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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 33 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.

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 change 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 most abundant and 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, the second most abundant poly-ubiquitin chain, does not target degradation but 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].

The other poly-ubiquitin chains, termed "atypical ubiquitin chains" (reviewed in [8]), include poly-ubiquitin chains linked via the "unconventional" lysines in ubiquitin, branched ubiquitin 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 is less well understood, although they have been shown to drive signaling, provide novel binding sites for partner proteins and target proteolysis. Overall these unconventional modifications are of much lower abundance than K48 or K63 linked chains, but their impact 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 frequently 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. 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/X 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/X 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 not fully understood, but very likely to be dependent on the context of other modifications of p53 [57] and the choice of experimental model (Fig. 1) .

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

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