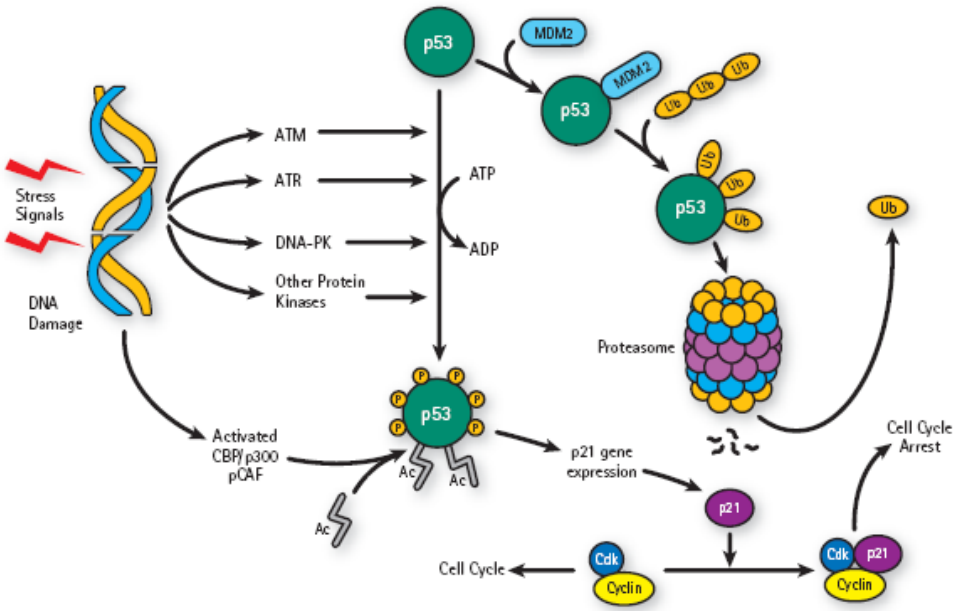
p53 and MDM2: Partners in Repair and Death

Chandra Mohan, Ph.D. EMD Chemicals, San Diego, California

p53, a well-conserved phosphoprotein, is one of the best known tumor suppressors. Human p53 consists of 393 amino acids assembled into four structurally and functionally different domains: an acidic N-terminal region that contains the 42 amino acids transactivation domain followed by a hydrophobic proline-rich region (amino acids 64 to 92), a central sequence-specific DNA-binding domain (amino acids 102 - 292), a tetramerization domain (amino acids 324 - 355), and a highly basic C-terminal region regulatory domain (amino acids 363 - 393). p53 is a sequence-specific nuclear transcription factor that binds to defined consensus sites within DNA as a tetramer and represses transcription of a set of genes involved in cell growth stimulation, while activating a different set of genes involved in cell cycle control. It causes growth arrest before either DNA replication in the G_1 phase or mitosis in the G_2 phase. This provides a window for DNA repair or elimination of cells with severely damaged DNA strands. Hence, p53 is considered as an important regulator of DNA repair that ensures genomic integrity. Agents that damage DNA induce p53 to become very stable by a post-translational mechanism, allowing its concentration in the nucleus to increase dramatically.

In unstressed cells, p53 is latent and is maintained at low levels by targeted degradation mediated by MDM2. Through its binding to p53, MDM2 can shuttle p53 out of the nucleus into the cytoplasm for degradation. When normal mammalian cells are subjected to stress signals, such as hypoxia, radiation, and chemotherapeutic drugs, p53 is phosphorylated at multiple sites, including those involved in it's binding to MDM2. This leads to its activation and blockage of its ubiquitin-dependent degradation. Activation of p53 can result in cell cycle arrest, presumably to allow for DNA repair before replication or mitosis. In some cell types, however, p53 activation results in apoptosis as means of eliminating severely damaged cells. The final outcome of p53 activation depends on many factors, and is mediated largely through the action of downstream effector genes transactivated by p53.

Human p53 is phosphorylated at least at 23 different sites by stress-activated protein kinases, DNA Protein kinase (DNA-PK), casein kinase I and II, and cyclin-dependent kinases. Although the exact functions of specific phosphorylation at various sites is still controversial, evidence indicates that phosphorylation of p53 provides stability by promoting its dissociation from MDM2 and enhancing its transcriptional activity. Most of the p53 phosphorylation sites are clustered within the 40 amino acids at its



N-terminal region. ATM and ATR kinases promote phosphorylation of human p53 at Ser^{15} and Ser^{20}, which are essential for the activation of p53 following DNA damage. DNA-PK phosphorylates Ser^{15} within the critical N-terminal region of p53, which controls the interaction of p53 with the transcriptional apparatus and with the MDM2 protein. DNA-PK also phosphorylates Ser^{9} and Thr^{18}; however, phosphorylation at these sites is dependent upon the presence of the full-length p53, but is independent of phosphorylation at other sites. Phosphorylation at Thr^{18} alters the structure of the amphipathic α-helix with which MDM2 interacts. Studies have shown that when p53 co-localizes with DNA-PK and ssDNA, there is a 10-fold enhancement of p53 phosphorylation. Casein Kinase I can also phosphorylate Ser^{9} and Thr^{18}, however, these phosphorylations are dependent upon prior phosphorylation of Ser^{6} and Ser^{15}. All types of tumor cells exhibit higher levels of p53 phosphorylation when compared to normal non-transformed cells. These phosphorylations offer greater stability to p53 regardless of p53 mutations.

In spite of extensive studies on p53 phosphorylation, it is now known that phosphorylation is not the only mechanism that regulates activation of p53. Following cellular stress, p53 is shown to be acetylated by CBP/p300 at multiple lysine residues ($Lys^{370, 372, 373, 381, and 382}$) and by pCAF at Lys^{320}. The physiological relevance of p53 acetylation is still controversial, although acetylation does correlate well with increased cellular stress. Additional support for the role of acetylation comes from studies that show that increasing the level of p53 acetylation with deacetylase inhibitors prevents p53 from degradation. Over-expression of MDM2 is also shown to effectively reduce p300-dependent p53 acetylation.

p53 is shown to be either non-functional or mutated in most human cancers. The most common anomaly of p53 in human cancers is mutation of the *p53* gene. A large number of mutations are caused by single base substitutions, and about 30% of these mutations are reported to occur in hotspot codons. Functional p53 provides a protective mechanism against tumor growth, and a loss of p53 function is a key step in the neoplastic cascade. In addition, the function of p53 is critical to the success of many cancer treatments since radiation and chemotherapy act in part by triggering cell suicide in response to DNA damage. A successful response to therapy is greatly reduced in tumors where mutant p53 is present, and these tumors are often very difficult to treat. The p53 network in normal, non-activated situations is non-functional, but is activated in cells as a response to various signals that take place in the carcinogenic process. Carcinogen-induced DNA damage, abnormal proliferative signals, hypoxia, and loss of cell adhesion are some of the most common signals that activate p53.

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