

P022 Regulation of p53 stability and activity by Multiple Mono Ubiquitylation

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The ubiquitin-ligase Mdm2 plays a role in maintaining low levels of p53 by promoting its ubiquitylation and proteasome-mediated degradation. To explore the contribution of p53 sequences in its ubiquitylation, degradation by Mdm2, subcellular localization and transcription during DNA damage, without interference of the transcription-mediated regulatory feedback, we used a transfer-of-signals strategy. Our results show that, Mdm2-mediated degradation requires multiple N- and C-terminal p53 sequences to drive ubiquitin-proteasome-dependent degradation. As for WT p53, p53-fusions able to oligomerize, are also poly-ubiquitylated and efficiently degraded by Mdm2. However, non-oligomerizable p53-fusions exhibit a multiple mono-ubiquitylation-dependent degradation. While the Mdm2 binding site and a region containing the 6 most C-terminal lysine residues are sufficient to promote degradation, artificial nuclear localization of p53-EGFP-fusions favors Mdm2 binding, multi-ubiquitylation and degradation of non-oligomerizable p53-fusions suggesting that degradation is primed in the nucleus. Identified signals promote the p53 early degradation step mediated by Mdm2 and activated by genotoxic insults. Single point mutations in the oligomerization domain of the p53 fusions are only multi-ubiquitylated and show an efficient Mdm2-mediated degradation similar to WTp53. Our results support a model where multi-ubiquitylation driven degradation is a main process controlling stability of p53 monomers which might contribute to the one regulated by poly-ubiquitylation of the transcriptionally active p53 tetramers.