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Post-translational Modifications Modulate p53 Tumor Suppressor Functions

The multi-domain, tetrameric p53 protein was discovered in 1979¹⁻³, first mistakenly described as an oncogene, before its true function as a powerful tumor suppressor was realized^{4,5}. p53 consists of two N-terminal transactivation domains (TAD1 and TAD2), a proline-rich domain (P-rich), a sequence-specific DNA binding domain (DBD), a linker domain, tetramerization domain (TD), and a lysine-rich, basic C-terminal regulatory (REG) domain⁶ (Fig. 1). As a transcription factor, p53 can regulate the expression of up to 3000 genes involved in apoptosis, senescence, cell cycle arrest, DNA repair, apoptosis, tumor microenvironment, autophagy, and invasion/metastasis⁶⁻⁸. p53 functionality is spatiotemporally regulated by up to fifty post-translational modifications (PTMs) that occur within multiple domains⁹⁻¹² (Fig. 1). Here, regulation of p53 by ubiquitination, phosphorylation, and acetylation is discussed.

Ubiquitination

During normal cell growth and development (i.e., non-stress conditions), the MDM2 (mouse double minute 2 protein, mouse homolog of human protein HDM2) E3 ligase maintains p53 expression at a minimal level by ubiquitin-proteasome-regulated degradation through a protein complex consisting of MDM2, p53, and p300/CREB binding protein (p300/CBP)^{13,14}. MDM2 binds p53's TAD1 and negatively regulates p53 as it mediates poly-ubiquitination on at least six different lysine residues within p53's REG domain¹⁵ (Fig. 1). Importantly, MDM2

directly mediates mono-ubiquitination at multiple sites on p53¹⁶, but is not directly responsible for poly-ubiquitination. Instead, p300/CBP, which has both ubiquitin ligase and acetyltransferase activity, subsequently utilizes these mono-ubiquitinated lysines as substrates for poly-ubiquitination^{13,14}. Notably, numerous other E3 ligases, such as Pirh2, COP1, CHIP, ARF-BP1, E6-AP, TOPORS, TRIM24, and MKRN1, are also capable of regulating p53 turnover⁹.

Phosphorylation

Phosphorylation of serine/threonine (Ser/Thr) residues stabilizes and activates p53 and is triggered by cellular stresses, including genotoxic damage, oncogene activation, hyperproliferative signals, hypoxia, and nutrient starvation^{17,18}. When activated, tetrameric p53 forms active transcriptional complexes on target gene promoters to regulate expression of genes involved in cell cycle arrest and apoptosis. Arguably, the most important phosphoepitope governing p53 activation is Ser15 as its phosphorylation is required for subsequent phosphorylation of other p53 residues (e.g., Thr18 and Ser20)^{9,19}. In fact, Thr18 phosphorylation is the critical catalyst for disruption of MDM2 binding. Phosphorylation of neighboring Ser residues (Ser20, -33, -37, -46, and -55) within the TAD1 further reduces MDM2 binding affinity to p53^{9,19-26} (Fig. 1). Concomitant with this loss of MDM2 binding is the recruitment and binding of the co-transcriptional activator p300/CBP^{9,19}, whose binding with p53

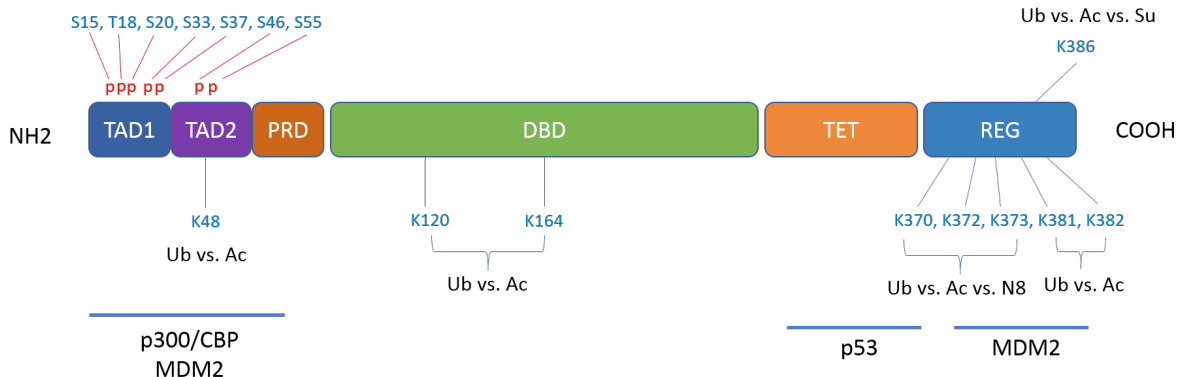


Figure 1. PTMs of p53. p53 is a substrate of approximately 50 PTMs within multiple domains. The PTMs show interdependence, cooperativeness, and/or mutual inhibition.



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is also critically reliant upon Ser15 and Thr18 phosphorylation^{21,22}. p300/CBP has four structurally similar domains (TAZ1, KIX, TAZ2, and NCBD) that bind p53's TADs with varying degrees of affinity²⁴⁻²⁶. Upon di-phosphorylation of p53, bound MDM2 dissociates and p53's affinity for the KIX domain is mildly enhanced, and affinity for both KIX and TAZ1 domains is significantly increased by sequential phosphorylation of the neighboring Ser residues (~80 fold, compared to Thr18)^{9,24-26}. Indeed, p300/CBP binding to, and activation of, p53 occurs along a gradient which increases with multi-site phosphorylation of p53^{22,24,26}.

Acetylation

Besides phosphorylation, p300/CBP-mediated acetylation is essential for p53 stabilization and activation in response to cellular stresses²⁷⁻²⁹. At least six lysine residues (Lys370, Lys372, Lys373, Lys381, Lys382, and Lys386) within the REG domain of p53 are targeted for acetylation by p300/CBP. This prevents MDM2-mediated ubiquitination of these lysines and maintains p53 transactivation capacity^{9,28}. Also, p53 acetylation is indispensable for destabilizing the p53-MDM2 interaction, regardless of phosphorylation status. Acetylation of two lysines (Lys120 and Lys164) in p53's DBD and six lysines in the REG domain blocks the recruitment of MDM2 to the p53 complex binding promotor and enables the p53-mediated stress response²⁹ (Fig. 1). MDM2 promotes p53 deacetylation through the recruitment of a HDAC1 complex²⁸, which allows for poly-ubiquitination of p53 and eventual p53 degradation by the proteasome.

Summary

The tetrameric p53 tumor suppressor protein regulates the expression of thousands of genes involved in cell cycle arrest, apoptosis, and associated pathways. Thus, it is tightly negatively regulated by MDM2- and p300/CBP-mediated poly-ubiquitination. Cellular stresses disinhibit p53 through phosphorylation- and acetylation-mediated stabilization and activation. HDAC1-mediated deacetylation returns p53 to a quiescent state until the arrival of the next cellular stress. To better understand how these and other PTMs can regulate p53 and other cancer-related proteins, Cytoskeleton, Inc. offers Signal-Seeker Enrichment Kits to study the endogenous levels of tyrosine phosphorylation, ubiquitination, acetylation, and SUMOylation in cell and tissue lysates, as well as PTM antibodies validated for multiple applications.

PTM Antibodies, Beads, Etc

Kit or Affinity Beads	Type	Size	Cat. #
Acetyl-Lysine Antibody Mouse Monoclonal (7B5A1)	MAB	2 x 100 ul 1 x 25 ul	AAC02 AAC02-S
Acetyl-Lysine Antibody Mouse Monoclonal (19C4B2.1)	MAB	2 x 100 ul 1 x 25ul	AAC03 AAC03-S
Acetyl-Lysine-HRP Antibody Mouse Monoclonal (19C4B2.1)	MAB	1 x 100 ul 1 x 25 ul	AAC03-HRP AAC03-HRP-S
Acetyl-Lysine Affinity Beads	Beads	4 x 500 ul	AAC04-beads
Phosphotyrosine Affinity Beads	Beads	4 x 330 ul	APY03-beads
Phosphotyrosine-HRP Antibody Mouse Monoclonal (27B10)	Beads	4 x 330 ul	APY03-HRP
SUMO 2/3 Affinity Beads	Beads	2 x 400 ul	ASM24-beads
Ubiquitin Antibody Mouse Monoclonal	Beads	40 reactions	AUB01
Control for IgG Beads	Beads	10 reactions	CIG01-beads
Control for Acetyl-Lysine Beads	Beads	10 reactions	CIG02-beads
Control for Ubiquitin Affinity Beads	Beads	10 reactions	CUB02-beads

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Signal Seeker™ Kits

Kit or Affinity Beads	Type	Reactions	Cat. #
Signal-Seeker™ Acetyl-Lysine Detection Kit	Kit	30	BK163
		10	BK163-S
Signal-Seeker™ Phosphotyrosine Detection Kit	Kit	30	BK160
		10	BK160-S
Signal-Seeker™ Ubiquitin Detection Kit	Kit	30	BK161
		10	BK161-S
Signal-Seeker™ SUMO 2/3 Detection Kit	Kit	30	BK162
		10	BK162-S