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# p53 regulation upon genotoxic stress: intricacies and complexities

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**Abbreviations:** miRNA, microRNA; ROS, reactive oxygen species; UTR, untranslated region

p53, the revered savior of genomic integrity, receives signals from diverse stress sensors and strategizes to maintain cellular homeostasis. However, the predominance of p53 overshadows the fact that this herculean task is no one-man show; rather, there is a huge army of regulators that reign over p53 at various levels to avoid an unnecessary surge in its levels and sculpt it dynamically to favor one cellular outcome over another. This governance starts right at the time of p53 translation, which is gated by proteins that bind to *p53* mRNA and keep a stringent check on p53 protein levels. The same effect is also achieved by ubiquitylases and deubiquitylases that fine-tune p53 turnover and miRNAs that modulate p53 levels, adding precision to this entire scheme. In addition, extensive covalent modifications and differential protein interactions allow p53 to trigger a tailor-made response for a given circumstance. To magnify the marvel, these various tiers of regulation operate simultaneously and in various combinations. In this review, we have tried to provide a glimpse into this bewildering labyrinth. We believe that further studies will result in a better understanding of p53 regulation and that new insights will help unravel many aspects of cancer biology.

Regulation of any cellular pathway is essential to coordinate the heterogeneity and complexity of functions in multicellular organisms. Among the tumor suppressors, decoding the bewildering number of pathways that p53 is involved in has long been the holy grail of scientists. p53 is a master regulator that integrates signals from diverse nodes and thus it is of no surprise that it is the most commonly mutated gene in a huge array of cancers with varied origins. p53 has many weapons at its disposal to combat stress including cell cycle arrest, senescence, apoptosis, autophagy, and metabolic reprogramming. Paradoxically, some of the outcomes of p53 activation are disparate and contradictory, such as cell cycle arrest, which is prosurvival, versus apoptosis and senescence, which are directed toward eliminating irreversibly damaged cells. This indicates that p53 needs to be educated to sense the extent and type of damage and make an appropriate choice of the kind of response it is going to elicit. Extensive research on the regulation of p53 under diverse kinds of stresses including genotoxic stress, starvation, hypoxia, and oncogene activation clearly indicate that p53 protein is regulated at diverse levels, including synthesis, degradation, covalent modifications, subcellular localization, and differential interaction with other proteins. Moreover, all possible permutations and combinations of these are employed to modulate p53 specificity, tissue heterogeneity, and diversity of function. In light of this, we restrict this review to exclusi<!——>vely discussing the myriad layers of p53 regulation upon genotoxic stress (**Fig. 1**).

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## It Begins at the Beginning: Translational Regulation of p53

Transcriptional regulation of p53 is not a major contributor to the modulation of p53 levels. There are reports of various factors that bind to the p53 promoter and can activate its transcription under stress conditions, such as cAMP responsive element binding protein (CREB)[1] and Myc.[2] p53 is also known to transcriptionally activate itself.[3] However, p53 levels are predominantly regulated by a slew of post-transcriptional and post-translational mechanisms. Initially, it was thought that the increase in p53 protein levels upon genotoxic stress is the result of inhibition of its degradation, but translational regulation was later recognized as being critical for maintaining p53 levels. Both the 5'UTR and 3'UTR of *p53* mRNA play a significant role in negative regulation of p53 translation.[4,5] Moreover, the ribosomal protein RPL26 binds to the 5'UTR of *p53* mRNA, and hastens p53 translation under genotoxic stress to elicit a rapid p53 response.[6] On the other hand, MDM2 binds to RPL26 and triggers its polyubiquitylation and proteasomal degradation, thereby turning down p53 translation.[7] As MDM2 is a p53 transcriptional target, this constitutes an autoregulatory feedback loop that modulates p53 translation. Further exploration of RPL26-dependent translational regulation of p53 showed that 5'UTR–3'UTR base pairing of *p53* mRNA is a critical determinant of RPL26 binding and can be manipulated by mutating these sites.[8] Providing proof of concept, short oligonucleotides targeting this region inhibit p53 translation and can be used to inhibit expression of mutant p53 protein implicated in tumor progressive mechanisms. This has exciting implications on the clinical front and could be used

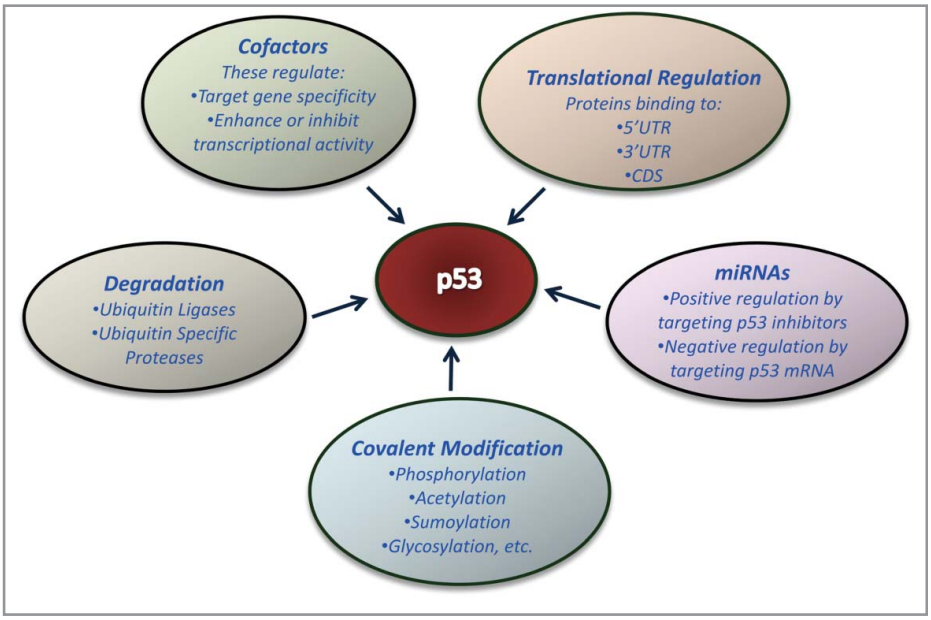


**Figure 1.** Diverse modes of p53 regulation. The multitude of ways by which p53 can be regulated include regulation at the level of translation and mRNA stability, cellular proteins that bind to and regulate p53 function and stability, and post-translational modifications.

in targeted therapy to combat many pathologic conditions. The nucleolin protein also binds to the *p53* mRNA 5'UTR; however, this interaction inhibits p53 translation under unstressed conditions.[6] Pdcd4 performs a similar function to nucleolin to repress p53 translation under normal conditions.[9] Furthermore, thymidylate synthase, a folate-dependent enzyme that catalyzes the conversion of dUMP to dTMP, binds to the coding sequence of *p53* mRNA and suppresses its translation.[10,11] Moreover, the RNA binding proteins HuR and Hzf work hand in glove to bind to the 3'UTR of *p53* mRNA in the presence of p19Arf and export it from the nucleus to the cytoplasm in conjunction with its increased translation.[12] RNPC1, GAPDH, hnRNP A/B, and hnRNP D also regulate p53 translation by binding to the cytoplasmic polyadenylation elements of *p53* mRNA.[13,14]

In essence, different regulators bind to *p53* mRNA 5'UTR, 3'UTR, and coding sequence and collaborate to fine tune p53 abundance in accordance to stress cues. This in turn largely excludes undamaged cells from the ravaging consequences of abundant p53 while ensuring that damaged cells do not escape surveillance.

## Auditing p53 Levels: Regulation by Degradation

Ubiquitylases and deubiquitylases are indispensable for protein turnover and are key mediators of signal transduction and thus important players in cancer development.[15] Ubiquitin is attached to target proteins through different lysine linkages, namely K6, K11, K27, K29, K33, K48, and K63. K48-linked polyubiquitylation mediates degradation and K63-polyubiquitylation coordinates signaling. In this section we have focused on the ubiquitin-mediated degradation of p53; the role of ubiquitin as a signal modifier and determinant of p53 cellular localization will be discussed later in this review.

MDM2, an E3 ligase, has the honor of being the first regulator of p53 to be investigated. It keeps p53 levels in check under conditions of no stress through polyubiquitylation and proteasomal degradation.[16] MDM2 binds to the N-terminal transactivation domain of p53 and ubiquitylates its 6 C-terminal lysines,[17] thus targeting it for proteasomal degradation. As MDM2 is in turn a p53 transcriptional target this constitutes an autoregulatory feedback loop.[18] Upon genotoxic stress various DNA damage sensors (e.g., ATM kinases, DNA-PK, Chk2, HIPK2) relieve p53 of association with MDM2 through phosphorylation of p53 N-terminal serine residues, resulting in an increase in p53 levels.[19] After DNA damage ATM-mediated phosphorylation of MDM2 elicits its autoubiquitylating activity, decreasing levels of ATM and MDMX, which in turn increases p53 levels.[20,21] Although MDM2 is considered the key E3 ligase for p53, continued research has revealed the involvement of a myriad of other E3 ligases in maintaining p53 levels. Of these, Cop-1 and Pirh-2 E3 ligases function similarly to MDM2 in that they are also transcriptionally induced by p53 and constitute an autoregulatory loop. ARF-BP1, Trim24, Trim28, Trim39, E4F1, the cullin family of E3 ligases, Hades, Carp1 and 2, synovilin, and SUMO E3 ligase also degrade p53 under basal conditions.[22] Trim24 has been shown to function in a similar manner as MDM2 in regulating p53 levels and is inactivated by ATM-kinases.[23]

Deubiquitylases remove the chains of ubiquitin that are attached by E3 ligases. To date, USP10, USP42, USP7 (HAUSP), and USP2a have been studied in detail with respect to p53 deubiquitylation. USP7 has a dualistic role in the context of p53 stabilization and degradation. MDM2 and p53 have both been shown to be deubiquitylated and stabilized by USP7, highlighting the complexity of p53 regulation.[24] Further studies suggest that following genotoxic stress TRIM21 ubiquitylates GMPS, a nucleotide metabolism enzyme, causing it to be translocated to the cytoplasm where it associates with USP7. This interaction causes a shift in substrate specificity of USP7 from MDM2 to p53.[25] Unlike USP7, USP10 is a p53-specific deubiquitylase. Under normal conditions, it is localized in the cytosol where it deubiquitylates monoubiquitylated p53 sequestered in the cytoplasm and sends it back to the nucleus for degradation by MDM2. However, under genotoxic stress conditions ATM-mediated phosphorylation of USP10 promotes its nuclear import where it protects polyubiquitylated p53 from degradation by deubiquitylating it.[26] USP42 has also been shown to deubiquitylate p53 in response to genotoxic stress but its regulatory role needs to be further explored.[27] On the other hand, USP2a

downregulates p53 by deubiquitylating its E3 ligases MDM2 and MDMX.[28]

Individual interactors of p53 and MDM2 also influence the p53–MDM2 interaction as well as p53 ubiquitylation, thus affecting p53 levels. p14Arf was the first known example of this type of regulation; it binds to MDM2 and inhibits MDM2-mediated p53 degradation.[29] Ribosomal protein L11 also binds to and inactivates MDM2 by inducing its nucleolar localization.[30] However, Pax3, Twist, Niban, Smurf1/2, and TCTP aid p53 ubiquitylation by enhancing MDM2 activity.[22] WIP1 is a phosphatase that dephosphorylates MDM2 thereby increasing its stability and affinity for p53 and resulting in enhanced p53 ubiquitination and degradation.[31] p53 also interacts with other transcription factors that modulate its ubiquitylation status such as ATF3, which binds to the C-terminus of p53 and inhibits its ubiquitylation,[32] and TFII31, which binds to the p53 N-terminus of p53 and prevents it from binding to MDM2.[33] Recently, Bouafia et al. reported that the stress sensor USF1 functions as a stabilizer of p53 by inhibiting the p53–MDM2 interaction after DNA damage. Being present in cells at a high level all times, USF1 binds to p53 as soon as genomic danger is encountered. Studies have shown that USF1 is equally as efficient as Nutlin-3a treatment and superior to other transcription factors such ATF3 and TFII31 in p53 stabilization during genotoxic stress.[34]

Taken together, these findings indicate that ubiquitylation-dependent pathways ensure minimal levels of p53 in normal cells whereas inhibition of these factors by messengers of DNA damage induces p53 to protect cells from transformation-inducing alterations.

## Different Strokes for Different Folks: p53 Regulation Through Covalent Modifications

One of the most important mechanisms for regulating p53 function and stability is post-translational modification. Phosphorylation, acetylation, and ubiquitylation are the prominent modifications of p53 while ubiquitin-like modifiers (e.g., SUMOylation and NEDDylation), glycosylation (O-linked N-acetylglucosamine), prolyl isomerization, and ADP-ribosylation play niche roles in p53 regulation (**Fig. 2**). The importance of these modifications is brought to the forefront when context-dependent p53 effector functions need to be executed. These modifications also act as a barcode that is read by cellular proteins for association with p53.

Phosphorylation was the first functionally relevant post-transcriptional modification of p53 to be discovered[35] and since then 23 phosphorylation sites on p53 have been uncovered. Several serines and threonines of p53 have been shown to be differentially phosphorylated by kinases, some under genotoxic stress (S6, S9, S15, S20, S46, S215, S366, S376, T388, S392) and others under basal conditions (T55, S376). The extensive redundancy of phosphorylation sites and the respective kinases involved make the implications of phosphorylation even more mystifying, for example S15 is phosphorylated by at least 8 kinases and CHK2 phosphorylates p53 at 7 different sites. ATM, ATR, and their downstream kinases CHK1 and CHK2 play a central role in genome surveillance and mediate p53 S9, S15, S20, S46 phosphorylations.[36] S15/S20 phosphorylations play an important role in disrupting the binding between p53 and MDM2 to stabilize p53 and facilitate its transcriptional activity.[37] This observation is supported by defective apoptosis and delayed tumor development in mice expressing the p53 S15/20A mutant.[38] S6 and S9 phosphorylations have importance in both development and cancer and are thought to be mediated by CK1 family members.[39] S46 phosphorylation, which is important for the induction of apoptosis[40] is primarily regulated by HIPK2,[41] DYRK2,[42] and PKCδ[43] in response to DNA damage. S392 phosphorylation, which is increased by UV radiation, stabilizes tetramer formation of p53 and hence increases its transcriptional activity.[44] Additionally, the immediate stabilization of p53 in response to UV-induced damage has been attributed to phosphorylation of p53 T18 by the serine-threonine kinase VRK1. VRK1 remains associated with p53 even in the absence of damage signals and phosphorylates p53 as soon as cells are exposed to UV,[45] thus providing an immediate regulatory response against DNA damage. Phosphorylation events also have an inhibitory effect on p53 activation. ATM-dependent dephosphorylation of S376 activates p53[46] and TAF1-mediated phosphorylation of T55 prevents binding of p53 to its target promoters.[47] Further studies have shown that TAF1 coordinates with cellular ATP fluctuations caused by DNA damage and facilitates global inhibition of p53 target genes in unstressed cells.[48] Consistent with this study, the T55A p53 mutant shows enhanced apoptosis compared to wild-type p53.

Ablation of these phosphorylations by distinct phosphatases adds another layer of complexity to the regulation of p53. DUSP26, PP1, and PP2A are among the key phosphatases that balance the DNA damage response by regulating the p53–MDM2 interaction and p53 transcriptional target preferences. These phosphatases are mainly required to reinitiate the cell cycle after DNA repair is accomplished. DUSP26 specifically dephosphorylates S20 and S37 and inhibits apoptotic functions of p53.[49] On the same note, PP1 dephosphorylates S15 and S37 of p53 thereby downregulating its transcriptional activity and apoptotic functions.[50] Ionizing radiation-induced S46 phosphorylation of p53 is reversed by PP2A, resulting in attenuation of p53-mediated apoptosis.[51] Recent studies show the requirement for different phosphatases to overcome G1 and G2 arrest upon resolution of DNA damage. PP4 rescues cells from G1 arrest by dephosphorylating KAP1, which then associates with p53 and acts as a transcriptional repressor to inhibit p21 expression. WIP1, on the other hand, dephosphorylates p53 at S15 and relieves CCNB1 repression to rescue cells from G2 arrest of the cell cycle.[52]

Acetylation of p53 at key DNA binding and C-terminal regulatory domain lysine residues (K120, K164, K320, K370, K372, K373, K381, K382, and K386) is a critical factor in determination of cellular outcome upon genomic insult. Acetylation plays an important role in transcriptional regulation by p53 and influences the recruitment of repressors, activators, and other modulators.[53] Histone acetyl transferases (HATs), including p300/CBP, PCAF,

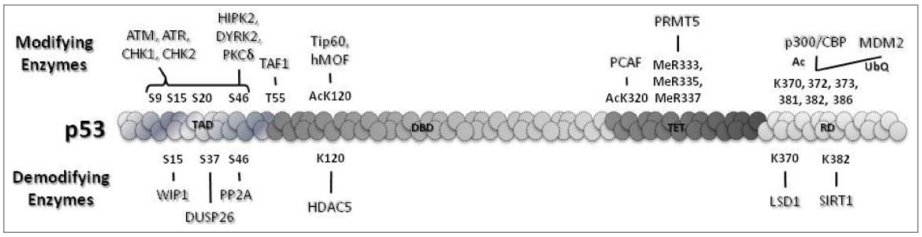


**Figure 2.** Selected modifiers and demodifiers of p53. These modifications act as determinants of p53 levels and p53-mediated cell fate decisions.

hMOF, and Tip60, and the deacetylases SIRT1 and HDACs (HDAC1 and HDAC5) harmonize to bring about cell type-specific and stimulus-specific responses. p300/CBP acetylates all 6 C-terminal lysines that are targeted by MDM2 and promotes p53-mediated transactivation, emphasizing the competitive nature of binding of these proteins at the same site. p53 K320 acetylation by PCAF promotes preferential activation of cell cycle arrest as a DNA damage response and decreases apoptosis.[38] p53 acetylation by Tip60/hMOF at K120 promotes cell death as a response to DNA damage.[54,55] Mutation of lysine to a non-acetylable arginine (R) residue has become the benchmark method to study the functional consequences of each acetylation separately (e.g., $p53^{K120R}$), C-terminal acetylation of K370, K372, K373, K381, K382, and K386 all at once ($p53^{6KR}$), core domain containing K120 and K164 together ($p53^{2KR}$), or all key acetylations (K120, K164, K370, K372, K373, K381, K382, and K386) at once ($p53^{8KR}$).[56] $p53^{8KR}$ is completely inert *in vivo*, showing the relevance of acetylation for p53 stability and transcriptional activity. $p53^{2KR}$ cells show complete loss of p53 transactivation function and stability compared to $p53^{6KR}$ cells, which show relatively increased apoptosis after IR radiation exposure. $p53^{K120R}$ cells undergo cell cycle arrest and senescence upon genotoxic stress but p53-mediated apoptosis is completely abrogated.[57]

To maintain homeostasis, p53 is also regulated by deacetylases that counteract the effect of different acetylations. SIRT1 is known to deacetylate p53 at K382 and hence inhibits DNA damage-mediated apoptosis.[58] The intricacies of the relationship between SIRT1 and p53 are highlighted by the presence of hyperacetylated p53 in mice lacking SIRT1[59] and tumors overexpressing SIRT1 with inactivated p53.[60,61] Another deacetylase that plays a critical role in modulating the p53 response to genotoxic stress is HDAC5, a class IIa deacetylase. In the early phase of genotoxic stress, HDAC5 binds to and deacetylates p53 at the K120 residue. This induces cell cycle arrest and clearance of reactive oxygen species (ROS). At the late phase of genotoxic stress, high levels of ROS lead to CamKII-mediated nuclear exit of HDAC5, which facilitates p53 K120 acetylation and induction of apoptosis; in contrast, restraining HDCA5 nuclear export promotes senescence. Thus, in mice subjected to genotoxic stress inhibition of HDCA5 nuclear export extends protection from genotoxic stress whereas abrogation of HDAC5 expression accelerates the onset of p53-mediated apoptosis.[62]

Methylation is another modification of p53 lysines. There are 3 lysine methyl transferases (KMTs) that monomethylate p53 and 2 KMTs that dimethylate p53. Monomethylation of p53 by the SET family of proteins (SET7, SET8, and SET9) and Smyd2 and dimethylation by G9a and GLP are known to modulate p53 transcriptional activity. K370 methylation by Smyd2[63] and K382 methylation by SET8 repress p53 transactivation.[64] Methylation of K372 by SET7/9 has been shown to positively regulate acetylation of K120 by Tip60 upon DNA damage.[65] However, this observation is contradicted by an another study showing a lack of significance of SET7/9 methylation in p53 acetylation and transcriptional functions.[66] Dimethylation of K370 by a currently unidentified methylase is known to promote association of p53 to 53BP1 upon DNA damage. The demethylase LSD1 inhibits this association by removing methyl moieties from K370.[67] G9a and GLP specifically methylate K373 and interfere with apoptotic functions of p53.[68] Arginine methylations in the tetramerization domain of p53 (R333, R335, R337) have also been shown to be catalyzed by PRMT5.[69] Arginine methylation regulates p53 target gene specificity thereby promoting apoptosis over cell cycle arrest.

In addition to modulating degradation, ubiquitylation also determines the endocytosis, transcriptional regulation, and subcellular localization of p53.[70] Monoubiquitylated p53 shuttles from the nucleus to cytoplasm, which lowers p53 transactivation activity. MDM2 levels in the cell also act as a determinant for subcellular localization of p53. Low levels of MDM2 monoubiquitylate p53 and result in its sequestration in the cytoplasm.[71] MSL2 specifically monoubiquitylates K351 and K357 of p53 independent of MDM2, and mediates its nuclear export.[72] Similarly, other E3 ligases such as CUL9/PARC and WWP1 ligase monoubiquitylate p53 and facilitate its nuclear export.[73,74] Paradoxically, monoubiquitylation by E4F1 facilitates association of p53 with chromatin, eliciting its cell cycle arrest functions.[75]

Modification by SUMOylation and NEDDylation involves the conjugation of small ubiquitin-like proteins. While SUMOylation of K386 of p53 by the PIAS family and TOPORS enhances transcriptional activity,[76] NEDDylation of p53 at different C-terminal residues by MDM2 (K370, K372 and K373) and FBXO11 (K320 and K321) represses its activity.[77,78] Prolyl isomerization by Pin1[79] and glycosylation[80] also direct p53 transactivation after DNA damage. In response to DNA damage, p53 also becomes poly(ADPribosyl)ated (PARylated) within its core domain (E255, D256, and E268), which inhibits its nuclear export and hence contributes to increased transactivation.[81]

## It Takes Two To Tango: the p53 Interactors

The p53 interactome includes many proteins that bind to p53 to regulate its transactivation function. Some of these proteins play a crucial role in target selection whereas others mediate assembly of the transcription complex. An important regulator of p53 apoptotic functions is the ASPP family. ASPP family members ASPP1, ASPP2, and iASPP bind to the DNA-binding domain of p53 to modulate selectivity of binding to p53 target promoters. ASPP1 and ASPP2 guide p53 toward induction of the apoptotic response whereas iASPP competes for the same site to inhibit p53 binding to apoptotic targets and promote cell cycle arrest.[82,83] The p53 target Hzf also binds to the DNA-binding domain of p53 and enhances p53 selectivity for cell cycle arrest genes in response to genotoxic stress.[84] Brn3 family members Brn3a and Brn3b, APAK, YB1, Muc1, hCAS/CSE1L, p18/Hamlet, c-Abl, and the p52 subunit of NF-kB are other guiding partners of p53 that add heterogeneity to its functional outcomes.[53] Brn3a, YB1, and Muc1 direct p53 toward cell survival mechanisms by either inducing cell cycle arrest or senescence. In contrast, Brn3b, hCAS/CSE1L, p18/Hamlet, and c-Abl channel p53 toward apoptosis induction. APAK is unique among these proteins as it binds to p53 and facilitates repression of its transcriptional activity by recruiting HDAC1 under normal conditions.[85] NF-Y occupies a special place among transcriptional regulators of p53 in that it has a dual function. On one hand, NF-Y binds CCAAT sequences and subsequent binding of p53 to the NF-YA subunit of the NF-Y complex induces transcription of apoptotic genes lacking a p53 response element. On the other hand, NF-Y recruits HDAC corepressors to repress genes involved in the rescue of G2/M arrest.[86] CBP/p300, pCAF, JMY, MAML1, TAFII-32/70, GPS2, and ADA3 coactivate p53 transcriptional activity but do not partake in target selection.[53] These proteins play an important role in histone modification and chromatin remodeling, and also facilitate the recruitment of components of the transcription initiation machinery.

Interplay between post-translational modification and cofactor binding also determines cell fate decisions. S46 phosphorylation of p53 upon stress induces binding of Pin1 to its N-terminal domain, displacing iASPP from the core domain of p53 and thereby triggering apoptosis.[87] K120 acetylation inhibits binding of MDM2 as a corepressor and induces binding of p53 to apoptotic targets.[56] Association of p53 with p68 subunit of Drosha microRNA processor is also enhanced when p53 is acetylated on K120 and is important for nuclear primary miRNA processing of miRNA-203. Subsequently, miRNA-203 degrades antiapoptotic Bcl-w, leading to increased apoptosis.[88] Moreover, DNA damage induces K382 acetylation and S392 phosphorylation of p53, which augments the interaction of p53 with MDC1, a mediator of the DNA damage response.[89]

## Custodial Custody: miRNAs Regulating p53

MicroRNAs (miRNAs) act as insurance in the mesh of regulatory networks that maintain discipline at the molecular level. These endogenously expressed 20–25 nucleotide long RNAs can base pair to the 3'UTRs of target mRNA (mRNA), inhibiting their translation and/or affecting their stability. Interestingly, miRNAs both target and are targeted by p53. p53 takes a multivalent approach to the induction of miRNAs; it not only transactivates the miRNA coding genes but also has the potential to steer the processing machinery in favor of a particular miRNA family (e.g., mir-16–1, mir-143, and mir-145). It accomplishes this by enhancing the interaction of DEAD BOX RNA helicase p68 with the DROSHA complex, thereby facilitating the processing of microRNAs.[90] To further strengthen the network, p53 regulates miRNA target selection. p53 induces the RNA binding protein p38, which binds to the 3'UTRs of target mRNAs including p53 targets such as p21 and DR5 and obscures the miRNA binding site, thereby competing with miRNAs for binding and inhibiting their activity.[91]

miRNAs also play a key role in maintaining p53 levels (**Fig. 3**). miRNAs that positively regulate p53 include miR-29, which targets CDC42 (a Rho GTPase) and p85α (the regulatory subunit of phosphatidyl inositol 3 kinase) and increases p53 levels through a mdm-2–dependent mechanism.[92] miR-34 targets Sirt1 and potentiates p53 by inhibiting its deacetylation.[93] miR-542–3p contributes to p53 stability by weakening the interaction between p53 and MDM2.[94] miR-506 inhibits expression of the NF-κB p65 subunit, leading to ROS accumulation and subsequent p53 activation.[95]

miRNAs that negatively regulate p53 and further enhance the precision of the system include miR-21 that targets HNRPK, which is known to stabilize p53 by interfering with MDM2 activity. Downregulation of HNRPK thus results in increased MDM2-mediated ubiquitylation and degradation of p53.[96] miRNAs such as miR-125b, a brain enriched miRNA, can also directly target the 3'UTR of p53 and lead to its mRNA degradation. Upon genotoxic stress, miR-125b is downregulated to allow accumulation of p53.[97] The p53 3'UTR has 2 response elements for miR-504, and overexpression of miR-504 leads to reduced p53 protein levels and tumor suppressive functions.[98] miR-138 also targets the 3'UTR of *p53* mRNA, significantly reducing its expression.[99] More recently, miR-25, miR-30d,[100] and miR-19b[101] have also been recognized as negative regulators of p53 that bind directly to its 3'UTR. miRNAs thus play a significant role as agonists and antagonists of p53, allowing buffering of the p53 response and preventing extreme responses in its execution.

## Conclusion and Future Direction

Among the diverse stresses known to activate p53, genotoxic stress has achieved benchmark status in research endeavors to understand p53 regulation. Genotoxic drugs have been effectively used as a weapon to kill tumor cells by eliciting heightened p53 levels. p53, a savior for stressed cells and messenger of death for irreparable cells, is regulated in every probable dimension to make life and death decisions. An enhanced understanding of the kinetics of these regulatory mechanisms using systems biology approaches is an important approach that might bring clarity to

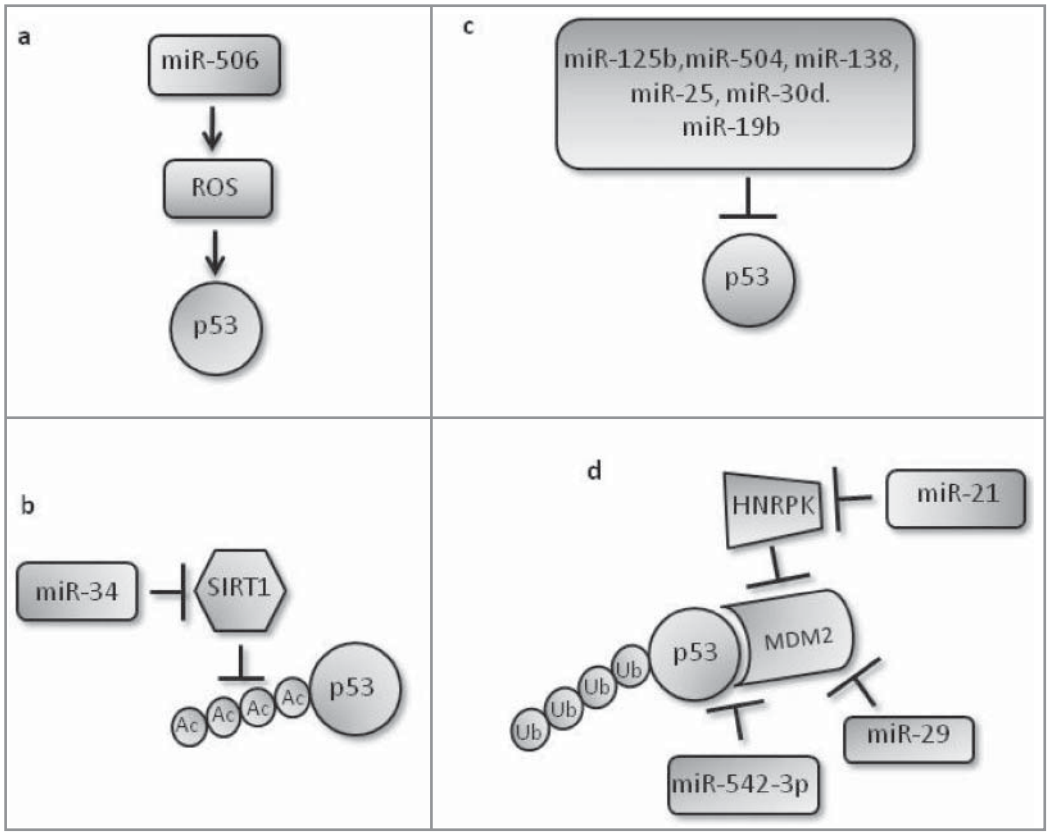


**Figure 3.** Various mechanisms by which miRNAs positively and negatively regulate p53 to maintain cellular homeostasis. (**a**) miR-506 leads to DNA damage via ROS generation and hence activates p53 through the DNA damage pathway. (**b**) miR-34 positively regulates p53 by inhibiting its deacetylase SIRT1. (**c**) Various miRNAs negatively regulate p53 through direct base pairing with its 3′UTR. (**d**) Several miRNAs target MDM2-mediated p53 ubiquitylation: miR-542–3p and miR-29 positively regulate p53 by targeting p53-MDM2 interaction and MDM2, respectively; miR-21 negatively regulates p53 by inhibiting HNRPK, an inhibitor of MDM2, leading to increased MDM2 activity and p53 ubiquitylation.

p53 regulation. Moreover, p53 regulatory mechanisms provide the opportunity to exploit its function in cancer cells by regulating the selectivity of p53 toward its target promoters. The emergence of p53 post-translational modifications and new interaction partners as important mediators of its functions lends credence to the search for small molecules that specifically target the enzymatic activities of regulators of post-translational modifications and the binding affinities of interaction partners. Such agents could be used in combination with genotoxic drugs for better therapeutic outcomes.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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