activation domain; BD: Basic domain; DBD: DNA-binding domain; NLS: Nuclear localization sequence; TD: Transactivation domain.