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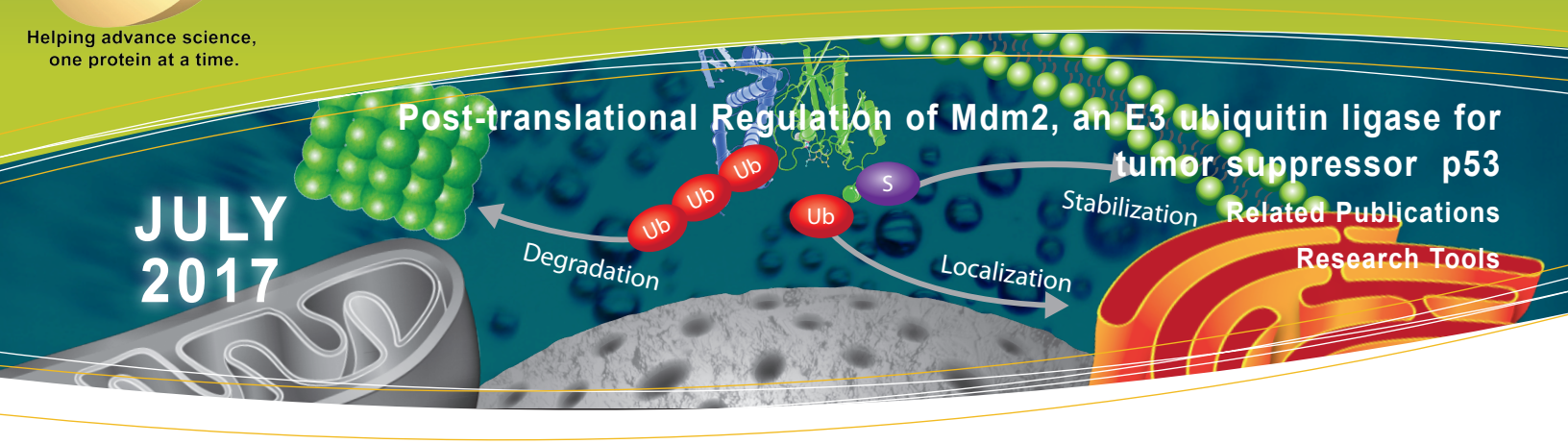
JULY 2017



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Post-translational regulation of Mdm2, an E3 ubiquitin ligase for tumor suppressor p53

The ubiquitin E3 ligase Mdm2 (murine double minute 2; human homolog, Hdm2) is well known for its oncogenic activities and as the master regulator of the powerful tumor suppressor p53[1,2]. Moreover, Mdm2 may function as an oncogenic protein independent of p53[3]. Mdm2 is able to inhibit p53-mediated gene expression through two pathways: inhibition of transcriptional activity by direct binding and ubiquitin-mediated degradation via its E3 ligase activity[4]; however, the effectiveness of p53 inhibition by direct Mdm2 binding has been questioned[5].

Under normal, non-stress conditions, Mdm2 maintains p53 expression and activity at a minimal level to tightly regulate its apoptotic/cell death transcriptional activities. Under conditions of cellular stress, Mdm2-mediated ubiquitination of p53 ceases, allowing p53 to activate transcription of apoptotic genes and those involved in inhibiting cell growth. Much research concludes this is due to Mdm2's auto-inhibition by self-ubiquitination[1-3]. However, this story appears to be more complex than originally thought[5] and involves multiple post-translational modifications (PTMs). Here, we discuss Mdm2's regulation by ubiquitination, SUMOylation, phosphorylation, and acetylation[6-8] (Fig. 1).

Ubiquitination and SUMOylation of Mdm2

Mdm2 is ubiquitinated, at least partially by Mdm2 itself (auto-ubiquitination, common to most E3 ligases), which leads to its destabilization and degradation[1-3] (Fig. 1). However, Mdm2's E3 ubiquitin ligase activity is not necessary for Mdm2 degradation, suggesting that auto-ubiquitination is not the only means by which Mdm2 is degraded[5]. There are other candidate proteins with E3 ligase activity such as p300-CBP-associated factor (PCAF) which ubiquitinates Mdm2 and regulates its stability[9]. The role of ubiquitination in regulating Mdm2 activity is further complicated with the report that poly-ubiquitination of Mdm2 activates its E3 ligase activity as this modification significantly enhanced the capacity to poly-ubiquitinate p53[10]. Interestingly, poly-, but not mono-, ubiquitination upregulated Mdm2's E3 ligase activity[10]. These findings challenge the assumption that auto-poly-ubiquitination only signals degradation[10].

SUMOylation confers proteins with differential stability, localization, inter-protein interaction, and DNA binding/transactivation[11]. Mdm2 is SUMO1-modified by SUMO E3 ligases Ubc9, PIAS1, and RanBP2[12,13] (Fig. 1). Upon nuclear entry, Mdm2

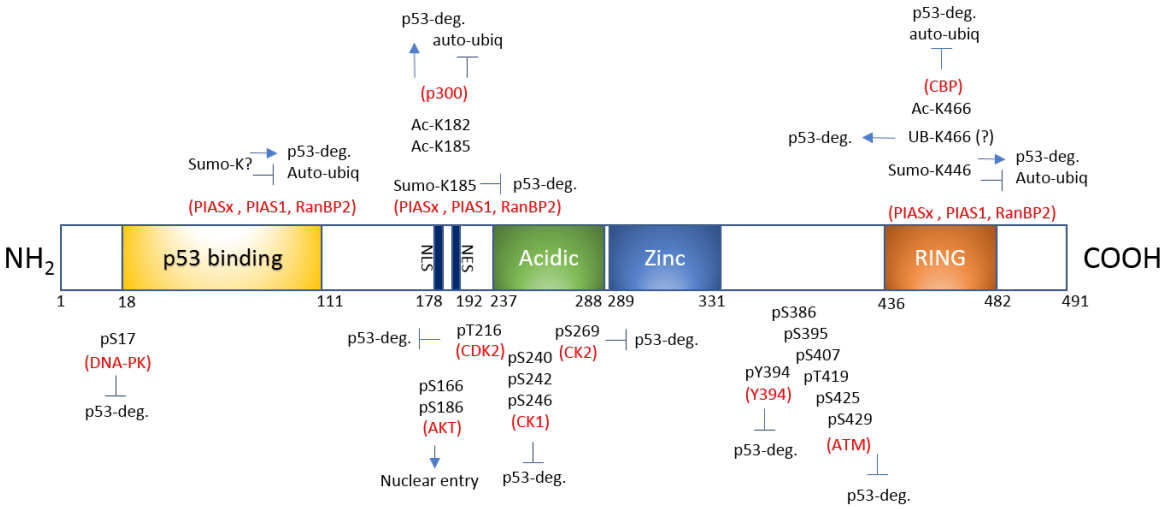

Fig 1. PTMs of Mdm2. Mdm2 undergoes multiple site modifications to regulate its binding to p53 and proteasomal degradation of either p53 or Mdm2 itself. Acidic: acidic domain; Zinc: zinc finger; RING: RING finger; NLS: nuclear export signal and NES: nuclear localization signal.

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is SUMO1-modified by RanBP2 and further SUMOylated by PIAS, which favors p53 stability and abundance in the nucleus[13]. This modification can be reversed by stress induction as UV irradiation decreases Mdm2 SUMOylation[12]. SUMOylated Mdm2 minimizes auto-ubiquitination and maximizes its E3 ligase activity toward p53[12]. This is consistent with the idea that SUMO and ubiquitin modifications may be mutually exclusive and antagonistic.

Multi-site Phosphorylation of Mdm2

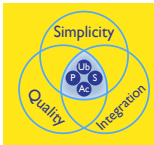
Serine and threonine residues constitute almost 20% of Mdm2's amino acids and many of these are phosphorylated by multiple kinases (Fig. 1). They are heavily clustered in two distinctive sites, the N-terminal and Central domains[14]. The phosphorylation of Mdm2 fine-tunes mainly the interaction with p53. Ataxia-telangiectasia mutated (ATM) kinase-mediated phosphorylation of Mdm2's S395 residue destabilizes Mdm2, thus reducing p53 degradation. Conversely, PI3-K/Akt-mediated phosphorylation of S166 and S186 residues stabilizes Mdm2 and destabilize p53[3,6,15] (Fig. 1). Upon genotoxic stress, DNA-dependent protein kinase (DNA-PK) phosphorylates the S17 residue of Mdm2, which dissociates p53 from Mdm2[16]. As might be expected, protein phosphatase 1D (PPM1D; a.k.a. wild-type p53-induced phosphatase 1)-mediated dephosphorylation of phosphorylated S395 stabilizes Mdm2 and facilitates p53 degradation[17] (Fig. 1).

Acetylation of Mdm2

Mdm2 is also acetylated by CREB-binding protein (CBP) and p300 *in vitro* and CBP *in vivo*, and the acetylation primarily occurs in the C-terminal RING finger domain[8,18]. These data suggest that acetyltransferases may positively modulate cellular p53 activity indirectly by inactivating Mdm2. *In vitro* acetylation by p300 also occurs within a central domain (124-246 amino acids) of Mdm2[18]. More recently, acetylation on two lysine residues (K182 and K185) in the nuclear localization sequence has been identified[7] (Fig. 1). Under normal, non-stress conditions, Mdm2 is acetylated by p300 on K182 and K185, facilitating binding of the deubiquitinase HAUSP (herpes virus-associated ubiquitin-specific protease) to Mdm2. These modifications (acetylation, presumed de-ubiquitination) stabilize Mdm2 and protect it from auto-ubiquitination, which correlates with increased inhibition of p53 activity. Genotoxic stress activates the deacetylase SIRT1 and SIRT1-mediated deacetylation of these sites promotes Mdm2 auto-ubiquitination, thereby increasing p53 stability and subsequent activity[7,19]. Therefore, p300-mediated acetylation and SIRT1-mediated deacetylation on K182 and K185 regulate the functional stability of Mdm2 (with help from HAUSP) and serve as molecular switches for control over p53 activity[7] (Fig. 1).

Summary

PTMs are well known regulators of a protein's function, localization, and stability. In the case of Mdm2, the ubiquitination, SUMOylation, phosphorylation, and acetylation modifications regulate Mdm2's function and stability both independently and via cross-talk with one another. In so doing, precise and quick Mdm2-mediated control over p53-stimulated cell death and survival is possible under various types and intensities of cellular stress. Cytoskeleton, Inc. offers many different Signal Seeker™ research tools for dissecting and analyzing the role that PTMs play in the regulation of virtually any protein, including clinically relevant proteins in neurobiology, cardiology, rheumatology, and oncology.



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Ubiquitin Affinity Beads	Beads	40 reactions	UBA01-beads
Control for IgG Beads	Beads	10 reactions	CIG01-beads
Control for Ubiquitin Affinity Beads	Beads	10 reactions	CUB02