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p53 post-translational modification: deregulated in tumorigenesis

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Abstract

The p53 tumor suppressor protein has well-established roles in monitoring various types of stress signals by activating specific transcriptional targets that control cell cycle arrest and apoptosis although some activities are also mediated in a transcription-independent manner. Here, we review the recent advances in our understanding of the wide spectrum of post-translational modifications that act as epigenetic-like codes for modulating specific functions of p53 *in vivo* and how deregulation of these modifications might contribute to tumorigenesis. We also discuss future research priorities to further understand p53 post-translational modifications and the interpretation of genetic data in appreciation of the increasing evidence that p53 regulates cellular metabolism, autophagy and many unconventional tumor suppressor activities.

Coordinating stress response through p53

p53, often regarded as the “guardian of the genome”, exerts tumor suppressive capacities by centrally coordinating a regulatory circuit that monitors and responds to a variety of stress signals, including DNA damage, abnormal oncogenic events, telomere erosion and hypoxia [1,2]. p53 is a sequence-specific transcription factor and responds to these stress events via regulating cell cycle progression, apoptosis, DNA repair, senescence, cellular metabolism or autophagy [3]. Recently discovered transcription-independent functions of p53 in the cytosol add another layer to p53-mediated stress response.

In order to coordinate a wide variety of cellular processes, p53 demands a refined and complicated regulatory network consisting of many positive and negative regulators. At homeostasis, the steady-state level of p53 is kept low and p53 function is repressed mainly by the negative regulators Human Double Minute 2 (HDM2, mouse ortholog is mdm2) and Human Double Minute X (HDMX, mouse ortholog is mdmX)[3]. Under stress conditions, however, p53 is stabilized and released from repression, and further activated in a promoter-specific fashion.

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Mutations that disrupt p53 function occur in about half of all human cancer cases, and abrogation of other components of the p53 pathway is prevalent in the remainder [4]. p53 possesses an amino (N)-terminal transactivation domain, a proline rich domain, a central DNA-binding core domain, a tetramerization domain and a carboxy (C)-terminal regulatory domain. The vast majority of p53 mutations found in tumors are missense mutations within the DNA binding domain, resulting in p53 proteins with altered conformation and attenuated sequence-specific binding to DNA [5]. The significance of p53 mutations in tumorigenesis is 3-fold: (i) they abolish wild-type p53 function, (ii) they create dominant negative activity through tetramer formation with wild-type p53, (iii) they convey “ oncogenic” function through the selective growth advantages of cells with the mutations, the transactivation of new target genes or via inappropriate interaction with other cellular proteins [6].

p53 harbors many conserved sites that can be regulated by a multitude of covalent post-translational modifications, including phosphorylation, ubiquitination, acetylation, methylation, sumoylation and neddylation (Figure 1) [7]. Here, we focus on recent advances in our understanding of p53 post-translational modifications and how deregulated p53 modification contributes to tumorigenesis.

Phosphorylation

Human p53 harbors an array of serine (S)/threonine (T) phosphorylation sites that span the entire protein but are concentrated in the N-terminal transactivation domain and the C-terminal regulatory domain (Figure 1). The majority of these sites are rapidly phosphorylated following cellular stress, although a few (e.g. T55 and S376) are constitutively phosphorylated in unstressed cells and dephosphorylated following stress [8,9].

p53 phosphorylation at the N terminus shows significant redundancy; a single site can be phosphorylated by multiple kinases and a single kinase can phosphorylate multiple sites [3]. The most extensively studied N-terminal p53 phosphorylation sites are S15 and S20. S15/S20 phosphorylation reduces p53 affinity for its primary negative regulator Mdm2 and promotes the recruitment of transcriptional co-activators [5]. Studies with mice containing single and double S to alanine (A) mutations reveal redundancy in the physiological importance of these two phosphorylation sites. Although the individual mutations in gene knock-in experiments in mice only marginally change p53 stability and transactivation activity, the mice bearing p53 with both S15A and S20A mutations display a more severe phenotype including tissue-specific defective pro-apoptotic capacity, mildly compromised replicative senescence and a latent development of a spectrum of tumors [5,10]. Nevertheless, the phenotypes of these mice are more subtle than predicted by transfection studies, suggesting that p53 activation *in vivo* is controlled by a regulatory network more complex than S15/S20 phosphorylation.

S46 phosphorylation has recently attracted much attention. Phosphorylation of S46 is critical for p53-mediated induction of pro-apoptotic genes such as p53-regulated Apoptosis-Inducing Protein 1 (p53AIP1) but is not required for the induction of cell cycle arrest targets [11,12]. Indeed, the resistance of a human oral squamous cell carcinoma cell line HSC-3 to p53 is attributed to deficiency in S46 phosphorylation, and the introduction of the exogenous phospho-mimic p53S46D (aspartic acid) mutant enhanced transcription of the pro-apoptotic target *Noxa* and restored apoptosis in HSC-3 cells [13]. A study with knock-in mice expressing the human *TP53* gene with the S46A mutation partially supports the idea that S46 has a physiological role in differentially regulating cell cycle arrest and apoptosis. The mutant mice, compared to knock-in mice expressing the wild-type human *TP53* gene, displayed modestly reduced p53 transcription of some pro-apoptotic targets and compromised apoptosis but not cell cycle arrest, although the effects were tissue-specific [14].

Phosphorylation of C-terminal S392 in response to Ultra-Violet (UV) light activates specific DNA binding through the stabilization of the p53 tetramer [6]. Knock-in mice with a S389A (human S392A) mutation displayed normal p53 stability but an increased predisposition to UV-induced skin cancer [15] as well as altered expression of p53 target genes compared to wild-type mice [16,17], supporting a physiological role for S392 phosphorylation in the tumor suppressive responses of p53 to UV. However, some studies report a correlation between S392 hyper-phosphorylation and poor prognosis, advanced tumor stage and tumor grade in p53-positive cancers [18–20]. How does a tumor-suppressive modification acquire tumor-promoting functions? Perhaps S392 phosphorylation enhances the tetramer formation of certain gain-of-function p53 mutants, turning these mutants into more potent oncoproteins. Further investigation is needed to determine whether S392 phosphorylation is common to both wild-type and mutant p53, and if so, how it might contribute to tumor progression.

The relatively mild and tissue/cell-specific phenotypes of mice with S to A mutations suggest redundancy in p53 regulation by phosphorylation. It is highly probable that no one specific p53 phosphorylation functions as a “switch” for p53 stability or transcriptional activity. Instead each phosphorylation might help to fine-tune and regulate p53 function in a tissue- and promoter-specific fashion.

Ubiquitination

Ubiquitination refers to the covalent conjugation of one or more ~8 kD ubiquitin molecules to a protein substrate, and requires the consecutive function of three enzymes: an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin-ligating enzyme. Many E3 ligases harbor a Really Interesting New Protein (RING) domain or a Homologous to the E6-AP Carboxyl Terminus (HECT) domain. Ubiquitination plays a key role in regulating p53 stability and localization (Figure 2). Poly-ubiquitination primarily targets p53 for proteasomal degradation, whereas mono-ubiquitination facilitates p53 cytoplasmic translocation [21,22]. Both mechanisms contribute to ensure p53 latency and low steady-state level under homeostasis. Although cytosolic localization was previously thought to passively block p53 function by excluding it from the nucleus where it transactivates target genes, it is now understood that cytosolic p53 has transcription-independent roles in triggering apoptosis and inhibiting autophagy, although the mechanism for the latter process is largely unknown [4,23,24].

HDM2 is the pivotal E3 ubiquitin ligase and negative regulator of p53. HDM2 targets six p53 lysine (K) residues within the C-terminal regulatory domain (K370, K372, K373, K381, K382, and K386; Figure 2). p53 is poly-ubiquitinated by high levels of HDM2 and mono-ubiquitinated by low levels of HDM2. HDM2-mediated suppression of p53 is 2-fold: (i) HDM2 as an E3 ligase ubiquitinates p53 and targets it for proteasomal degradation and (ii) HDM2 inhibits transcriptional activation by directly binding to and repressing p53. The critical role for HDM2 suppression of p53 is best illustrated by the rescue of embryonic lethality in *mdm2* null mice by the loss of p53 [22]. Furthermore, mice expressing a cysteine (C)462A mutated version of *mdm2* (equivalent to C464A in HDM2), which has no E3 activity but retains p53 binding capacity, die during embryogenesis but can be rescued by the loss of p53 [25], demonstrating that the E3 ligase activity of *mdm2* is indispensable for the repression of p53.

Importantly, the gene encoding HDM2 is a p53 transcription target, and the stress-induced increase in p53 levels induces the expression of *HDM2*, which in turn downregulates p53, creating a negative feedback loop [22]. The p53/HDM2 feedback loop is regulated by multiple factors including the Alternate Reading Frame of the INK4a/ARF locus (ARF) tumor suppressor [26], the E3-ligase activity-lacking HDM2 homolog HDMX (also known as HDM4) [3], the deubiquitinating enzyme Herpesvirus-Associated Ubiquitin-Specific Protease

(HAUSP, also known as Ubiquitin-Specific Protease 7 (USP7)) [27], and post-translational modifications of HDM2 such as phosphorylation [28,29] and acetylation [30]. Importantly, HDM2 and HDMX physically interact with p53, forming a protein complex on target gene promoters that represses p53 function by preventing access to the general transcriptional machinery [31,32]. The repression of p53 by HDM2 and HDMX is non-overlapping, because neither regulator can compensate for the embryonic lethality caused by the loss of the other [5]. The importance of HDM2 and HDMX in repressing p53 tumor suppressor function is further supported by the prevalence (around 1/3 of human tumors) of *HDM2* or *HDMX* gene amplification or overexpression in human tumors retaining wild-type p53 [5,33].

In addition to HDM2, other E3 ligases regulate p53 function by affecting its degradation and cytoplasmic localization through HDM2-independent ubiquitination. In cell culture, the RING domain containing p53-Induced protein with a RING-H2 domain (PIRH2), Constitutively Photomorphogenic 1 (COP1), Carboxy terminus of Hsp70p-Interacting Protein (CHIP), Caspase 8/10-Associated RING Proteins (CARPs) and SYNOVIOLIN [21,34,35], the HECT domain containing ARF-Binding Protein 1 (ARF-BP1) [36] as well as Ubiquitin-Conjugating enzyme 13 (UBC13) (containing neither domain) [37] poly-ubiquitinate p53, targeting it for proteolysis, although whether these E3 ligases regulate p53 stability *in vivo* needs further genetic validation. Other E3 ligases such as WW domain-containing Protein 1 (WWP1) [37] and Male-Specific Lethal-2 (MSL2) [38] affect p53 nuclear export. Recent studies also support the existence of E4 ubiquitin ligases that specifically target mono-ubiquitinated p53 in the cytosol for homeostatic proteolytic degradation [39], possibly antagonizing the transcription-independent apoptotic functions of cytosolic p53, which requires mono-ubiquitinated p53 in the mitochondria. The presence of multiple ubiquitin ligases that control p53 stability and subcellular distribution suggests a “fail-proof” redundancy in negative regulation. The capacity of these ligases to repress p53 function predicts that these p53-specific E3 ubiquitin ligases could be oncogenes. Indeed *PIRH2*, *COP1* and *WWP1* are amplified or overexpressed in certain cancers [40–43].

The ubiquitination of p53 is counteracted by at least two enzymes, HAUSP and Ubiquitin Specific Protease 10 (USP10) [27,44], both belonging to a large deubiquitinase (DUB) family [45]. The deubiquitinase activity of HAUSP and USP10 exist in different compartments: HAUSP deubiquitinates and stabilizes p53 primarily in the nucleus [27], whereas USP10 largely deubiquitinates cytoplasmic p53 during homeostasis, although it retains deubiquitinase activity upon translocation to the nucleus following DNA damage [44]. HAUSP deubiquitinates p53, auto-ubiquitinated HDM2 and ubiquitinated HDMX [46], thereby playing a dynamic role in the p53 regulation pathway. USP10, by contrast, has not been shown to deubiquitinate HDM2 or HDMX. Rather, USP10 reverses HDM2-induced p53 nuclear export, thereby recycling cytoplasmic p53 back to the nucleus [44].

The physiological relevance of HAUSP in p53 regulation is supported by a recent *hausp* knock-out study in mice [47], where the early embryonic lethality caused by *hausp* knock-out mutation could be partially rescued by loss of p53. The role of HAUSP in tumorigenesis is complexed by the non-linear HAUSP-mediated effects on the p53-HDM2 pathway. Moderate down regulation of HAUSP reduces the deubiquitination of p53, leading to p53 destabilization and therefore favors cell proliferation [48]. These data lend support to the finding in a study of patient samples of Non-small Cell Lung Cancer (NSCLC) that nearly 50% of NSCLC samples with wild-type p53 display reduced *HAUSP* mRNA expression [49]. By contrast, complete loss of HAUSP function through a robust small interfering (si)RNA knockdown or knockout of the *HAUSP* gene destabilizes HDM2 and HDMX, therefore stabilizing p53 and would inhibit tumor growth [46]. This is consistent with the observation that no HAUSP mutation was identified in the *TP53*^{+/+} NSCLC samples [49]. Inhibition of HAUSP, therefore, presents a promising therapeutic approach for treating cancers that retain wild-type p53. Indeed, a small

molecule inhibitor HBX 41,108 was identified for HAUSP by high-throughput screening [50]. In tissue culture, treatment with HBX 41,108 stabilizes p53 and inhibits tumor cell growth, warranting further studies to confirm the anti-tumor effect *in vivo*.

Using human Renal Cell Carcinoma (RCC) cell lines, Yuan and colleagues showed that USP10 is capable of stabilizing both wild-type and mutant p53; therefore USP10 might have different roles in tumorigenesis depending on the p53 status [44]. In RCC cell lines that retain wild-type p53, USP10 behaves like a tumor suppressor and upregulation of USP10 is favorable for repression of cancer growth. In RCC cell lines that have mutant p53, USP10 promotes cancer cell proliferation, and downregulation of USP10 would be beneficial for the inhibition of cancer growth. *usp10* knockout mice studies would facilitate our understanding of the physiological role of USP10 in tumorigenesis. It is perceivable that discovery of USP10-activating or -inhibiting drugs would offer promising treatments for cancers with wild-type or mutant p53.

The continual discovery of novel p53 E3 ubiquitin ligases and our understanding of how they regulate p53 stability and localization raise interesting questions: (i) how are diverse E3 ligases preferentially activated in response to different stress stimuli or in different tissues?; (ii) does crosstalk occur between different ubiquitin ligases, allowing synergistic regulation of p53 function?; (iii) given the compartmentalization of HAUSP and USP10, the two DUBs are probably not redundant; however, the low substrate specificity of HAUSP for p53 suggests there might be additional DUBs that regulate p53. By combining *in vitro* deubiquitination assays with siRNA knock-down screens targeting members of the DUB family, these missing DUBs can be identified and their physiological roles can be characterized.

Ubiquitin-like modifications

p53 is targeted by two other ubiquitin-like proteins, Small Ubiquitin-like Modifier (SUMO) and Neural precursor cell Expressed Developmentally Down-regulated protein 8 (NEDD8), both of which are evolutionarily conserved in eukaryotes and resemble ubiquitin in both their three-dimensional structure and their mechanism of conjugation through lysines [51–53]. p53 is sumoylated at a single site K386 by members of the Protein Inhibitor of Activated Stat (PIAS) family and Topors [54,55]. Neddylation of p53 is mediated by HDM2 and F-box protein 11 (FBXO11): Mdm2 catalyzes the neddylation of three C-terminal lysines (K370, K372 and K373) that are also targeted for ubiquitination [56], FBXO11 neddylates two lysines (K320 and K321) [57]. Unlike ubiquitination, neddylation and sumoylation have not been demonstrated to affect p53 stability or localization. Neddylation inhibits p53 transcriptional activation activity [56,57], whereas the functional consequences of K386 sumoylation is interesting, albeit not well-defined; some reports link it to increased p53 transcriptional activity [58] and premature senescence [54,59,60].

It is noteworthy, that the low abundance of SUMO- or NEDD- modified p53 *in vivo*, normally less than 5% of total cellular p53, poses a challenge for defining the cellular roles of these modifications. Reconstituted systems allow robust testing of the roles of these ubiquitin-like modifications *in vitro*, but are unlikely to recapitulate the physiological conditions in which these modifications occur. It remains to be determined under what circumstances sumoylation and neddylation might affect p53 function.

Acetylation

The acetylation of p53 is a powerful mechanism for activating function. The significance of p53 acetylation is 3-fold: (i) it promotes p53 stabilization by excluding ubiquitination on the same site, (ii) it inhibits the formation of HDM2/HDMX repressive complexes on target gene promoters, and (3) it recruits cofactors for the promoter-specific activation of p53 transcriptional activity.

Nine acetylation sites have been identified for p53, and the Histone Acetyltransferases (HATs) responsible for these modifications are the structurally related p300 (also called KAT3B) and CREB-Binding Protein (CBP, also known as KAT3A), –P300/CBP-Associated Factor (PCAF, also known as KAT2B) and the MYST (named for members MOZ, Ybf2/Sas3, Sas2, and Tip60) family HATs, –Tat-Interactive Protein of 60 kDa (TIP60, also known as KAT5) and human Males absent On the First (hMOF, also known as MYST1/KAT8) [7,61,62] (Figure 1).

Six lysine residues (K370, K372, K373, K381, K382 and K386) in the C-terminal regulatory domain are acetylated by CBP/p300 and ubiquitinated by HDM2 [7] (Figure 1). Acetylation in tissue culture systems activates sequence-specific binding of p53 to DNA and its transcriptional activation activity and enhances the stability of p53, owing to the mutual exclusion of acetylation and ubiquitination. Nevertheless, despite some cell-type specific differences in transcriptional profiles, mice expressing C-terminal acetylation-deficient p53 (p53^{6KR} and p53^{7KR} knock-in mice) generally exhibited no major difference in cell cycle control, apoptosis or tumor suppression [63,64], which is in line with the fact that mutation in the p53 C-terminal regulatory domain is rarely found in human cancers (UMD_TP53 Mutation database <http://p53.free.fr/>)

K320 in the tetramerization domain is acetylated by PCAF [7]. K320 acetylation favors cell survival by promoting p53-mediated activation of cell cycle arrest target genes such as *Cyclin-Dependent Kinase inhibitor 1A* (*CDKN1A*, commonly known as *p21*) [65]. This is supported by studies using mice with a K317R (arginine; human K320R) mutation in p53 [66] that have increased expression of pro-apoptotic target genes and enhanced p53-mediated apoptosis upon irradiation, suggesting that K320 acetylation negatively regulates p53 apoptotic activities upon DNA damage.

The recent discovery of two additional acetylation sites, K120 (acetylation mediated by TIP60/hMOF [61,67]) and K164 (acetylation by CBP/p300 [32]) in the DNA binding domain where the vast majority of p53 mutations are found in cancer, predicts that they might have physiological roles in the control of p53 function. K120 acetylation is indispensable for the activation of target genes involved in apoptosis but not cell cycle arrest [61,67], suggesting a means for controlling promoter specificity and hence cell fate. Additionally, K120 acetylation might be required for p53 to effectively displace the pro-apoptotic protein BCL2-Antagonist/Killer 1 (BAK) from the oncoprotein Myeloid Cell Leukemia sequence 1 (MCL-1) at the mitochondria [68]. Therefore, it is likely that K120 acetylation by TIP60 contributes to both transcription-dependent and transcription-independent apoptotic functions of p53. K164 acetylation, by contrast, appears to be important for the activation of the majority of p53 targets. If K120, K164 and the six CBP/p300-targeted C-terminal lysine residues are collectively mutated to arginine, p53 is rendered inert [32]. Although an individual K to R mutation can be compensated for by acetylation at other sites, the collective mutation of all eight sites (p53^{8KR}) completely abolishes p53-mediated cell cycle arrest and apoptosis [32], demonstrating that acetylation is indispensable for p53 activation. Mechanistically, acetylation allows p53 to evade HDM2 and HDMX repression by blocking recruitment of HDM2 and HDMX to target gene promoters. Importantly, both K120 and K164 are in the DNA binding domain and are recurrently mutated in cancer (UMD_TP53 Mutation database <http://p53.free.fr/>), implying that these two modifications might have profound and non-redundant effects on p53 function. It would be interesting to test the collective importance of these eight lysine residues and the importance of K120 and K164 acetylation *in vivo* in knock-in mouse models.

Equilibrium in the acetylation of p53 is maintained by the Histone Deacetylases (HDACs), HDAC1 and Sirtuin 1 (SIRT1) [69,70]. SIRT1 preferentially deacetylates p53 at K382 and has a profound negative impact on the capacity of p53 to induce the expression of target genes

involved in apoptosis, such as *BCL2 Binding Component 3* (*BBC3*, commonly known as *PUMA*) and *BCL2-associated X* (*BAX*). Thymocytes of Sirt1-deficient mice exhibit p53 hyperacetylation and increased radiation-induced apoptosis compared to wild-type thymocytes [71]. *SIRT1* is negatively regulated at the transcriptional level by Hypermethylated In Cancer 1 (*HIC1*) and at the translational level by the microRNA (miR)-34a [72,73], both of which are targets of p53. *SIRT1* expression is elevated in leukemia [74], prostate cancer [75] and skin cancer [76], and it is negatively regulated by Deleted in Breast Cancer 1 (*DBC1*) [77,78], supporting a role for *SIRT1* in tumorigenesis. However, the suppression of intestinal tumorigenesis and colon cancer growth in a β -catenin-driven mouse model of colon cancer by ectopic induction of Sirt1 [79] suggests that it has tumor-suppressive properties as well.

The evidence that *SIRT1* harbors both tumor-promoting and tumor-suppressing functions generates interest in developing *SIRT1*-targeted drug therapies for cancer treatment [80]. The most promising *SIRT1* inhibitors discovered to date are tenovin-1 and its more water-soluble derivative, tenovin-6 [81]. At low micromolar concentrations, tenovins potently inhibit the deacetylase activities of *SIRT1* and *SIRT2*, significantly increase the level of p53 K382 acetylation in tissue culture, and decrease tumor growth in xenograft mouse tumor models. Studies on activators of *SIRT1* focus on resveratrol, which is abundant in grapes. Although dietary intake of resveratrol delays aging in mice [82], more studies are in need to assure that resveratrol activation of Sirt1 does not impose cancer susceptibility.

Methylation

The large number of lysine and arginine residues in p53 presents the potential for regulation by methylation (Figure 1). Arginine methylation has only been shown for one methyltransferase, Protein Arginine N-Methyl Transferase 5 (*PRMT5*) [83,84], which targets R333, R335 and R337 in the tetramerization domain, and methylation of these residues differentially affect the target gene specificity of p53 [84]. p53 lysine methylation is better understood. p53 is mono-methylated by three different Lysine Methyl Transferases (*KMTs*) and di-methylated by at least two *KMTs* [85]. The functional consequences of p53 lysine methylation can be either activating or repressive, depending on the location of the modification and the number of methyl groups attached.

Mono-methylation at K372 is mediated by *SET7/9* (also known as *KMT7*) and this modification promotes the transactivation of target genes [86]. *SET8* (also known as *KMT5A*)-mediated K382 mono-methylation and *SMYD2* (also known as *KMT3C*)-mediated K370 mono-methylation repress p53 transcriptional activity [87,88]. *G9A* (also known as *KMT1C*) and *G9A*-like Protein (*GLP*, also known as *KMT1D*) di-methylate p53 at K373, thereby negatively regulating p53-mediated apoptosis [89]. Interestingly, however, conjugation of a second methyl group to K370 (*K370me2*), by a currently unknown enzyme, leads to a distinct functional consequence than mono-methylation. *K370me2* increases in response to DNA damage and promotes p53 function by facilitating the association of p53 with the coactivator p53 Binding Protein 1 (*53BP1*) [90]. Lysine Specific Demethylase 1 (*LSD1*, also known as *KDM1*) preferentially removes this positive-acting second methyl group, thereby repressing p53 function by inhibiting the association of p53 with *53BP1* [90]. These findings suggest that p53 methylation and demethylation dynamically regulate p53 function, at least in part by allowing or disallowing p53 binding to coactivators.

Interestingly, there appears to be crosstalk between p53 methylation at different sites and between p53 methylation and acetylation. Activating methylation of K372 inhibits the repressive methylation of K370 by preventing *SMYD2* binding to p53 [87]. Moreover, the repressive methylation of K382 normally prevents acetylation at this same site by *CBP/p300* [88]. Upon DNA damage, the level of methylation at K382 decreases, reversing its inhibitory

effect and allowing CBP/p300 acetylation of K382, and thereby promoting p53 activity. Together, the interplay between p53 methylation sites as well as between p53 methylation and acetylation provide mechanisms for triggering a rapid increase in p53 transcriptional activity in response to stress.

The presence of negatively-acting lysine methylation sites and KMTs that normally maintain p53 in an inactive state suggests the possibility that abnormally high levels of KMTs could be oncogenic. Indeed, the SET domain containing methyl transferase G9A is upregulated in many cancers cell types and its homolog GLP is also overexpressed in brain tumors and multiple myeloma [89].

Concluding remarks and future directions

The modest phenotypes of many mice expressing p53 with point mutations that disrupt post-translational modifications suggest functional redundancy in these modifications, perhaps important for the “fail-proof” regulation of p53 considering its central role in tumor suppression. The presence of multiple modification possibilities (acetylation, methylation, sumoylation and neddylation), some activating and some repressing, on many residues in the p53 C terminus might mask the effects of a single modification when the K to R mutations are introduced. The extensive crosstalk between p53 modification sites or between the different modifications adds further complications. Future work should elucidate the interplay between numerous p53 regulators, as well as take advantage of modification-mimicking mutants or other methods that specifically affect one type of modification while retaining other types.

Although each site/modification might only fine-tune p53 function, the numerous possible combinations of different modifications could dictate p53 activity in a promoter-specific fashion, allowing p53 to exert a spectrum of functions (Figure 3). Importantly, altering single-site modifications might produce tissue or cell-type specific and stimulus-specific changes in p53 function. The specific and subtle phenotypic changes resulting from alterations in post-translational modifications, therefore, require extremely careful study of the mouse models.

Although the best studied function of p53 is its control of cell cycle arrest and apoptotic cell death, increasing evidence demonstrates that p53 regulates cellular metabolism and autophagy [91–94]. *TP53-Induced Glycolysis and Apoptosis Regulator (TIGAR)* and *Guanidino Acetate Methyl Transferase (GAMT)* are two newly identified p53 target genes that regulate glucose metabolism and nutrient stress responses, respectively [91,93]. By switching the cellular metabolic pathways away from glycolysis and towards the pentose phosphate shunt, TIGAR protects cells from Reactive Oxygen Species (ROS)- associated apoptosis, allowing cell survival in mild stress. GAMT, by increasing fatty acid oxidation in response to nutrient deprivation, contributes to p53-mediated apoptosis induced by genotoxic stress and starvation. Nuclear p53 induces autophagy following genotoxic stress by transcriptionally upregulating the mammalian *Target Of Rapamycin (mTOR)* inhibitors, *Phosphatase and Tensin homolog (PTEN)* and *Tuberous Sclerosis 1 (TSC1)*, or the p53-regulated autophagy and cell death gene *Damage-Regulated Autophagy Modulator (DRAM)* [92,94], whereas basal levels of cytoplasmic p53 inhibit autophagy through transcription-independent mechanisms such as AMP-Activated Protein Kinase (AMPK) activation and mTOR inhibition [23,94,95]. Because the role of p53 in regulating metabolism and autophagy has only recently begun to be appreciated, *in vitro* and *in vivo* studies of p53 post-translational modifications and their mediators have generally overlooked these aspects of p53 function. Therefore, it is important that the previously generated p53 mutant mice and any future mouse models be carefully investigated for p53 transcription-dependent and -independent functions on metabolism and autophagy.

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Abbreviations: TAD, transactivation domain; PRD, proline rich domain; DBD, DNA-binding domain; TD, tetramerization domain; CRD, C-terminal regulatory domain.

Figure 2. Regulation of p53 stability and localization by ubiquitination

Nuclear p53 is targeted by Mdm2 for monoubiquitination promoting cytoplasmic translocation or polyubiquitination promoting proteosomal degradation. The abundance of Mdm2 and MdmX are also regulated by ubiquitination and deubiquitination. HAUSP stabilizes p53, Mdm2, and MdmX through deubiquitination. In the cytoplasm, USP10 deubiquitinates monoubiquitinated p53, reversing nuclear export and recycling p53 into the nucleus. Monoubiquitinated p53 in the cytoplasm can possibly be further ubiquitinated by E4 ubiquitin ligases and targeted for degradation. Cytoplasmic p53 also has transcription-independent roles in activating apoptosis through permeabilization of the mitochondrial outer membrane and the inhibition of autophagy through mechanisms yet to be discovered.

Abbreviations: U, Ubiquitination

Figure 3. Three-step activation of p53 transcriptional activity

p53 transcriptional activity is activated through three sequential steps: (i) DNA binding, (ii) anti-repression and (iii) cofactor recruitment. Under homeostasis, p53 is bound to target gene promoter DNA but is repressed by Mdm2 and MdmX. Cellular stress triggers phosphorylation and acetylation at key p53 residues and facilitates the release of p53 from Mdm2 and MdmX mediated repression. The exact combinations of cofactors and post-translational modifications present on p53 provide promoter specificity. Anti-repression alone is sufficient for the induction of the p53 negative feedback loop. Cell cycle control requires partial activation of p53 through further modifications. Apoptotic activation requires the full activation of p53 activity via specific cofactors and an array of modifications. The control of p53 transcriptional regulation of metabolism and autophagy remains to be understood.

Important p53 target genes for each cellular outcome are listed on the right.

Abbreviations: TFs, transcription factors; P, phosphorylation; Ac, acetylation.