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Review

The role of ubiquitin modification in the regulation of p53☆

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ABSTRACT

The p53 tumor suppressor protein is involved in regulating a wide variety of stress responses, from senescence and apoptosis to more recently discovered roles in allowing adaptation to metabolic and oxidative stress. After 34 years of research, significant progress has been made in unraveling the complexity of the p53 network, and it is clear that the regulation of p53 protein stability is critical in the control of p53 activity. This article focuses on our current understanding of how the level and activity of p53 is controlled by this seemingly simple mechanism. This article is part of a Special Issue entitled: Ubiquitin–Proteasome System. Guest Editors: Thomas Sommer and Dieter H. Wolf.

1. Ubiquitination — a complex signal

While ubiquitination was initially identified as the key mechanism in marking misfolded or surplus protein molecules for degradation, it soon became clear that it is far more than just a general mechanism to mark obsolete proteins for degradation. Indeed, ubiquitination is now recognized as a highly regulated, flexible and reversible process that can signal multiple responses, from degradation to changes in activity, re-localization or changes in the histone code. This high bandwidth in signaling power is achieved by the complex nature of the ubiquitin signal itself, which reflects not only the position of the ubiquitin mark on the substrate protein, but also the length and architecture of the ubiquitin chain.

While conjugation of a single ubiquitin to a target protein can provide a signaling tag (for example to alter subcellular localization or mark membrane proteins for recycling), the formation of ubiquitin chains provides greater diversity in signaling potential. Ubiquitin modifications are assembled by a hierarchical cascade comprising ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin-ligating enzymes (E3) [1]. The E3 ligase is responsible for substrate and target lysine specificity, and also determines the linkage type within the poly-ubiquitination chain, aided to some extent by the E2 enzyme [2].

Ubiquitin can be interlinked via any of its lysines (K6, K11, K27, K29, K33 K48 and K63) and through the amino terminal methionine. The best-characterized poly-ubiquitin chain is formed via K48 linkages. Chains of four or more ubiquitin molecules interlinked via K48 lead to degradation of the marked protein [3]. In contrast, poly-ubiquitination via K63 predominantly regulates signaling through other pathways that can lead, for example, to NF-κB activation [4], the regulation of different steps in the DNA repair program [5] and the control of membrane trafficking [6,7]. In addition to this, K63 ubiquitin chains may also act as a 26S proteasome targetting signal.

Other poly-ubiquitin chains (reviewed in [8]) include linkages via the other lysines in ubiquitin, branched chains formed through the use of mixed lysines and linear ubiquitin chains linked through the N-terminal methionine and the C-terminal glycine of adjacent ubiquitins. The functional outcome of these modifications — some of which may be relatively abundant — is less well understood, although they have been shown to drive signaling, provide novel binding sites for partner proteins and target proteolysis. Overall, the impact of these modifications on substrate proteins is likely to be profound, and their identification has opened new and exiting areas of research.

2. p53: it's all about stability

The primary function of p53 is as a transcription factor, activating and repressing the expression of a large number of target genes [9]. Non-transcriptional activities of p53, for example in the regulation of apoptotic signals at the mitochondria, have also been described [10]. In healthy cells, p53 plays a pivotal role in responding to oncogenic stress signals and helps to keep cells metabolically stable [9]. The importance of p53 is highlighted by the fact that it is frequently altered in human cancers [11,12], indeed even tumors that retain wild type p53 are often compromised in their ability to activate the p53 pathway. Acute activation of p53 leads to numerous responses that prevent further cell division, including cell cycle arrest,

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senescence and apoptosis. In this way, p53 can prevent the outgrowth of incipient cancer cells. However, these activities of p53 must be carefully regulated under normal unstressed conditions to allow cell growth and division. Various aspects of p53 expression, subcellular localization and activity are actively regulated, and a large number of post-translational modifications on p53 have been shown to modulate these functions [13]. Key to the regulation of p53 is the control of the stability of the p53 protein, orchestrated mainly through a network of ubiquitination reactions (Fig. 1). In addition, there is evidence of continuous degradation of p53 by the 20S core catalytic chamber of the proteasome, which can be inhibited by the detoxifying enzyme NQO1 (reviewed in [14]). This relationship links the stress response to ROS (which induces NQO1 expression) with stabilization of p53, which is itself a potent regulator of intracellular ROS. However, while this mechanism of p53 degradation depends on the proteasome, it is independent of ubiquitin.

Constantly cycling between producing and then degrading p53 is an energy costly way of maintaining low levels of p53, but it allows for a very short response time after a stress signal. The p53 pathway therefore remains poised to an extent that would not be possible if p53 induction depended on regulated transcription, splicing, translation and folding. Spontaneous pulses of p53 accumulation can be detected in normal proliferating cells, although these do not reach a threshold necessary for full activation of a p53 response [15]. This system has been proposed to allow growth under normal conditions while ensuring a rapid reaction to stress that might otherwise prove harmful to both the cell and – ultimately – the whole organism.

3. MDM2/MDMX

A key negative regulator of p53 is MDM2 and its close homolog MDMX. Complete loss of either MDM2 or MDMX results in an early embryonic lethality that is p53 dependent [16–18], demonstrating the importance of these regulators of p53 function. While the lethality of both mice indicates that the activities of MDM2 and MDMX are not redundant, MDMX deficient animals can be rescued by overexpression of MDM2, suggesting some overlap in function [19], although the absence of MDM2 cannot be compensated by overexpression of MDMX [20]. Furthermore, loss of MDMX appears to be somewhat less deleterious than loss of MDM2, with some adult tissues showing no phenotype following MDMX deletion [21].

Both MDM2 and MDMX bind to the N-terminal transactivation domain of p53, and can inhibit p53's transcriptional activity directly by blocking the binding of co-activators such as p300 and recruitment of repressors such as histone deacetylases and lysine methyltransferases [22–24]. MDM2 binding has also been shown to promote a conformational shift in p53, rendering it unable to bind DNA and so carry out its normal transcriptional activities [25,26]. However, much more efficient regulation of p53 activity is achieved by the ability of the MDM2 to function as a RING finger E3 ligase and target p53 for degradation [27,28]. While MDM2 can homodimerize and poly-ubiquitinate p53, at physiological concentrations the MDM2 homo-dimer seems to predominantly mono-ubiquitinate p53 [29]. MDMX also contains a RING domain, and although it has no intrinsic ubiquitin E3 ligase activity, MDM2 and MDMX dimerize efficiently through RING/RING interactions. Importantly, this heterodimerization of MDM2 and MDMX plays an important role in the regulation of p53 stability, at least in the embryo [30,31]. So, although both MDM2 and MDMX can exert independent regulation on p53, there is growing evidence to support the idea that MDMX contributes to the degradation of p53, and that the MDM2/MDMX complex constitutes the principal active E3 ligase for p53 [29]. MDM2 modifies p53 predominantly on six lysine residues located at the C-terminus of the protein (K370, K372, K373, K381, K382, and K386 [32]) to target it for degradation. Both the RING domains and C-terminal tails of MDM2 and MDMX are critical for this activity [33], and either deletion [34] or extension [35] of the MDM2 tail substantially inhibits E3 activity. The exact role of the C-terminal tail is not fully established, although by analogy with other RING domain E3s it seems possible that the tail of MDM2 or MDMX docks into the RING of the partner protein in the dimer, to form a binding site for the ubiquitin loaded E2 [36]. Finally, MDM2 is also involved in the subsequent post-ubiquitination step that brings p53 to the proteasome [37].

Interestingly, despite the clear evidence supporting a role for MDM2/MDMX in the negative regulation of p53 activity, a number of studies suggest that under some circumstances p53 function could be stimulated by MDM2 or MDMX. MDM2 can bind p53 mRNA, resulting in enhanced p53 expression — an activity that also depends on the RING domain of MDM2 [38]. An MDM2 RING domain point mutant, which lacks E3 activity, serves to enhance p53's activity towards several target genes by enhancing the recruitment of p300 — a transcriptional co-activator [39].

It is also important to remember that MDM2/MDMX specific ubiquitination of p53 does not necessary lead to degradation of p53, but can have different outcomes depending on the chain length and chain linkage. Lower levels of MDM2, or maybe the availability of MDM2 homodimers, causes mono ubiquitination [29] and nuclear export of p53 [40]. MDMX can also independently help to promote the stabilization of cytoplasmic p53 in an active conformation [41]. Interestingly the accumulation of cytoplasmic p53 is an activity that is exhibited by several E3s (see below and Table 1), consistent with the importance of the regulation of p53's subcellular localization in the control of the p53 response. Clearly, removal from the nucleus inhibits p53's transcriptional activity, and once in the cytoplasm, p53 can be further ubiquitinated and degraded by p300, an E4 ligase, as discussed later. However, a number of different functions for cytoplasmic p53 have also been described that play a positive role in regulating processes such as apoptosis, autophagy and metabolism [42–44]. Cytoplasmic p53 also interacts with the ubiquitin ligase CUL9/PARC [45], resulting in the cytoplasmic sequestration of p53. However, this activity is not dependent on ubiquitination of p53, and again leads to enhanced apoptosis [46].

MDM2/MDMX can also promote the modification of p53 with other ubiquitin-like proteins. Neddylation by MDM2 occurs on three C-terminal lysines (K370, K372, K373) of p53, resulting in the inhibition of transcriptional activity [47] and nuclear export [48]. However, this modification does not seem have a significant effect on the degradation of p53. As with ubiquitination, the MDM2/MDMX heterodimer seems to be the preferred Nedd8 E3 ligase complex and MDMX can rescue E3 ligase deficient point mutations of MDM2 [49]. Apart from MDM2/MDMX, FBXO11 has also been reported to modify p53 with Nedd8, again leading to reduced transcriptional activity of p53 [50].

p53 is also modified specifically on lysine 386 with the small ubiquitin like modifier SUMO, with evidence that various SUMO E3s, including the PIAS family and Topors, can target this modification of p53 [51]. Interestingly, MDM2 has also been shown to promote both the SUMO-1 [52] and SUMO-2/3 conjugation of p53 [53], in a process that does not require the RING domain of MDM2 and which can be further increased by MDM2 binding proteins like p14ARF and L11 [52,54]. The consequences of p53 SUMOylation remain unclear, with evidence for both a promotion and inhibition of transcriptional activity [54–56] and regulation of subcellular localization [52,54,57,58]. Overall, only a small fraction of p53 (probably less than 5%) is found to be modified by SUMO-1 at a steady state in cells [55,59,60] and the overall outcome of SUMOylation on p53 is likely to be dependent on the context of other modifications of p53 [57] and the choice of experimental model.

Taken together, therefore, it seems clear that MDM2 and MDMX can modulate p53 through several mechanisms, both independently and working in partnership. Mice carrying mutations in MDM2 or MDMX that specifically inhibit E3 activity and dimerization without preventing the interaction of these proteins with p53 show phenotypes similar to the complete deletion of MDM2 or MDMX, [30,31,61], indicate that simply the binding of MDM2 or MDMX to p53 is not enough to keep it

Table 1
Ubiquitin ligases and de-ubiquitinating enzymes that modify p53.

Enzyme	Class	Selected references (also see text)	Type	Chain	Lysine modified	Effect on p53	Activity observed		In vivo model
							In vitro	In cells	
MDM2	E3	[27,28], see text	RING	K48	K370, K372, K373, K381, K382, and K386, *more* in vitro	Repressed transcriptional activity, degradation, nuclear export	✓	✓	Mouse
MDMX	(E3)	[18], see text	RING	K48	K370, K372, K373, K381, K382, and K386	Repressed transcriptional activity, degradation (with MDM2)	✓	✓	Mouse
Arf-BP1	E3	[196]	HECT			Degradation	✓	✓	Mouse
Carpi	E3	[210]	RING			Degradation		✓	
Carp2	E3	[210]	RING			Degradation		✓	
CHIP	E3	[198]	U box			Degradation	✓	✓	Mouse
Copi	E3	[125]	RING	K48		Degradation	✓	✓	
Cul1	E3	[217]	RING			Degradation	✓	✓	Mouse
Cul4a	E3	[219,220,226]	RING			Degradation		✓	Mouse
Cul5	E3	[221]	RING	K48		Degradation	✓		
Cul7	E3	[223]	RING			Repressed transcriptional activity	✓	✓	
Cul9/PARC	E3	[46]	RING			Cytoplasmic sequestration, apoptosis			Mouse
Hades	E3	[208]	RING	K24		Degradation	✓	✓	
MSL2	E3	[207]	RING	K351 and K357		Nuclear export		✓	
Pirh2	E3	[124,193,195]	RING	K48	K101, K164, K292, K305, K357 K382 and K386	Degradation	✓	✓	Mouse
Synoviolin	E3	[211]	RING			Degradation, nuclear export	✓	✓	Drosophila
Topors	E3	[212]	RING			Degradation	✓	✓	
Trim24	E3	[200]	RING			Degradation	✓	✓	Drosophila
Trim28	E3	[214]	RING			Degradation	✓		
Trim39	E3	[215]	RING			Degradation	✓		
wwpl	E3	[206]	HECT			Stabilization, nuclear export	✓	✓	
UBC13	E2	[209,269]		K63		Stabilization as monomer (less activity)		✓	
E4F1	E4	[204]		K48	K319, K320, K321	Modulated transcriptional activity	✓	✓	
Gankyrin	E4	[179]				Degradation	✓	✓	
p300/CBP	E4	[176,268]				Degradation	✓	✓	
UBE4B	E4	[177,270]	U-Box			Degradation	✓	✓	
Yin Yang 1	E4	[180]				Degradation	✓	✓	
Hausp	DUB	[233,235,237]				Degradation (stabilization of MDM2/X)	✓	✓	Mouse
Otubain1	DUB	[230]				Stabilization	✓	✓	
USP10	DUB	[227]				Stabilization	✓	✓	
USP29	DUB	[229]				Stabilization		✓	
USP42	DUB	[228]				Stabilization	✓	✓	

under control. These results are also entirely consistent with a critical role for the ubiquitination function of MDM2/MDMX in the regulation of p53, although the present information makes it difficult to distinguish a role for the E3 activity from an alternative function for dimerization — such as the regulation of MDMX subcellular localization [62,63], for example. Furthermore, while an intact MDMX RING is required in the embryo, this domain appears to be unnecessary in adult tissue [30]. Indeed, MDM2 can restrain p53 activity without any MDMX in the adult, to some extent [64], but the reciprocal is not true [65], suggesting that MDM2 has the predominant role in this partnership. Nevertheless, overexpression of either MDM2 or MDMX can play a clear role in preventing p53 function and enhancing malignant development in several tumor types [66–69].

4. Regulation of MDM2/MDMX activity

Given the key role played by MDM2/MDMX in controlling p53, it is not surprising that the regulation of their activity is controlled through multiple systems. MDM2 is a transcriptional target of p53, resulting in a negative feedback loop where p53 can induce the expression of its own negative regulator. Interestingly, however, maintenance of low basal p53 levels does not depend on the ability of p53 to transcriptionally activate MDM2, and other transcriptional regulators of MDM2 such as NFAT1 have been described [70]. While p53 function is not required for MDM2 expression in normal cells, an elegant study in zebrafish showed that the transcriptional activity of p53 (and presumably the activation of MDM2) is necessary to reduce p53 levels following the resolution of a stress response [71]. MDMX has also been shown to be regulated by p53, although the extent of activation is far weaker [72] and less general [73] than that seen with MDM2. MAPK signaling has been reported to induce MDMX transcription [74], but in general the mechanisms regulating MDMX expression are not well understood. Interestingly, MDMX has also been shown to contribute to the ability of p53 to activate the expression of MDM2 [75], thereby enhancing p53 degradation through an indirect mechanism. Polymorphisms in MDM2 [76] and MDMX may regulate their expression, while splice variants of each protein are also associated with differential activities [77]. There is, therefore, ample opportunity for the regulation of MDM2 and MDMX at the level of gene expression. However, it seems that critical control is exerted at the level of post-translational modifications, which regulate protein function, interaction, localization and stability.

5. Modifications of MDM2/MDMX

A multitude of post-translational modifications on both p53 and MDM2/MDMX, contribute to the regulation of p53 stability. In addition to the regulation of p53 with ubiquitin and other ubiquitin-like proteins already discussed, MDM2 and MDMX are also actively controlled through these modifications.

The E3 activity of MDM2/MDMX is not confined to the targeting of p53, and ubiquitination and degradation of all the players in this system have been described. MDM2 can ubiquitinate itself, although E3 defective MDM2 proteins remain unstable in cells, suggesting that another E3 can also drive the turnover of MDM2 (discussed further below). MDMX is efficiently ubiquitinated when in complex with MDM2 [78], although p53 is the preferred target of the E3 activity, and MDMX becomes stabilized in the presence of p53. High levels of MDMX can inhibit the degradation of p53 by MDM2, and the E3 activity is likely to be limited by the level of MDM2 in most cells [62]. In many cases it seems that the regulation of p53 stability results not from a complete inhibition of

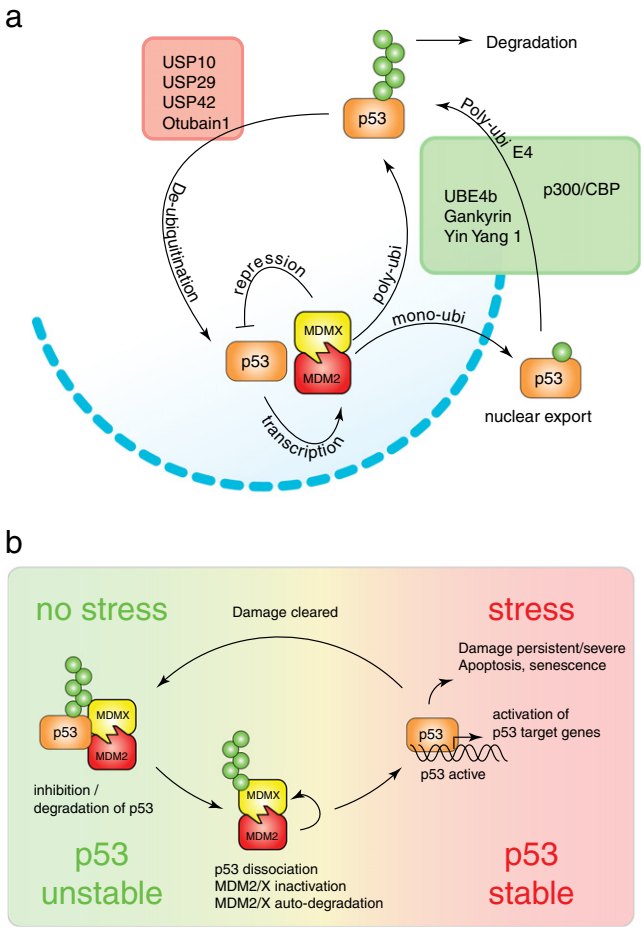

Fig. 1. a: Regulation of p53 by ubiquitination. p53 and MDM2 form an auto-regulatory feedback loop, where p53 induces transcription of MDM2, which dimerizes with MDMX, forming an active E3 ligase. Both MDM2 and MDMX can repress the transcriptional activity of p53 (independently of ubiquitination), promote p53 nuclear export via mono-ubiquitination or poly-ubiquitinate p53 (in some cases aided by E4 ligases) causing its degradation. Cytoplasmic p53 can exert a variety of activities (discussed in the text) or be poly-ubiquitinated and degraded by E4 ligases (E4s). Ubiquitination of p53 can be counteracted by several de-ubiquitinating enzymes. b: Feedback loop between p53 and MDM2/MDMX during stress response. In unstressed cells, p53 is kept inactive by both transcriptional inhibition and proteasomal degradation. Stress causes MDM2/MDMX to release p53 and increase auto-degradation. This leads to p53 accumulation, resulting in cell cycle arrest and other p53 responses. If the stress can be resolved, the cells may return to the pre-stress state. If the p53 activating signal cannot be alleviated, p53 initiates apoptosis or senescence.

MDM2's E3 activity, but from a switch in target from p53 to MDM2/MDMX.

MDM2 is also modified by other ubiquitin like proteins. SUMO-1 modification of MDM2 targets its N-terminus (between amino acids 134 and 212) through a mechanism that does not require the RING domain of MDM2 and is promoted by p14ARF [79]. Nedd8 modifications of MDM2 have also been reported and are thought to increase MDM2 stability. This is counteracted by the NEDP1 which de-neddylates MDM2 leading to its destabilization [80]. MDMX has also been shown to be SUMO modified [81], although the significance of this modification is not yet clear.

6. Phosphorylation in the regulation of p53 stability

p53, MDM2 and MDMX can be phosphorylated on many sites by numerous kinases and these modifications can be key in the activation of the p53 signaling network. The regulation and consequences of these phosphorylation events are a complex field that we can only briefly touch on here. However, several excellent reviews on this topic that provide a much deeper description of this area have been published recently [82–85].

An excellent example of the complexity of phosphorylation pathways in the control of p53 is provided by the response to DNA damage, which promotes phosphorylation of several sites on p53, including serine 15 by DNA-PK [86] and ATM [87], and serine 20 via Chk2 [88,89]. While these phosphorylation events have been implicated in the stabilization of p53, abrogating these modifications by mutating the target amino acids on p53 – including serine 15 and 20 – does not prevent p53 stabilization after induction of DNA damage in tissue culture [90–92]. Furthermore, point mutation of serine 18 in mice (homologous to human serine 15) does not have a strong effect on p53 stabilization or tumor formation in vivo [93,94]. Knock in mice expressing p53 with a serine 23 to alanine substitution (homologous to human serine 20) show reduced accumulation of p53 after gamma irradiation in several tissues and increased incidence of long latency B cell lymphomas, although again the expression of this phosphorylation site mutant p53 has only a modest phenotype [95,96]. Mutation of both these residues (18 and 23) to alanine in a knock in model also causes partial defects in the p53 response and a spectrum of tumors distinct from p53 loss [97]. From these and a large number of additional studies (e.g. analysis of serine 389 [98–101]) it seems clear that individual phosphorylation modifications of p53 contribute to, but are not essential for the activation of p53 after DNA damage.

DNA-damage induced phosphorylation of MDM2 also plays an important role in the regulation of the p53 response. For example, phosphorylation of MDM2 on S17 (by DNA PK [102]) reduces the affinity of MDM2 towards p53, preventing the degradation and nuclear export of p53, and so allows for the induction of a p53 response. Phosphorylation of MDM2 on multiple sites (S386, S395 S407, T419, T425 and S429) by ATM [103] does not alter the interaction with p53, but reduces the ability of MDM2 to homodimerize by regulating the function of both the RING and the acidic domain [104,105]. Substituting all 6 ATM target residues to alanine (6A) delays the stabilization of p53, while the substitution of aspartic acids at these 6 sites (6D), which can mimic phosphorylation, reduces p53 poly-ubiquitination — again without altering the affinity of MDM2 to p53 [104]. Furthermore, phosphorylation of S395 alone has been reported to inhibit MDM2 dimerization [104]. In vivo, the ability to phosphorylate S394 in mice (homologous to human S395) is necessary for DNA damage-induced p53 activation. Mutating this residue to alanine (S394A) renders knock in mice radioresistant to a similar extent as p53 null mice and these animals also develop T cell derived lymphomas [106]. Interestingly the corresponding phosphomimic mice (S394D) are viable and respond essentially normally to DNA damage, suggesting that either the aspartic acid substitution does not completely mimic phosphorylation, or that further DNA damage induced signals are required for a complete activation of the DNA damage response. However, the defect in ubiquitination of p53 does not seem to reflect an overall reduction in E3 ligase activity, since both MDM2 6A and 6D degrade MDMX as efficiently as wild type MDM2, and show a comparable level of auto-ubiquitination [103].

Interestingly, the ATM-mediated phosphorylation at S395 also increased the binding of MDM2 to p53 mRNA, promoting p53 synthesis and suppressing MDM2's E3 ligase activity towards p53 and so resulting in a switch from inhibition to activation of p53 [38].

Finally, phosphorylation of MDMX also plays an important role in signaling to p53. Stress induced phosphorylation of MDMX by Chk2 results in 14–3–3 binding and nuclear accumulation of MDMX [107,108], which – unlike MDM2 – does not contain its own nuclear localization signal. Both Chk2 and ATM phosphorylation of MDMX can promote the MDM2-mediated degradation of MDMX [109,110] — which is normally a stable protein in undamaged cells. Interestingly, mutations of phosphorylation sites in MDMX can inhibit the ability to be degraded by MDM2, without impeding MDM2's binding. These mutants lead to a situation where MDMX is no longer sensitive to degradation by MDM2, but the ability of the MDM2/MDMX complex to target p53

remains intact. As might be predicted, this resulted in abnormally efficient p53 degradation under both basal and stressed conditions [111].

The complexity of regulation by phosphorylation is further enhanced by the action of the Wip1 phosphatase, itself the product of a p53-inducible gene. Wip1 has been shown to dephosphorylate both MDM2 and MDMX [112–114] resulting in decreased p53 levels and activity.

7. Other modifications to regulate p53

In addition to phosphorylation, several other post-translational modifications play an important role in fine-tuning the p53 response.

While mono-methylation of p53 on K370 (by SMYD2 [115]) and K382 (by SET8 [116]) suppresses its transcriptional activity, di-methylation of K370 or K382 activates p53 by binding of PHF20, which protects it from MDM2 mediated ubiquitination [117]. Acetylation of lysines on p53 by p300/CBP prevents the subsequent ubiquitination of p53 by MDM2, since these modifications on the same lysine are mutually exclusive [118]. Further modifications of p53, such as prolyl isomerization [119] and glycosylation [120], may also contribute to the regulation of p53 stability and activity. Knock in studies have shed some light on the role of isomerization of p53, which in vivo seem to be dispensable for the tumor suppressive activity [121]. The extent to which MDM2 and MDMX may be controlled by these modifications has not yet been explored.

8. Chasing the tail of p53 regulatory loops

Many of the regulators of p53 are themselves regulated by p53, forming complex positive and negative feedback loops [122]. As mentioned, p53 directly activates the transcription of MDM2 [123] and to a lesser extent MDMX [72,73]. PirH2 [124] and Cop1 [125] transcription is also induced by p53 and, as discussed above, the phosphatase Wip1 is a p53 target gene and can dephosphorylate both MDM2 and MDMX [112–114]. All these establish negative feedback loops to induce efficient p53 turnover and allow cells to return to an unstressed state once the cause of p53 induction is removed. An interesting example of a positive feedback loop is the ability of the p53 inducible protein PIDD to promote caspase 2-dependent cleavage of MDM2 [126]. Removal of the RING domain of MDM2 by PIDD results helps to sustain p53 activity following DNA damage.

9. MDM2/MDMX binding partners that inhibit p53 degradation

While DNA damage signaling provides an interesting example of how p53 can become activated, it is clear that other stress signals can control MDM2 and MDMX activity through quite independent mechanisms. Several proteins that bind MDM2 and play a role in regulating its activity in response to oncogene activation or ribosomal stress have been described. Of these, the best studied are p14ARF (p19ARF in mouse) and various ribosomal proteins, which can all directly inhibit MDM2's E3 activity towards p53.

Aberrant signaling by several oncogenes including Ras [127], c-Myc [128], E2F1 [129], ß-catenin [130] and NMI [131] leads to an induction of ARF expression, either through transcriptional activation or stabilization of the ARF protein. While ARF is a highly stable protein in tumor cells, it is rapidly degraded in normal cells through the activity of ULF, which promotes ubiquitin dependent degradation of ARF [132] — a function that is inhibited by the oncogenic activity of Myc. The induction of ARF leads to its binding to MDM2, and stabilization and activation of p53. The importance of this function of ARF is supported by the observation that tumors arising in response to the loss of ARF do not require p53 loss [133]. Interestingly, while p53 ubiquitination is inhibited by ARF, MDM2 ubiquitination is not affected, suggesting that ARF binding does not completely incapacitate the E3 activity of MDM2 [134].

Along similar lines, stress caused by an imbalance in ribosome biogenesis or ribosomal stress (which can be induced by perturbation of nuclear import of ribosomal proteins [135], expanded CAG RNAs associated with polyglutamine toxicity [136] and metabolic fluctuations [137,138]) can lead to the release of free ribosomal proteins (RPs) in the nucleus. Like ARF, these can bind MDM2 and inhibit p53 ubiquitination, although the binding requirements on p53 for ARF and the different ribosomal proteins are subtly different. As with phosphorylation, the full wealth of publications about the impact of ribosomal subunits on p53 activity lies beyond the scope of this review, but has been more fully discussed in several recent publications [138–140]. A large number of ribosomal proteins can influence MDM2 activity, including RPL5 [141], RPL11 [142,143] (which can also be regulated by increased translation in response to ribosomal stress [144]), RPL23 [145], RPL26 [146], RPS7 [147,148], RPS7L [149], RPS14 [150] and RPS25 [151]. As observed with ARF, binding or association with RPs does not necessary inactivate MDM2 but, much like phosphorylation after DNA damage, alter its specificity. For example association of RPL11 with MDM2 leads to a switch in activity of MDM2 from p53 towards MDMX, resulting in MDMX degradation [152]. While depletion of these MDM2-interacting ribosomal proteins can dampen the ability to induce p53, inhibition of one ribosomal subunit in isolation can also trigger ribosomal stress, and thereby lead to the stabilization of p53 through the activity of other ribosomal components. The protection of free ribosomal proteins from degradation – in particular L5 and L11 – has been shown to be critical for the activation of p53 [153]. Importantly, the ability of MDM2 to bind L5 and L11 is necessary for the induction of p53 in response to ribosomal stress (but not DNA damage), and is required for full protection from Myc-induced cancers [154]. Mutations disrupting RPL5 and RPL11, but not ARF binding to MDM2 have been observed in human cancers [155], again supporting the importance of signaling through this pathway in the protection from tumor development. Interestingly some of these ribosomal proteins (RPL26 [146] and RPS7 [148]) are themselves degraded by MDM2, and in the case of RPL26 further complexity is introduced by the observation that this ribosomal protein controls p53 mRNA translation, an activity that is inhibited by MDM2 [146]. Other nucleolar proteins also contribute to the regulation of p53 stability, including nucleostemin [156] and PICT1 (GLTSCR2), which functions by both enhancing the RPL11/MDM2 interaction [157] and direct p53 binding [158]. Nucleophosmin has also been reported to bind MDM2 after UV-induced DNA damage, reducing the p53–MDM2 interaction and thereby further stabilizing p53 [159].

While the nucleolar proteins and ARF can function to inhibit MDM2 independently in response to different signals, there is evidence for cooperation between different ribosomal proteins [160] as well as between ARF and L11 [161]. Indeed, there is evidence that ARF can trigger nucleolar stress that ARF functions, at least in part, through L11. Exactly how ARF and the ribosomal proteins inhibit MDM2 is not clear. All these proteins bind to the central region (and not the RING domain) of MDM2, a domain of MDM2 that is known to be important for p53 degradation [162], and can inhibit MDM2's E3 activity towards p53. Furthermore, the interaction of MDM2 with ARF can lead to the dissociation of the MDM2/p53 complex by relocalizing MDM2 to the nucleolus [142]. ARF also promotes enhanced SUMOylation of MDM2 [79], although the extent to which this modification is necessary to mediate the ARF repressive effects on MDM2 remains to be determined. Interestingly, while de-SUMOylation of MDM2 by SUSP4, a SUMO specific protease, resulted in an increased MDM2 activity, this led to increased auto-ubiquitination and degradation of MDM2, and so the accumulation of p53 [163]. Whether ribosomal proteins have a similar ability to drive the SUMOylation of MDM2 is not known.

While ARF and ribosomal proteins are the best understood inhibitors of MDM2, numerous other proteins that can bind p53 and protect it from MDM2-mediated degradation have been described. One of these is NUMB, which was initially described as a MDM2 interacting protein [164] but has since been shown to bind to both p53 and MDM2, thereby preventing ubiquitination and degradation of p53

[165]. NUMB functions through binding MDM2 in a similar way as p53 [166], leading to the dissociation of the p53–MDM2 complex, stabilization of p53 and degradation of NUMB instead.

Like NUMB, other important regulators of cell behavior, such as PML [167,168], Smad1 [169], SnoN [170], Pin1 (which functions through proline isomerization of p53 [119]), HEXIM1 [171], and TRIAD1 [172] have also been described to impact MDM2's E3 ligase activity. Each of these regulators is subject to control mechanisms, and the existence of loops involving the mutual regulation of various components of these systems creates a dizzyingly complicated network through which the p53 response can be controlled.

Less is known about the binding partners that control MDMX, although the importance of phosphorylation-dependent 14–3–3 binding has already been mentioned. Interestingly, although the ribosomal proteins have not been described to bind MDMX, ARF can interact with MDMX, causing its sequestration to the nucleolus and resulting in the activation of p53 [173]. Also, 5S rRNA binds efficiently to the RING domain of MDMX, but only very weakly to MDM2, and is important for protecting MDMX from MDM2-mediated degradation. However, the regulation of this mechanism during the stress response remains unclear [174].

10. MDM2/MDMX binding partners that promote p53 ubiquitination

Mounting evidence in the literature supports the notion that several E3 ligases are assisted by a ubiquitin chain assembly factor (E4), which supports E3 ligases by extending ubiquitination chains to increase overall performance. Although it is technically difficult to differentiate between an E3 ligase co-factor and an E4 ligase, several E4 enzymes for p53 have been proposed. Other MDM2/MDMX binding proteins may also enhance the ubiquitination of p53 through different mechanisms. One of those is TCTP, which acts as an antagonist to NUMB by competing with NUMB for binding to p53–MDM2, thereby inhibiting auto-ubiquitination of MDM2 and promoting degradation of p53 [175]. Interestingly TCTP is itself transcriptionally repressed by p53, adding to the intricacies of the feedback regulation.

10.1. p300

p300 and its close paralog CBP have been described to exhibit E4 activity towards p53 in vitro and in cells [176] by accepting mono-ubiquitinated p53 as a substrate, rapidly extending the ubiquitin chain and causing increased turnover of p53 [176]. This activity is independent and separable from p53 acetylation by p300/CBP and is exclusive to the cytoplasm. These observations are nicely consistent with a model in which at low levels MDM2 promotes mono-ubiquitination, SUMOylation by PIASy and nuclear export of p53 [57], which then can subsequently be poly-ubiquitinated by p300, leading to its proteasomal degradation in the cytoplasm. By contrast, high levels of MDM2 promote the polyubiquitination and nuclear degradation of p53 [40].

10.2. UBE4B

UBE4B, the mammalian homolog of UFD2 (the first E4 ubiquitin ligase to be identified in yeast), has been shown to interact with both MDM2 and p53, and can extend the poly-ubiquitin chain assembled by MDM2 on p53 both in vitro and in cells [177]. A RING-like domain, known as a U-box, in UBE4B is indispensable for its function as an E4 ligase towards p53. Deletion of UBE4B in vivo results in early embryonic death that is accompanied by induction of apoptosis in the heart, where UBE4B is exclusively expressed during embryogenesis [178]. However, it is not clear whether this apoptosis can be attributed to stabilization of p53.

10.3. Gankyrin

Gankyrin, an akyrin repeat containing protein, associates with the ATPase subunit of the 26S proteasome and can also control the ubiquitin ligase activity of MDM2 on p53. In cells, overexpression of Gankyrin increases the ratio of poly- versus mono-ubiquitinated p53 as well as increasing the interaction of ubiquitinated p53 and MDM2 with the proteasome. Therefore, Gankyrin probably enhances delivery of ubiquitinated p53 to the proteasome, in addition to increasing MDM2 activity [179].

10.4. Yin Yang 1

Yin Yang 1 not only enhances poly-ubiquitination of p53 to a similar extent as p300, but also increases the affinity of MDM2 towards p53, thereby potentiating its ability to promote the ubiquitination of p53 [180]. Furthermore, Yin Yang 1 binds p300 [181] and may therefore recruit p300 to further boost p53 poly-ubiquitination.

11. Signaling to enhance p53 turnover

While stress signals function to inhibit MDM2 and so stabilize p53, the ability to dampen or impede p53 function may also be important under certain conditions. Phosphorylation of MDM2 by AKT can induce MDM2 function and so prevent stabilization of p53 under conditions of growth factor survival signaling [182–184]. AKT can also phosphorylate MDMX to promote 14–3–3 binding, although unlike DNA damage-induced induction of MDMX-14–3–3 binding, the result is the stabilization of MDMX and MDM2 [185]. While the details of how 14–3–3 controls MDMX remain to be elucidated, the overall message is that AKT functions to enhance MDM2 activity, and so inhibit p53. Interestingly, a recent study has shown that the AKT signaling pathway is important to prevent p53-induced inhibition of cell cycle progression under conditions of normal cell growth [186]. The phosphorylation of the central region of MDM2 by kinases such as GSK-3 may also help to promote p53 degradation by enhancing the interaction of MDM2 with the proteasome [187].

Other protein interactions can promote the ability of MDM2 to degrade p53 include Pax3 [188], Twist [189], Niban [190] (which works by releasing MDM2 from nucleophosmin), Smurf1/2 [191] (which functions by enhancing the MDM2/MDMX interaction) and TCTP [175] (which binds MDM2, preventing NUMB binding and inhibiting auto-degradation). In another variation on this theme, the E3 ligase RFWD3 can synergize with MDM2 in the ubiquitination of p53, but this results in the formation of shorter ubiquitin chains that cannot be recognized by the proteasome [192]. RFWD3 therefore contributes to the DNA-damage induced stabilization of p53 by – paradoxically – enhancing ubiquitination.

12. Additional E3s for p53

The molecular and genetic evidence supporting the major role for MDM2/MDMX in targeting p53 for degradation is overwhelming. However, the complexity of p53 regulation suggests that additional factors can contribute to the control of p53 stability and several E3 ligases have been reported to fill this gap.

12.1. Pirh2

Like MDM2, Pirh2 is a RING E3 ligase that binds and ubiquitinates p53, and is itself transcriptionally activated by p53 [124]. Interestingly, Pirh2 ubiquitinates p53 on several lysine residues, including K101, K164, K292, K305, K357, K382 and K386 [193]. However, in vitro Pirh2 and MDM2 show differences in lysine specificities, with only Pirh2 able to modify K168, an activity that opposes the acetylation of this site by p300/CBP. Acetylation of K168 is a prerequisite for

p53-mediated cell growth arrest and apoptosis [194], so this activity is consistent with a role of Pirh2 in repressing p53 function. Mice deleted of Pirh2 [195] have highlighted a role for Pirh2 in the regulation of p53 turnover in response to DNA damage.

12.2. ARF-BP1

ARF-BP1, also known as MULE/HectH9/HUWE1, is a HectE3 ligase [196] that ubiquitinates and targets p53 for degradation. Deletion of ARF-BP1 leads to embryonic lethality around day E14.5, accompanied by increased levels of p53 and increased apoptosis in some tissues. Specific deletion of ARF-BP1 in pancreatic β-cells resulted in an age-dependent diabetic phenotype due to a reduction of the beta-cell population in a p53 dependent manner [197]. Interestingly, ARF-BP1 was identified as a binding partner for ARF, which acts as a negative regulator for both ARF-BP1 and MDM2.

12.3. CHIP

CHIP was initially described as an Hsp70 associated E3 ligase that has a role in the chaperone-assisted quality control, targeting – among other things – mis-folded and aggregated proteins for proteasomal degradation. Some p53 mutations cause conformational instability of the protein, suggesting that CHIP may have an effect on mutant p53 stability. Indeed, CHIP interacts with both mutant and wild-type p53, and can reduce mutant (and to a lesser extent wild type) p53 levels and half-life [198]. More recent studies have highlighted a role for CHIP regulating p53 in cardiomyocytes after hypoxic stress [199].

12.4. Trim24

Trim24 is another RING E3 that was identified as a p53 binding partner [200]. Ubiquitination and degradation of p53 by Trim24 are dependent on the RING domain, and depletion of Trim24 leads to an increase in p53 in various cell lines. Mutation of bonus, the drosophila homolog of Trim24, resulted in enhanced apoptosis in the wing imaginal disks, a phenotype that could be rescued by p53 depletion. These results suggest that Trim24 is necessary to down-regulate p53 activity in vivo and are particularly interesting in light of the fact that Drosophila does not have MDM2, despite the general assumption that MDM2 and MDMX binding domains co-evolved with p53 [201].

Further studies have shown that Trim24 can control p53 activity in human embryonic stem cells [202], where phosphorylation of p53 on lysine 373 in response to retinoic acid disrupts the degradation by Trim24.

12.5. E4F1

E4F1 is part of the polycomb repressive complex 1 (PRC1), and can induce cell-cycle arrest in a p53 dependent manner [203]. Interestingly, E4F1 ubiquitinates p53 through K-48 linked poly-ubiquitination [204], targeting lysines (K319, K320, K321) that are distinct from the target lysines of MDM2. The consequence of ubiquitination by E4F1 is not to drive degradation, but to promote the transcription of p53 dependent cell-cycle arrest genes. The involvement of E4F1 in several independent anti-proliferative pathways has made the analysis of in vivo phenotypes complicated, although recent analysis of conditional E4F1 knockout mice has shown that it is clearly involved in the BMI1/ARF/p53 pathway. However, as with all these E3s, the functions of E4F1 are likely to extend beyond the regulation of p53 [205].

12.6. Other ubiquitin ligases for p53

In addition to those discussed above, a number of other ubiquitin ligases have been implicated in the regulation of p53 stability. These include MSL2 and WWP1, which increase the cytoplasmic localization of p53 [206,207] but do not affect (MSL2) or even increase (WWP1) p53 stability, and Hades, a RING E3 ligase that interacts with p53 in the mitochondria and thereby inhibits a p53-dependent mitochondrial cell death [208]. Ubc13 is an E2 enzyme that directly assembles K63 chains on p53, leading to its monomerization and cytoplasmic localization [209]. Additionally, Carp1, Carp2 [210], the endoplasmatic reticulum located RING E3 ligase Synoviolin [211] and the ubiquitin/SUMO E3 ligase TOPORS [212] have all been described to ubiquitinate p53. Although Cop1 has been reported to be an E3 ligase for p53 [125], later studies have not found Cop1 mediated degradation of p53 in vivo [213]. Finally, the RING E3 ligases Trim28 [214] and Trim39 [215] have been shown to target p53 for ubiquitination. Biochemical evidence supports the role of each of these proteins in the fine-tuning of p53 activity, although their overall importance in vivo needs to be further investigated.

Finally, we come to the Cullin-RING ubiquitin-ligases. These are modular multi subunit enzymes comprising an active E3 ligase, either RBX1 (also known as ROC1) or RBX2, bound to one of the seven Cullin family members (Cullin-1, -2, -3, -4A, -4B, -5 and -7), and a substrate specific receptor, either directly or via adapter proteins. Cullin E3s are known to play important roles in the cell cycle, transcription, signal transduction and are often deregulated in cancer (reviewed in [216]). Several factors of the Cullin RING E3 ligase family have been implicated in influencing p53 activity, including Cullin 1 [217], Cullin 2 [218], Cullin 4a [219,220], Cullin 5 [218,221,222], Cullin 7 [223], Cullin 9 [46] and their common adaptor proteins Skp1 [217,224], Skp2 [225], the F-box protein JFK [217] and DDB1 [226]. However, the relevance of their respective impact on p53 remains to be determined.

13. De-ubiquitination of p53 and MDM2

It is clear that ubiquitination, like most other post-translational modifications, is a highly dynamic and reversible signal. Therefore it comes as no surprise that several de-ubiquitinating enzymes (DUBs) have been described that can balance the p53 ubiquitin equation, adding further possibilities for fine-tuning the p53 response.

13.1. USP10

USP10 was identified “by serendipity” [227] as a p53 interacting protein and functions to regulate p53 stability and localization by counteracting the effects of MDM2. Following genotoxic stress, USP10 is phosphorylated by ATM and translocated to the nucleus, where it can deubiquitinate and stabilize p53. Interestingly, USP10 also displays deubiquitinating activity towards mutant p53, and so may serve to promote the oncogenic effects of mutant p53 in tumor cells.

13.2. USP42

USP42 also targets p53 [228], but its main role appears to be to counteract residual p53 ubiquitination immediately after the induction of genotoxic stress, decreasing the response time of the p53 pathway. USP42 does not affect the basal levels of p53 in unstressed cells, presumably because of the high activity of the E3 ligases that target p53. During the initial phases of the stress response, USP42, accelerates but not increases the overall stabilization of p53. There is, as yet, no evidence that this function of USP42 can be regulated.

13.3. USP29

USP29 is transcriptionally activated in response to oxidative stress through a mechanism that involves the nuclear localization of JTV1. This DUB then de-ubiquitinates and stabilizes p53, so contributing to the full activation of the p53 target gene PUMA and consequent apoptosis [229].

13.4. Otub1

The mechanism of activation for Otub1, another de-ubiquitinating enzyme for p53, is less well understood [230], since the catalytic cysteine of Otub1 is not necessary for this deubiquitination. It is possible that the activity of Otub1 reflects the inhibition of UbcH5/MDM2, rather than a classic de-ubiquitination function.

14. Regulating the regulators

14.1. The E3s

Although it is clear that MDM2 can ubiquitinate and degrade itself, catalytically inactive MDM2 (C462A) retains the short half-life characteristic of the wild type protein [61], indicating that other E3 ligases can target MDM2. Several E3 ligases for MDM2 have been described, although none of them has been carefully investigated in vivo. Of specific interest is SCF β-TRCP, an F-box protein that binds and degrades both wild type and C464A (equivalent to C462A in mouse) MDM2 in a DNA damage and cell-cycle dependent manner [231]. Trim13, also known as RFP2, has also been shown to harbor E3 ligase activity towards MDM2 in vitro and in cells [232]. An increase in Trim13 levels after ionizing radiation could support a mechanism where upon induction of stress, Trim13 changes from auto-degradation to ubiquitination of MDM2, supporting the rapid stabilization of p53.

14.2. The DUBs

Just like p53, de-ubiquitination of MDM2 and MDMX also plays a role in the control of p53 stability. USP7, also known as HAUSP, was initially described to de-ubiquitinate and stabilize p53 [233]. However, soon it became clear that HAUSP functions mainly to de-ubiquitinate and stabilize MDM2 [234] and MDMX [235]. DNA damage induced phosphorylation of MDMX interferes with the MDMX/HAUSP interaction, so allowing the degradation of MDMX and stabilization of p53. The activity of HAUSP itself is also regulated by phosphorylation, with specific dephosphorylation by the DNA-damage induced phosphatase PPM1G leading to the down-regulation of HAUSP, destabilization of MDM2 and activation of p53 [236]. Deleting HAUSP causes early embryonic lethality [237], which is accompanied by elevated levels of p53 and reduced proliferation. Co-deletion of p53 did not completely rescue the HAUSP null phenotype, but resulted in significant developmental improvements. HAUSP therefore appears to function as a negative regulator of p53, through the de-ubiquitination and stabilization of MDM2, but also clearly functions through additional, MDM2-independent pathways.

USP2a is an MDM2 interacting DUB [238], which de-ubiquitinates and elevates the levels of MDM2, allowing for an increased ubiquitination of p53. Inhibition of USP2a in response to Frizzled 8-associated Antiproliferative Factor (APF) was shown to contribute to an increase in p53 levels and activation of growth arrest [239]. USP2a also de-ubiquitinates and stabilizes MDMX [240]. Finally, USP4 has been shown to de-ubiquitinate and stabilize ARF-BP1, leading to reduced p53 levels and a dampening of p53 activity [241].

14.3. MicroRNAs that regulate MDM2

MicroRNAs (miRNAs) are endogenously expressed short noncoding RNAs that can play important regulatory roles in gene silencing, translational repression, mRNA cleavage, and mRNA decay. They are involved in multiple biological processes, including the response to environmental stresses and cancer development. Several microRNAs targeting MDM2 have been identified, including miR143/145 [242], miR605 [243], miR25, and miR32 [244]. The transcription of miR25 and 32 is induced by E2F-1 and cMyc, leading to a reduction of MDM2 protein levels through direct binding to 3-UTR of MDM2 mRNA. This stabilizes p53 protein levels and results in decreased cell proliferation and cell cycle arrest. Both miR143/145 and miR605 can be induced by p53, leading to decreased MDM2 protein levels and increased p53 function, constituting a positive feedback loop that may help in allowing the robust activation of p53.

15. Drugging the p53 degradation pathway

Stabilization of p53 is critical for cells to respond to stress and limit cancer development. Cancer cells almost all lack the ability to activate a p53 response, and so continue to proliferate and survive despite their exposure to various forms of oncogenic stress, including oncogene activation, hypoxia and loss of normal environment. Indeed, numerous studies have shown that increasing p53 levels can lead to tumor regression through the activation of apoptosis or senescence [245–247], either in the incipient tumor cell or through non cell-autonomous pathways [248]. The stabilization of p53 in cancers that retain wild type p53 is therefore an attractive strategy for therapy.

The main focus of these studies so far has been to develop small molecule inhibitors of MDM2 dependent degradation of p53. There is substantial evidence that MDM2 and MDMX can contribute to tumor development, and that this reflects their ability to inactivate p53. Indeed several tumors that retain wild type p53 show evidence of MDM2 or MDMX overexpression, including gliomas, soft tissue tumors and melanoma [66,68,69,249,250]. The consequences of MDM2 and MDMX inhibition have been assessed using genetically engineered mouse models, using acute restoration of p53 in adult MDM2 or MDMX deficient mice [64,65]. Interestingly, the absence of MDM2 resulted in a fatal response to p53, raising some concerns about the general toxicity of systemic treatment with MDM2 inhibitors. However, while lack of MDMX resulted in the activation of p53, this was tolerated by the mice and could be reversed. Importantly, the absence of MDMX enhanced the therapeutic effect of p53 restoration in tumors, supporting the potential for MDMX as a therapeutic target.

A number of approaches to developing inhibitors of p53 degradation have been explored (again a broad topic that is more fully reviewed in [251]). These include the development of drugs that prevent the p53/MDM2 or p53/MDMX interaction, inhibit MDM2's E3 ligase activity, block the delivery of p53 to the proteasome or even modulate the activity of the DUBs that regulate p53 or MDM2.

A number of small molecules have been developed that can occupy a hydrophobic pocket in MDM2's p53 binding site, thereby disrupting the MDM2 p53 interaction. The first of these, Nutlin 3A, has proven to be highly specific in inducing arrest, apoptosis and cellular senescence in both tissue culture and xenographed mouse models [252,253] and a derivative of this drug has been advanced to phase I clinical trials for adults with leukemia [254]. A similar approach has led to the identification of small molecules such as RITA, which also prevent the p53/MDM2 interaction but function by binding the N-terminus of p53 [255]. Small molecule inhibitors of the p53/MDMX interaction have also been described [256].

Direct inhibitors of MDM2 or MDM2/MDMX E3 activity can also promote p53 stabilization and activation, without necessarily inhibiting the interaction of the two proteins [257–259]. The mechanism of action of such inhibitors is less well understood, although some have been shown to function by binding to the RING domain of MDM2. Small molecules that induce the dimerization of MDM2 and MDMX can also promote p53 stabilization and activation, and may simultaneously inhibit both MDM2 and MDMX [260]. Whether this will drive unacceptable levels of toxicity remains to be determined.

Stapled peptides based on the p53 transactivation domain have also been used to block both MDM2 mediated degradation [261,262] and MDMX mediated repression of p53 [263], and a phage display based approach has yielded novel peptide derived activators of p53 that display high binding affinity to both MDM2 and MDMX [264]. Finally, small

molecule inhibitors of MDMX expression [265] have also been shown to activate p53.

Most obviously, MDM2 inhibiting drugs could be used to activate p53 in tumors that retain wild type p53. However, an alternative approach harnesses the inability of tumor cells containing mutant p53 to cease proliferation in response to MDM2 inhibitors. In this approach, activation of p53 could be used to protect normal cells that retain wild type p53 from the toxic effects of mitotic poisons (through the induction of a reversible cell cycle arrest). The chemotherapy would therefore specifically target only the remaining cycling (mutant p53 containing) tumor cells [266,267].

Finally, development of inhibitors of p53, potentially by activating MDM2 E3 ligase activity, could also prove beneficial as a chemo- and radio-protective treatment where toxicity is related to the activation of p53. Again, the approach depends on the protection of normal tissue – in this case by transient inactivation of p53 – possibly allowing for higher doses of irradiation to target p53 mutant cancer cells. The potential benefit of such an inhibitor is supported by mouse studies, where a temporary inactivation of p53 renders normal tissues less sensitive to apoptosis by irradiation, while leaving them competent to antagonize oncogene activation by growth arrest and induction of senescence [245].

The complexity of the systems that regulate p53 stability will provide many other potential drug targets, including other E3s and DUBs. Although at an early stage, these studies have the potential to yield powerful tools to help fully comprehend the complexity of the interplay between p53 and ubiquitination, and develop new, targeted therapies for cancer treatment.

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