

■ IMMUNOLOGY

WIP-ing Down p53 During Thymic Ontogeny

Schito ML, Demidov ON, Saito S, Ashwell JD, and Appella E. Wip1 phosphatase-deficient mice exhibit defective T cell maturation due to sustained p53 activation. *J Immunol* 176: 4818–25, 2006.

In 1997, we reported the identification of a novel gene transcript whose expression was induced in response to ionizing radiation in a p53-dependent manner, and whose protein product showed homology to the type 2C protein phosphatases. We named the novel gene, wildtype p53-induced phosphatase 1 (*WIP1*), and it was later given the Genebank symbol, *PPM1D* (for protein phosphatase 1D magnesium-dependent, delta isoform). Preliminary results suggested that WIP1 might contribute to growth inhibitory pathways activated in response to DNA damage in a p53-dependent manner.

To investigate possible functions of WIP1, we evaluated its substrate specificity (Figure 1). We first found that WIP1 was able to specifically dephosphorylate the phosphatase p38 MAP kinase at phospho-Thr180, and its overexpression blocked UV-induced p53 activation in cultured human cells. Therefore, this phosphatase, which is induced by p53, can act in a negative feedback mechanism to turn off p53-activating signals. However, phosphatase activity is not usually directed to a single substrate. WIP1 was found to also specifically dephosphorylate the nuclear isoform of uracil DNA glycosylase (UNG2) at phospho-Thr6, leading to suppression of DNA base-excision repair. More recently, WIP1 was reported to dephosphorylate Chk1 at phospho-Ser345, Chk2 at phospho-Thr68, and p53 at phospho-Ser15. Because these signaling molecules activate DNA repair pathways and cell cycle checkpoints to maintain genomic integrity, suppression of these key homeostatic functions may, in part, reflect *WIP1*'s character as an oncogene. WIP1 is amplified or overexpressed in several types of human tumors, including breast tumors, neuroblastomas, and ovarian clear cell adenocarcinomas. In fact, the gene encoding WIP1 (*PPM1D*, at 17q22/q23) is amplified in human breast tumor cell lines and in approximately 11% of primary breast tumors, most of which harbor wild-type p53. This suggests that *PPM1D* overexpression contributes to the development of human cancers by suppressing p53 activation.

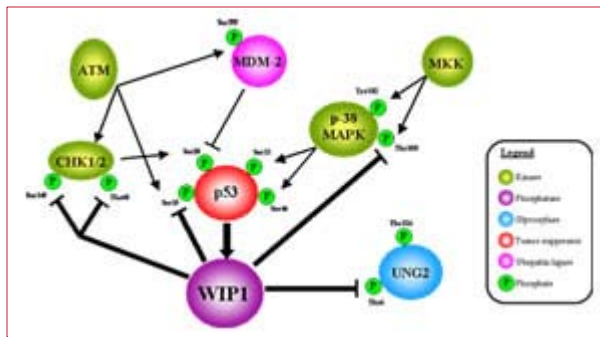


Figure 1. Substrate specificity of the type 2C protein phosphatase WIP1, which directly or indirectly modulates p53 activity or DNA repair.

To further determine the normal biological function of WIP1 in mammalian organisms, we generated WIP1-deficient mice. These mice were viable but showed a variety of postnatal abnormalities. Notably, mice lacking WIP1 showed increased susceptibility to pathogens and diminished T- and B-cell functionality. Our studies also determined that fewer lymphoid cells were present in the periphery, a finding that could not be accounted for by reduced proliferation or enhanced apoptosis. This prompted us to examine T-cell development in the thymus more closely.

T cells derived from the thymus provide the classical cell-mediated host response that controls intracellular pathogenic organisms. Although many of the players involved in thymic ontogeny have been identified, there still remain large gaps in our knowledge of the molecular pathways involved. In the late 1990s, the potential involvement of the tumor suppressor protein p53 in T-cell development was suggested by the partial rescue of certain immunodeficient mouse models by the elimination of p53. However, because p53-deficient animals exhibited normal T-cell development and were able to mount normal immune responses to pathogens, it was accepted that this tumor suppressor plays no role in T-cell development.

The number of T cells in the thymus of young WIP1-deficient mice is severely reduced, but as the animals age, the normal process of thymic involution does not occur. Therefore, the number of thymocytes in age-matched WIP1-deficient mice approaches that of normal mice as the animals age. Concentrating on young mice, we determined that the loss of T cells was occurring in α/β T cell-receptor cells between the double negative (DN)–to–double positive (DP) transition. Specifically, we observed a block at the last stage (DN4) of development in mice lacking WIP1 that corresponded to the maximal WIP1 mRNA expression found in normal mice. The absence of WIP1 resulted in defective cell cycle progression of the DN4 cells. The few cells that did acquire the DP phenotype were more susceptible to spontaneous apoptosis, possibly because of lower levels of the anti-apoptotic factor Bcl-x_L. Although cell cycle progression and apoptosis are controlled by a number of mechanisms, both can be controlled by p53. We determined that p53 protein levels, p53 phosphorylation status, and the levels of the downstream effector protein p21 were increased in DN4 and DP cell populations in the absence of WIP1. It is interesting to note that increased p53 activity occurs between the

DN-to-DP transition, because some T cell-deficient mice (those with severe combined immune deficiency, *Rag1/2^{-/-}*, and *CD3ε^{-/-}*), which have a severe defect in the same transition, can be partially rescued by the absence of p53. Because p53 was upregulated during the transition in the absence of WIP1, we crossed WIP1-deficient mice with those that were p53 deficient to determine if T-cell development can be rescued by eliminating p53 activity. Thymic development in these doubly deficient mice was normal, suggesting that elevated levels of p53 were detrimental to α/β T-cell development. Thus, WIP1 phosphatase appears to play an important role in T-cell development by turning off p53 at a key point in thymic development.

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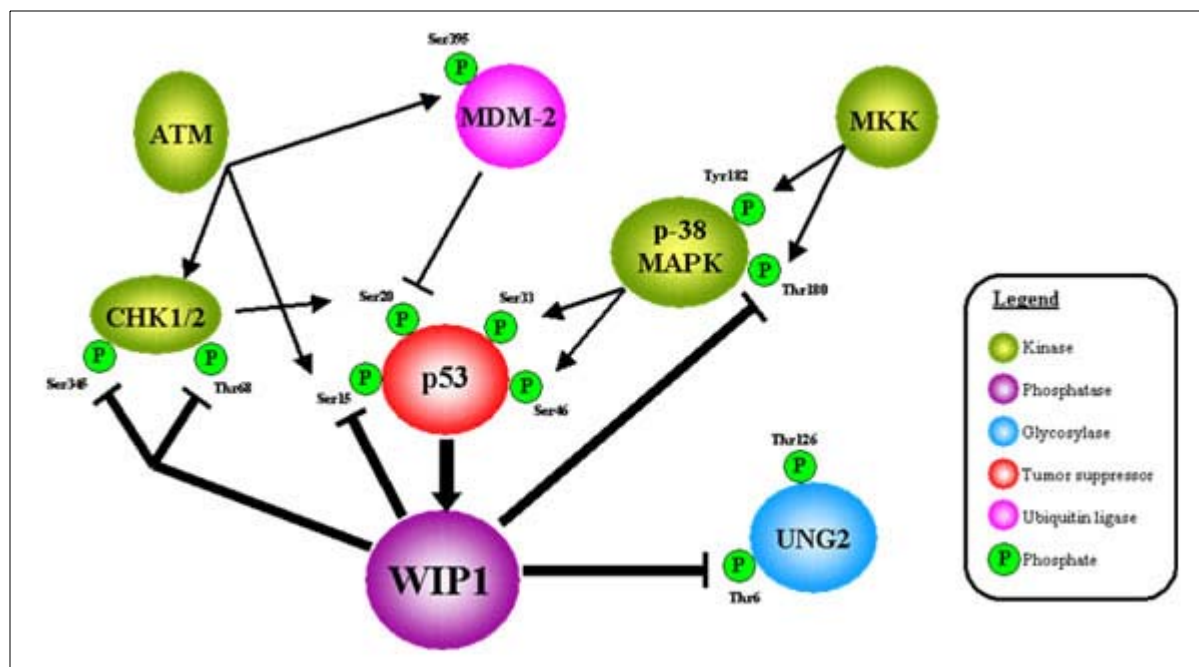


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