

Review

The multiple levels of regulation by p53 ubiquitination

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p53 is a central integrator of a plethora of signals and outputs these signals in the form of tumor suppression. It is well accepted that ubiquitination plays a major part in p53 regulation. Nonetheless, the molecular mechanisms by which p53 activity is controlled by ubiquitination are complex. Mdm2, a RING oncoprotein, was once thought to be the sole E3 ubiquitin ligase for p53, however recent studies have shown that p53 is stabilized but still degraded in the cells of Mdm2-null mice. Although the essential role of Mdm2 in p53 regulation is well established, there are an increasing number of other E3 ligases implicated in Mdm2-independent regulation of p53 by ubiquitination. The different types of ubiquitination on p53 by various E3 ligases have been linked to its differential effects on p53-mediated stress responses. In addition to proteasome-mediated degradation, ubiquitination of p53 acts as signals for degradation-independent functions, such as nuclear export. The function of ubiquitinated p53 varies in the nucleus and cytosol underlying the many potential contributions ubiquitinated p53 may have in promoting cell proliferation or death. Thus, p53 requires multiple layers of regulatory control to ensure correct temporal and spatial functions.

Cell Death and Differentiation (2010) 17, 86–92; doi:10.1038/cdd.2009.77; published online 19 June 2009

p53 Overview

p53, commonly referred to as ‘the guardian of the genome’, is a tumor suppressor that is capable of inducing cell senescence and apoptosis. The proliferation of oncogenic cells results in the repression of p53 expression and activation. A multitude of regulators and targets of p53 frames it as a central protein in a complex and divergent network involving numerous cellular pathways. The innate ability of p53 to regulate the cell cycle and activate apoptosis has led to p53 being extensively studied in the context of tumorigenesis and cell death.

Genotoxic stress, among others, induces p53 stabilization and activation setting off processes that result in the expression of various factors contributing to cell cycle arrest and death. On account of the rapid and concise p53 response to stressors, there is a need for tight regulations of p53 expression and activation for cells to respond appropriately to environmental stressors. Over the years, there have been identifications of numerous post-translational modifications associated with p53 and the role of each modification has been the subject of extensive studies.¹ For example, phosphorylation and acetylation of p53 have been shown to promote the expression of p53 transcriptional targets, whereas other modifications, such as ubiquitination, sumoylation, and neddylation have been associated with the suppression of p53-mediated transcription and p53 nuclear export. Methylation, in particular, has been associated with both the

enhancement and repression of p53 function, dependent on the specific site of methylation.² Many of these modifications have been shown to compete for lysine residues on p53, exhibiting an extraordinarily complex regulation of p53. p53 ubiquitination has been identified to regulate both p53 stability and localization and may be the master regulator of cell proliferation, death, or senescence.

Ubiquitination is associated with many different processes. Polyubiquitination, where four or more ubiquitin monomers are conjugated to a substrate, commonly targets it for proteasomal degradation. We now know that the specific linkage between the ubiquitins is important in regulating the eventual function of the ubiquitination. For example, substrates with K48-linked ubiquitination are unequivocally targeted to the proteasome for degradation, whereas substrates with K63-linked ubiquitination are linked to many other functions, including aggresome formation, lysosomal degradation, and protein–protein interactions.³ Furthermore, mono-ubiquitination and multiple mono-ubiquitination have been linked to almost everything in the cell, including histone regulation, endocytic trafficking, and inclusion body formation.^{3,4}

Mdm2/MdmX Regulation of p53 Ubiquitination

Mdm2 is a RING finger domain containing protein that exhibits E3 ubiquitin-protein ligase activity and is capable of regulating its own levels through auto-ubiquitination. Mdm2 was first identified to regulate p53 stability and was later shown to be

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Keywords: p53; Mdm2; Mdmx; HAUSP; ubiquitination; apoptosis

Abbreviations: Mdm, mouse double minute; ARF-BP1, ARF binding protein 1; HAUSP, herpesvirus-associated ubiquitin-specific protease; CHIP, C-terminus of Hsc70-interacting protein; MSL, male-specific lethal; CBP, CREB-binding protein; RING, really interesting gene; HECT, homologous to E6-AP C-terminus

Received 09.3.09; revised 07.5.09; accepted 07.5.09; Edited by J Silke; published online 19.6.09

the major E3 ubiquitin-protein ligase that effectively regulates p53 ubiquitination through its E3 ligase activity.^{5–8} Mdm2, in conjunction with E2 ubiquitin-conjugating enzyme, Ubc5, is capable of ubiquitinating p53 both *in vitro* and *in vivo*. The major lysine residues that are ubiquitinated by Mdm2 have been narrowed down to six lysines in the C-terminal of p53, K370, K372, K373, K381, K382, and K386.⁹ Although the mutations of these lysine residues to arginine, collectively referred to as 6KR, do not eliminate p53 ubiquitination, it largely prevents the degradation of p53 and modulates p53 activation as a transcriptional regulator. Moreover, it was shown that several lysines in the DNA-binding domain were also critical for p53 ubiquitination; however, the functions of these specific lysine ubiquitinations are unknown.¹⁰ Conversely, many of the same sites that are ubiquitinated by Mdm2 can also be acetylated by p300/CBP leading to p53 activation.¹¹ Acetylation of p53 is essential for the binding of p53 to p53-binding elements promoting various gene transcriptions, including *p21*, *PUMA*, *BAX*, and *Noxa*, which result in apoptosis or cell cycle arrest effectively halting tumorigenesis.¹² However, when Mdm2 ubiquitinates p53, p53 cannot be acetylated by p300/CBP and result in rapid proteasome-mediated degradation.¹¹ Interestingly, p300/CBP not only acetylate p53, but they can also acetylate Mdm2 and prevent Mdm2 from being able to ubiquitinate p53.¹³ The activation of p53 and inactivation of Mdm2 E3 ligase activity jointly prevent Mdm2-dependent ubiquitination of p53. Furthermore, Mdm2 expression is also regulated in a p53-dependent manner, thus the increased level of p53 will induce Mdm2 expression resulting in a reduction of p53 expression and activation creating a negative feedback loop.¹⁴ The complex regulation of p53 activation has on the Mdm2-mediated p53 ubiquitination and degradation is further reviewed in greater details in the following papers.^{15–17}

Genotoxic stress can induce high levels of p53 promoting the expression of many proteins associated with apoptosis and cell cycle arrest to counteract the stressors causing DNA damage. On the contrary, if p53 is overactive, the cell will die, so the p53-mediated increase in Mdm2 expression level helps to regulate p53 levels by polyubiquitination and targeting p53 for proteasomal degradation. However, Mdm2 can both mono- and polyubiquitinate p53 depending on Mdm2 protein levels,¹⁸ adding an extra layer of regulation to the Mdm2-p53 negative feedback loop. Furthermore, only the polyubiquitinated form of p53 is associated with p53 destabilization and proteasomal degradation, whereas the mono-ubiquitinated form of p53 is targeted for nuclear export.

One known Mdm2 regulator is ARF, a tumor suppressor known to be activated during the cellular stress and capable of promoting p53-dependent and independent apoptosis.^{19,20} ARF plays a central role in inducing p53 activity by inhibiting the function of two E3 ligases, Mdm2 and ARF-BP1.^{21,22} Although it was suggested that ARF recruits Mdm2 to the nucleolus,²³ an elegant study showed that ARF need not be in the nucleolus to bind Mdm2 and activate p53 activity.²⁴ In addition, it was shown that both Mdm2 and ARF-BP1 knockdown can further increase p53 levels in the presence of ARF suggesting both Mdm2 and ARF-BP1 are the links between ARF and p53.²¹

Another regulator of the Mdm2-p53 network is MdmX, a Mdm2 homolog (Figure 1). Mdm2 and MdmX are structurally similar with the same domains although retaining a high homology. Both Mdm2 and MdmX interact through their RING domains to form heterodimers,^{25,26} however, MdmX lacks a nuclear localization signal (NLS) and nuclear export sequence (NES) suggesting this interaction is critical for MdmX to travel to and from the nucleus. MdmX and Mdm2 can dynamically bind to p53 at the transactivation domain and prevent p53 target genes from being transcribed²⁷ (Figure 3). Although both Mdm2 and MdmX have C-terminal RING domains, the MdmX RING domain does not have E3 ligase activity. Mdm2 is also capable of ubiquitinating MdmX to regulate its expression level,²⁸ whereas MdmX and Mdm2 can synergistically enhance p53 ubiquitination and degradation.²⁹ Genetic knockout mouse models of both Mdm2 and MdmX have been shown to be embryonic lethal.^{30–32} p53 depletion rescues both knockout lines and allows for survival suggesting important roles for both Mdm2 and MdmX in regulating p53. This evidence also supports a physiological role for Mdm2 in regulating p53 function. Although MdmX also seems to be critical in the regulation of p53, only Mdm2 knockout mice exhibited increased p53 levels further supporting an *in vivo* Mdm2 function in regulating p53 stability.^{33,34} The further role of MdmX in p53 regulation still needs to be established to understand the essential role MdmX has on the Mdm2-p53 network.

After ubiquitination has served its purpose in targeting p53 for various functions, deubiquitinating enzymes remove ubiquitin from p53. HAUSP (USP7) was identified to deubiquitinate p53 in the nucleus resulting in p53 stability.³⁵ By preventing p53 degradation, there is an increase in cell arrest and apoptosis when HAUSP is overexpressed. HAUSP may also play a role in the cytosol by deubiquitinating mono-ubiquitinated p53 and affect p53 function.³⁶ Recently, HAUSP was shown to form a complex with Mdm2 and p53 resulting in the stabilization of p53, by deubiquitinating p53 *in trans*.³⁷ In addition to p53 deubiquitination, Mdm2 deubiquitination is also regulated by HAUSP.³⁸ HAUSP-mediated deubiquitination can result in both the increase in Mdm2 levels promoting p53 degradation and the increase in p53 levels directly. Further studies examining the exact temporal relationship among Mdm2, p53, and HAUSP would help to show the complex ubiquitination and deubiquitination mechanisms *in vivo*.

Mdm2-Independent Ubiquitination

The importance of Mdm2 as a bonafide E3 ubiquitin ligase for p53 is indisputable, however, there have been data suggesting Mdm2-independent ubiquitination may also be involved in p53 stability. Initially, this was shown in an experiment where p53/Mdm2 null MEF cells were transfected with p53 along with siRNA against ARF-BP1 and was able to show a significant increase in p53 level suggesting p53 may be stabilized by the knockdown of another E3 ligase.²¹ Moreover, an elegant study by another group was able to show Mdm2-independent mechanisms exist *in vivo* for p53 degradation.³⁹ They generated a mouse model with a p53ER inducible system in a Mdm2 null background and was able to achieve a

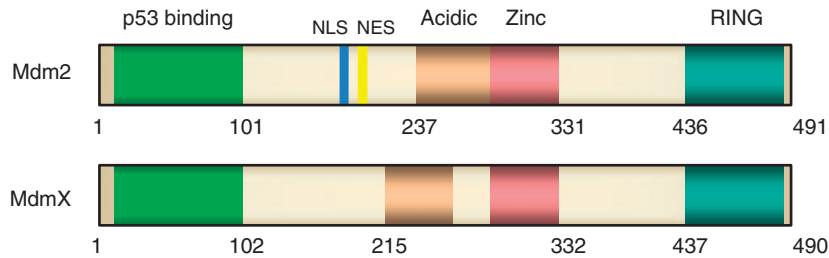


Figure 1 Domain structures of Mdm2 and MdmX. Domains: p53 binding domain, acidic domain, zinc finger domain, and RING finger domain. NLS, nuclear localization signal; NES, nuclear export sequence

similar p53ER level to endogenous p53 protein while maintaining inactivity during development allowing for the survival of the mice. When tamoxifen was administered to these mice, the p53ER is activated and translocated to the nucleus where it was shown to gradually degrade in a time-dependent manner suggesting Mdm2-independent mechanisms are actively responsible for p53 turnover in the nucleus.

Before the identification of Mdm2 as a critical E3 ligase for p53, the E3 ligase complex HPV-16 E6 and E6-AP was found to mediate p53 ubiquitination *in vitro*.⁴⁰ However, the next E3 ligase identified after Mdm2 to mediate p53 ubiquitination was Pirh2.⁴¹ The discovery of Pirh2 opened the gates for the characterization of a plethora of E3 ligases involved in Mdm2-independent p53 ubiquitination (Figure 2). Most of the E3 ligases are RING-type, including Mdm2 and Pirh2, whereas ARF-BP1 and WWP1 are HECT-type. E4F1 and Ubc13 are interesting E3 ligases because they lack a typical RING or HECT domain. The further analyses of these domains that coordinate p53 ubiquitination will help to identify the specific mechanism of ubiquitination. Although these E3 ligases are capable of mediating p53 ubiquitination *in vitro* and *in vivo*, genetic evidence is lacking to show their role of being anything more than accessories to Mdm2.

The many E3 ligases capable of ubiquitinating p53 suggest that the cell may have redundant regulations on p53 degradation, however, further analyses of these ubiquitination events show the specificity in place for p53 ubiquitination. Several E3 ligases, in addition to Mdm2, can mediate K48-linked polyubiquitination of p53 and target it to the 26 S proteasome for degradation. Other types of ubiquitination, including mono- or K63-linked polyubiquitinations, can lead to nuclear export and cytosolic localizations resulting in p53 stabilization. Ubiquitination can also disrupt p53 from binding to target gene recognition sequences in the nucleus that result in apoptosis and cell cycle arrest.

Proteasomal Degradation-Dependent p53 Ubiquitination

Non-ubiquitinated p53 is normally kept at a very low basal level in healthy cells. During tumorigenesis, p53 levels increase to slow down the progression of tumor growth. p53 is then activated through various kinases and acetyltransferases allowing for the binding of p53 to transcriptional targets, such as *p21*, *PUMA*, *BAX*, and *Noxa* (Figure 3).^{12,17,42} These targets will transcribe proteins that lead to the attenuation of tumor growth through cell senescence, cell cycle arrest, and/or apoptosis. Ubiquitination of p53, as discussed above, results in either targeting for proteasomal

degradation or for nuclear export and is a highly regulated process. In general, ubiquitinated p53 can decrease the target gene transcription levels. How does p53 ubiquitination regulate this? In the cases where p53 is polyubiquitinated and targeted for degradation, it is presumed that p53 levels are returned to the basal level of healthy cells. Decreased p53 levels and mutations in p53 have been linked to high incidences of cancer, thus p53 levels being elevated act as a mechanism of tumor suppression.⁴³ Mdm2-dependent and independent ubiquitinations that target p53 for degradation are considered oncogenic and prevent p53 from regulating the cell cycle or inducing apoptosis.

Although Mdm2 is the major E3 ligase responsible for regulating p53 polyubiquitination and targeting for proteasomal degradation, there are also Mdm2-independent ubiquitination performing similar regulation on p53. Topors can mediate p53 polyubiquitination and decrease p53 expression levels, however, the functional consequences of Topors regulation has yet to be determined.⁴⁴ Pirh2 and COP1 mediate Mdm2-independent p53 ubiquitination and target it for proteasomal degradation, whereas decreasing p53-mediated cell arrest as measured by p21-Luc activity.^{41,45} CARP1 and CARP2 can also polyubiquitinate p53, which reduces p53-transactivation of a PG12-Luc, a p53 reporter gene. CARPs can also specifically degrade phospho-S20, an activated p53, and unmodified p53 in stressed cells.⁴⁶ Furthermore, Pirh2 and COP1 overexpressions can rescue p53-mediated toxicity and Pirh2, COP1, and CARP2 knock-down induced a greater G1/S ratio. Pirh2 and COP1 are also transcriptional targets for p53 forming a negative-feedback loop with p53 similar to Mdm2.

As mentioned earlier, ARF can inhibit both the functions of Mdm2 and ARF-BP1. ARF was recently identified to regulate Mdm2-independent tumor suppression through ARF-BP1.²¹ N-terminal ARF can specifically block ARF-BP1 auto-ubiquitination and ARF-BP1-mediated p53 ubiquitination, whereas the knockdown of ARF-BP1 can induce an increase in sub-G1 phase. This supports a novel role of ARF regulation of p53 through ARF-BP1, however, it needs to be addressed the significance of ARF having redundancy in its function with ARF-BP1 and Mdm2.

Recently, synoviolin, an endoplasmic reticulum (ER) resident protein, linked p53 to ER-associated degradation (ERAD) by sequestering p53 to the ER.⁴⁷ p53 is polyubiquitinated by synoviolin both *in vivo* and *in vitro*, destabilizes p53, and requires ERAD as a mechanism to transport polyubiquitinated p53 to the proteasome for degradation. The knockdown of synoviolin was able to increase p53 levels, binding to

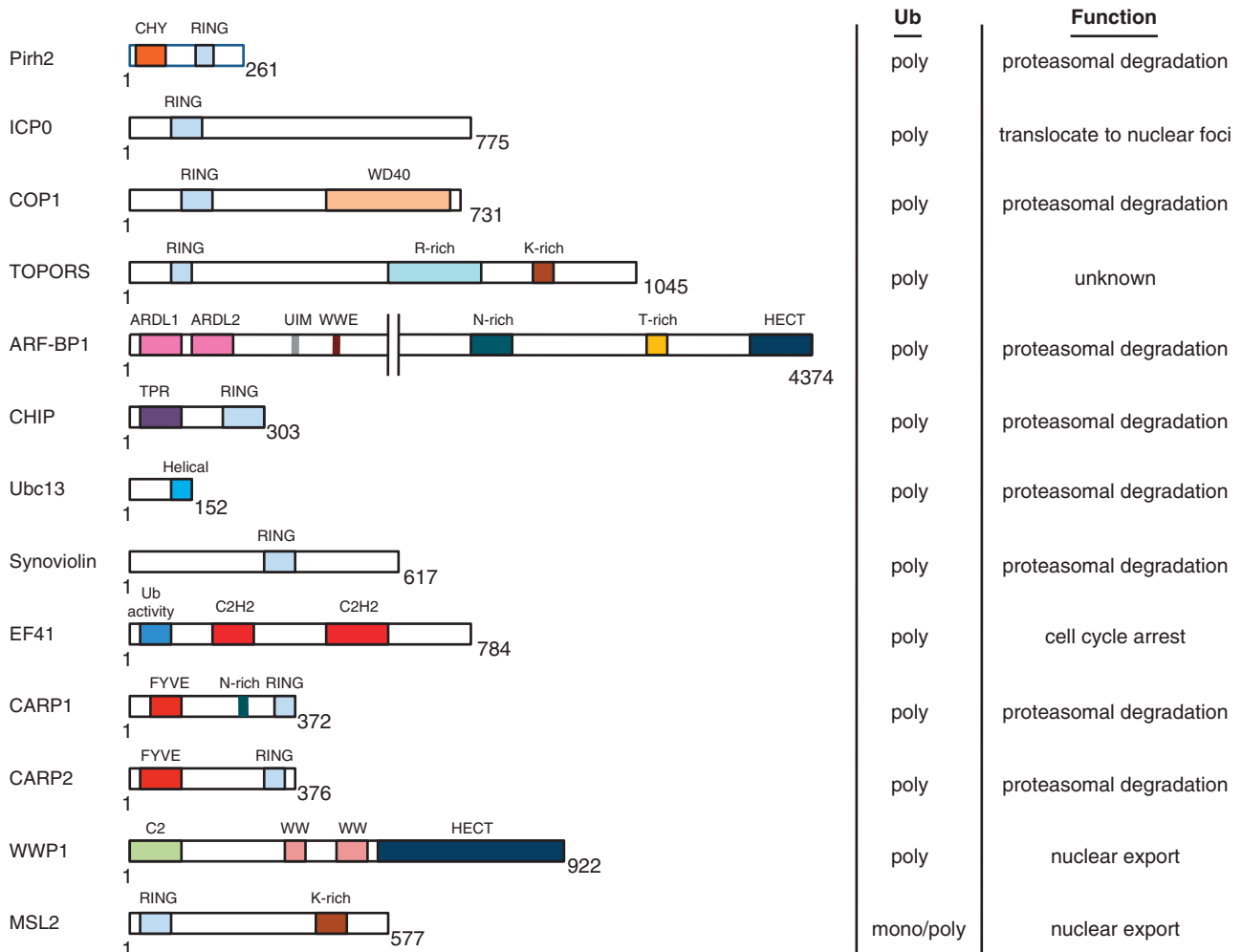


Figure 2 E3 ligases involved in Mdm2-independent p53 ubiquitination. Domains, ubiquitination type, and function of E3 ligases that regulate Mdm2-independent p53 ubiquitination. CHY, cysteine/histidine zinc finger; RING, RING finger domain; R-rich, arginine-rich domain; K-rich, lysine-rich domain; ARDL, Armadillo-like domain; UIM, ubiquitin-interacting motif; WWE, conserved sequence of two tryptophan residues followed by a glutamine residue; N-rich, asparagine-rich; T-rich, threonine-rich; HECT, HECT domain; TPR, tetratricopeptide repeat domain; Helical, helical region; Ub activity, required for ubiquitin ligase activity; C2H2, cysteine2/histidine2 zinc finger; FYVE, FYVE ring finger domain; C2, protein kinase C conserved region 2; WW, two conserved tryptophans domain; Ub, ubiquitination

p53-transcriptional targets, and cells in sub-G1 phase, supporting an antiapoptotic function for synoviolin. Further investigation as to the novel role of p53 in the ER would shed light on functional consequences of synoviolin-mediated p53 ubiquitination.

Some E3 ligases need to form complexes to mediate p53 ubiquitination. CHIP, an E4/E3 ligase, may participate in the degradation of p53 by recruiting other proteins, such as Hsp70.⁴⁸ Another E3 ligase complex of E4orf6 and E1B55K has also shown to ubiquitinate p53 *in vitro*.⁴⁹ Identification of their roles *in vivo* would provide evidence of the physiological roles of CHIP and E4orf6/E1B55K complexes in p53 ubiquitination.

Proteasomal Degradation-Independent p53 Ubiquitination

The ubiquitin linkages are responsible for the functional consequences of ubiquitination. As previously discussed, K48-linked ubiquitination targets p53 for degradation, whereas

mono-ubiquitination is linked to a multitude of proteasome-independent functions.^{3,4} Some E3 ligases involved in proteasome-independent p53 ubiquitination include Ubc13, WWP1, E4F1, and MSL2 (Figure 2). In addition to these E3 ligases, low levels of Mdm2 can also mediate proteasome-independent p53 ubiquitination by mono-ubiquitinating p53.¹⁸

Ubc13, an E2 conjugating enzyme, was shown to exhibit E3 ligase activity and was capable of mediating K48 and K63-linked p53 ubiquitination.⁵⁰ Ubc13 overexpression stabilized p53 suggesting proteasome-independent ubiquitination is responsible for the longer half life. Interestingly, K63R ubiquitin mutant ubiquitination of p53 led to a decrease in p53 stability possibly mediated through K48-linked ubiquitination. Ubc13 also promoted cytosolic localization of p53 and attenuated p53-induced apoptosis. Further investigation to the specific role of Ubc13-mediated p53 ubiquitination on p53-regulated transcription of gene targets or effects on apoptosis would shed light to the role of Ubc13 as a novel E3 ligase in cells.

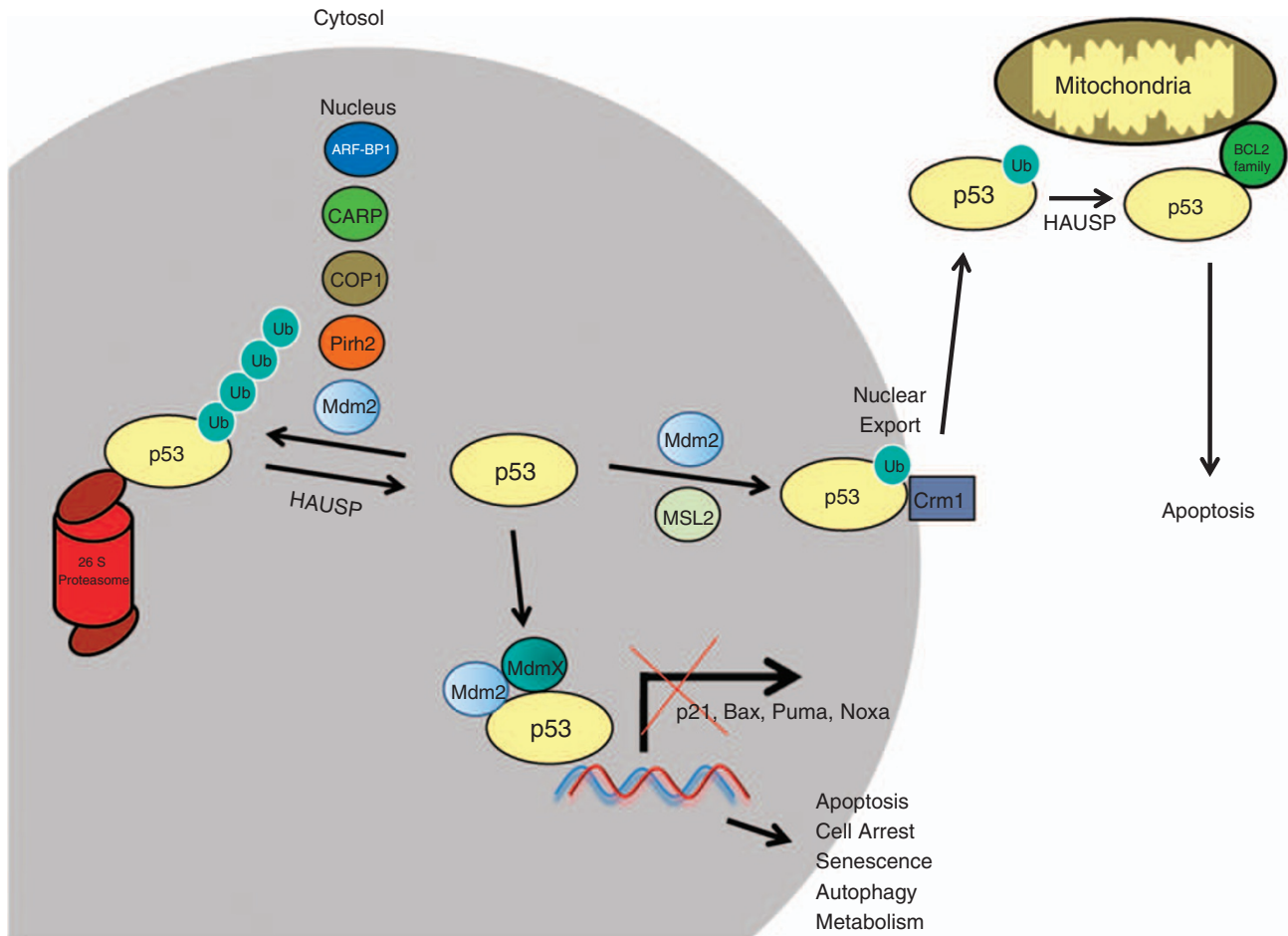


Figure 3 Ubiquitinated p53 functions and pathways. Ub, ubiquitin

WWP1 is also capable of polyubiquitinating p53. Although the ubiquitin linkage that regulates WWP1-mediated p53 ubiquitination is unknown, ubiquitinated p53 seems to be stabilized.⁵¹ In addition to stabilizing p53 levels, WWP1 overexpression causes a translocation of a portion of p53 from the nucleus to the cytosol. Besides p53 levels being regulated by WWP1, WWP1 is also regulated by p53 after genotoxic stress. These changes are similar to another p53 transcriptionally regulated protein survivin suggesting that WWP1 may also be transcriptionally regulated by p53 similar to Mdm2, COP1, and Pirh2. Although there is a correlation between WWP1 expression with WWP1-mediated p53 ubiquitination and p53 translocation to the cytosol, the identification of the ubiquitin-linkage mediating p53 stabilization in the cytosol may help identify the role of WWP1-mediated ubiquitination.

More recently E4F1, an atypical E3 ligase, was shown to ubiquitinate p53 mainly through a K48-linkage.⁵² E4F1, a transcriptional factor, does not contain a HECT or RING domain although its E3 ligase activity is dependent on amino acids 41–85, which share homology to SUMO E3 ligase RanBP2 catalytic core domain. Although E4F1 mediates K48-linked ubiquitination, this ubiquitination does not resemble typical polyubiquitination with high-molecular-

weight smears, but instead exhibits a mono-, di- or tri-ubiquitination. E4F1-mediated ubiquitination of p53 does not affect its stability or target it for nuclear export, but instead increases its localization to the chromatin fraction. This correlates with a selective enhancement in p21 and cyclin G1 transcription resulting in cell cycle arrest at G0/G1. Activation of growth arrest also prevents apoptosis and inhibits E4F1-mediated p53 ubiquitination after UV exposure. This suggests that E4F1-mediated p53 ubiquitination may regulate a very specific p53 function through its K48-linked oligo-ubiquitinated p53. Furthermore E4F1 ubiquitinates K320, a PCAF acetylation target, which suggests that E4F1 ubiquitination competes with PCAF acetylation for the same lysine residue providing a highly regulated response to UV exposure and subsequent DNA damage. Another group generated a K317R knock-in mouse, the corresponding residue to human K320 that is also acetylated by PCAF.⁵³ The K317R knock-in mouse exhibited increased levels of apoptosis after ionizing radiation, suggesting the lysine residue was essential for survival and does not require acetylation of K317 to activate apoptosis. These data would suggest that K317/320 residue is involved in both apoptosis and cell cycle arrest, which is a discrepancy that requires further investigation.

MSL2, an E3 ligase that is part of the mammalian MSL complex, was recently identified as a novel E3 ligase that mediates p53 ubiquitination.⁵⁴ MSL2 specifically ubiquitinates K351 and K357 residues and targets p53 for nuclear export, similar to Mdm2-dependent p53 mono-ubiquitination. p53^{QS} mutant, containing a QS mutation that normally prevents target gene transactivation and nuclear export by abrogating Mdm2 binding, was unable to prevent MSL2 from targeting it to the cytosol. MSL2 is capable of ubiquitinating both wild-type p53 and p53^{QS} mutants in the form of both mono-ubiquitination as well as polyubiquitination. Similar to E4F1-mediated p53 ubiquitination, MSL2 does not affect p53 stability. MSL2 relocalization of ubiquitinated p53, in an Mdm2-independent manner, may contribute to potential functions in the cytosol.

As previously described, low levels of Mdm2 have been shown to induce mono-ubiquitination and target p53 for nuclear export.¹⁸ p53 C-terminal ubiquitination mediated by Mdm2 was identified to be essential for the nuclear export.⁵⁵ A p53-ubiquitin fusion protein, with an ubiquitin fused to the C-terminus, was shown to localize mainly in the cytosol¹⁸ suggesting ubiquitin is somehow capable of exposing the NES that is normally masked when p53 is in a tetramer.⁵⁶ Subsequently, the NES is recognized by CRM1, a nuclear export regulator, and exported to the cytosol (Figure 3). There are two potential ways this could occur: (1) the ubiquitin could cause p53 to dissociate into monomers, thus exposing the NES; (2) the ubiquitin itself can change the p53 conformation in the C-terminal region and expose the NES. It was recently shown that ubiquitination does not affect tetramerization,⁵⁷ deducing the mechanism of nuclear export must involve the conformational change of the C-terminal to expose the NES. Indeed evidence points to the importance of ubiquitination of lysines in the C-terminus in exposing the NES allowing for proper nuclear export.⁵⁸ This hypothesis clearly elucidates the Mdm2-dependent and independent p53 mono-ubiquitination mechanism to induce nuclear export, however, the function of mono-ubiquitination in regulating p53 activity is still inconclusive.

Identifying the potential function of p53 in the cytosol is becoming an important endeavor to fully understand the role of mono-ubiquitinated p53 after nuclear export. p53 has been linked to transcription-independent signaling that also result in apoptosis. This apoptosis is carried out in the mitochondria and there have been several reports linking p53 directly with the mitochondrial apoptotic pathway.

The first report identified an interaction between p53 and BclXL, a Bcl2-family member, at the mitochondria, which promotes cytochrome c release.⁵⁹ Cytochrome c is released into the mitochondria after mitochondrial membrane integrity is compromised and subsequently activates the caspase cascade leading to apoptosis. BclXL, part of a BH3 complex, is a pro-survival outer membrane mitochondrial protein that functions to prevent cytochrome c release. The overexpression of BclXL can suppress p53-dependent apoptosis. When recombinant p53 was incubated with isolated mitochondrion, cytochrome c was released suggesting p53 and its interaction with BclXL is responsible for the cytochrome c release. Another protein, Bak, a pro-apoptotic Bcl-2 family member, was also identified to interact directly with p53 and permeabilize the mitochondrial outer membrane releasing

cytochrome c,⁶⁰ whereas BAX, another Bcl-2 family member, was shown to oligomerize in the presence of p53 resulting in cytochrome c release.⁶¹ Although p53 is capable of interacting directly or indirectly with these Bcl2-family members and induce cytochrome c release, it is yet unknown whether ubiquitinated p53 is required for these events to occur *in vivo*. Recently, it was shown that mitochondrial HAUSP can deubiquitinate p53 allowing for interaction with BclXL,³⁶ however, the abundance of mitochondrial HAUSP needs to be further investigated as HAUSP is mainly localized in the nucleus.⁶²

There may be other roles cytosolic ubiquitinated p53 may play in regulating cell fate. Recently, cytoplasmic p53 was linked to the mTOR pathway resulting in autophagy⁶³ and was also shown to directly regulate autophagy.⁶⁴ Moreover, p53 ubiquitination is enhanced by binding to nonactive JNK, whereas the activation of the JNK signaling pathway can stabilize p53.^{65,66} A more likely role of ubiquitinated p53 may be to recruit other proteins to p53 and regulate further post-translational modifications on p53 and its regulators,^{67,68} which then modulate p53 activity to promote either tumorigenesis or tumor suppression. Further research into the regulation of p53 ubiquitination in relationship to its different interacting partners and post-translational modifications will provide better insights into the regulations of E3 ligases and deubiquitinating enzymes in respect to p53 and tumorigenesis.

Acknowledgements. This study was supported by grants from NIH and the Leukemia and Lymphoma Society. JL is supported by NIH cancer biology training grant T32-CA09503. WG is an Ellison Medical Foundation Senior Scholar in aging.

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