PTEN and p53: Who will get the upper hand?

Mutations of *PTEN* and *p53* are very frequent, yet often mutually exclusive due to functional interdependence of the proteins and, according to a new study, the most intimate possible interaction: direct binding.

Tumor suppressor genes are conceptually regarded as autonomous anti-cancer units. Along this tenet, only their homozygous loss would lead to cancer pathogenesis (Knudson, 1971). At the cellular level, however, autonomy appears to be a remote concept since protein function and gene expression are well regulated through cooperating and antagonizing networks. Therefore, linking the regulatory mechanisms between tumor suppressors is key to understanding, predicting, and interfering with tumorigenesis. Given that previous work has demonstrated several layers of indirect crosstalk between the two most highly mutated tumor suppressors, p53 and PTEN, it would have certainly been tempting to speculate that PTEN also directly cooperates with p53 in tumor suppression. Mechanistically however, the role of PTEN in tumor suppression has been attributed to the cytoplasm while the site of action of p53 is definitely ascribed to the nucleus, thus rendering it problematic to envision how this would occur. According to a study published in this issue of *Cancer Cell* (Freeman et al., 2003), a direct functional crosstalk between PTEN and p53 does indeed exist, adding a novel twist to the emerging picture of the PTEN-p53 tumor suppressor network.

The p53 tumor suppressor acts as a transcription factor capable of regulating the expression of target genes critical for growth inhibition, cell senescence, and induction of apoptosis in response to DNA damage and oncogenic transformation (Sharpless and DePinho, 2002). Cells are therefore extremely sensitive to p53 levels and transcriptional activation. In the absence of stress, levels of p53 in normal cells are maintained low mostly through its constant proteasome-dependent degradation mediated by the mdm2 protein that acts as a p53 ubiquitin-ligase. A number of p53 posttranslational modifications that are subjected to a dynamic and tight regulation including phosphorylation, acetylation, and sumoylation lead to its stabilization and transcriptional activation upon cellular stress. The extraordinary multilayered complexity of the p53 regulatory network and its central importance in tumor suppression make it an ideal molecular platform on which other tumor-suppressive pathways could impinge.

PTEN (for phosphatase and tensin homolog deleted on chromosome 10) mutations and partial or complete loss have been frequently reported in tumors of various histological origins. *PTEN* is also found mutated in three related human autosomal dominant disorders characterized by developmental defects and high tumor susceptibility (Di Cristofano and Pandolfi, 2000).

The *PTEN* gene encodes a dual specificity phosphatase, which to some surprise, however, has been shown to primarily dephosphorylate the plasma membrane lipid phosphatidyl-inositol-3-phosphate (PIP3) whereas examples of protein targets have remained rare. Conversion of PIP3 to PIP2, however, enables PTEN to effectively antagonize the PI3 kinase pathway, resulting in inactivation of the many PH domain-containing downstream protein kinases, most notably Akt and PDK1 (Cantley, 2002), which in turn act on a variety of proteins to stimulate cell survival, proliferation, growth, and migration.

In the mouse, complete *Pten* inactivation is embryonic lethal while *Pten* heterozygous mice co-develop, after a latency period, multiple tumors of various histological origins in complete agreement with an important and pleiotropic tumor suppressive role (Di Cristofano and Pandolfi, 2000). In *Pten* heterozygous mutants that have also lost one or both copies of the $p27^{KIP1}$ gene, encoding a cell cycle inhibitor whose loss does not cause an overt cancer phenotype, *Pten*-specific tumors are markedly accelerated (Di Cristofano et al., 2001). Most notably, these tumors retain *Pten* expression, indicating that its tumor-suppressive function might be dose dependent, and Pten thus haploinsufficient in certain tissues in combination with loss of another tumor suppressor. These findings underscored the importance of establishing the regulatory mechanisms governing PTEN protein levels and activity as their tight regulation may be critical for tumor suppression.

The first report to link PTEN and p53 on a molecular level showed direct binding of p53 to a site in the *PTEN* promoter (Stambolic et al., 2001). On top of p53-independent basal expression, DNA-damaging events such as γ irradiation resulted in a p53-dependent increase of *PTEN* mRNA in several cell lines (Figure 1A, pathway 2). Moreover, in *Pten* null mouse embryonic fibroblasts (MEFs), p53-mediated apoptosis was impaired, underscoring the potential importance of *Pten* transactivation for this process. Altogether, this report positioned p53 upstream of *PTEN* in the regulation of its expression level.

However, it soon became apparent that PTEN could also regulate p53 function through its ability to antagonize PI3 kinase since Akt phosphorylation consensus sites were identified in mdm2, one of the major negative regulators of p53 (Mayo and Donner, 2001; Zhou et al., 2001). Upon phosphorylation of these sites, mdm2 translocated into the cell nucleus to ubiquitinate p53, resulting in its nuclear export and degradation. Since PTEN phosphatase activity is the major antagonist of Akt, it was therefore tempting to speculate that PTEN would prevent this p53 degradation pathway by keeping Akt inactive and that PTEN would therefore be an essential component of the p53 response upon DNA damage (Figure 1A, pathway 1). Indeed, the effect of PTEN on p53-mediated transcription was also soon reported (Mayo et al., 2002). PTEN, but not a phosphatase-dead mutant, was able to produce a moderate increase of p53-mediated transcription even in the presence of mdm2, indicating that PTEN by antagonizing PI3 kinase can indirectly protect p53 from the degradation pathway. Importantly, the above findings suggested that PI3 kinase inhibitors such as Wortmannin could sensitize refractory tumors with wt p53 to treatment with p53-inducing drugs such as etoposide by mimicking PTEN activity and thus increasing p53 stability (see Figure 1B).

In a study appearing in this issue of *Cancer Cell* (Freeman et al., 2003), the PTEN-p53 crosstalk is taken a step further by identifying a p53 binding domain in PTEN. By performing GST-pulldown

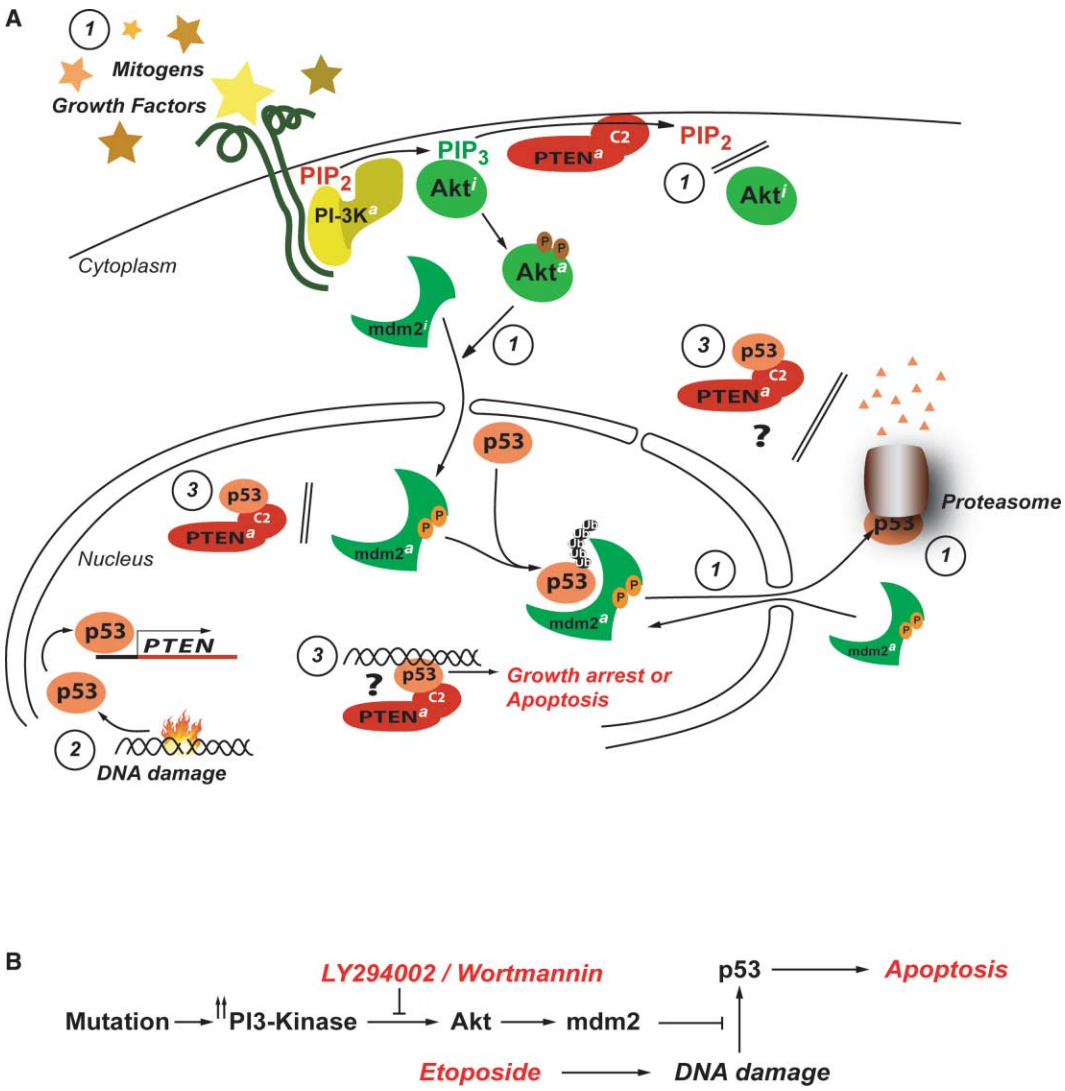

Figure 1. Functional crosstalk between PTEN and p53 and its therapeutic implications

A: PTEN and p53 interact on three levels.

1: The PTEN phosphatase inhibits activation of mdm2 by keeping Akt inactive. Akt, when activated through the PI3 kinase pathway, can phosphorylate cytoplasmic mdm2, resulting in its nuclear import. Nuclear mdm2 ubiquitinates p53 and targets it for proteasomal degradation in the cytoplasm.

2: DNA damage results in a sharp increase of p53 protein which, in turn, can enhance *PTEN* transcription by binding to the *PTEN* promoter leading to a positive feedback loop that protects p53 from degradation.

3: Pten binds to p53 resulting in increased p53 half-life. This interaction also stimulates p53-mediated transcription, although it is still unclear whether PTEN participates in a p53-DNA complex. Cytoplasmic PTEN may also antagonize p53 degradation by cytoplasmic proteasomes.

Active or inactive forms of enzymes are denoted by an "*a*" or "*i*" superscript, respectively.

B: Tumor cells can be resensitized to DNA damage-inducing agents.

Tumor cells can be incapable of a p53 response in spite of having the functional protein because loss of, e.g., PTEN function results in increased PI3 kinase activity. Thus, combination of DNA damage-inducing agents with PI3 kinase inhibitors can restore drug sensitivity.

experiments with p53 deletion mutants, the C terminus of p53 was found to bind PTEN. These in vitro binding studies could be confirmed by a series of co-immunoprecipitations in both human cell lines and in cells derived from a *Pten* conditional mouse knockout model. Intriguingly, on PTEN, the p53 binding activity was mapped to the C2 domain, which so far had only been implicated in stabilizing and properly positioning the catalytic phosphatase domain of PTEN at the plasma membrane (Georgescu et al., 2000; Lee et al., 1999). This finding led the authors to ask whether PTEN might regulate p53 activity independently of its enzymatic activity. The results indicate that phosphatase-dead mutants

of PTEN are capable of stabilizing p53 as well. In half-life experiments, catalytically inactive PTEN could even protect p53 from mdm2-independent degradation, indicating that it can counteract additional mechanisms aimed at degrading p53. In this respect, it could be speculated that PTEN can also compete with proteasomal degradation of p53 in the cytoplasm (Figure 1A, pathway 3). In human SAOS2 cells, it was furthermore observed that protection did not affect p53 mRNA levels, consistent with degradation occurring at the protein level. Next, the authors tested whether the interaction with PTEN might separately enhance p53-mediated transcription. In transcription transactivation assays adding back *PTEN* into *Pten* null MEFs, an increase in p53-mediated transcription could be observed in agreement with the previous findings by Mayo et al. However, in conflict with that report, add back of a phosphatase-dead PTEN mutant or the PTEN C2 domain only still resulted in some stimulation of p53 transactivation, consistent with a role for direct interaction in p53 stabilization. Given the multiple levels of crosstalk, it is not yet clear whether PTEN activates p53-mediated transcription per se in addition to protecting it from degradation. Also surprisingly, the study could be carried out in MEF cells that unexpectedly expressed high levels of p53, while usually such p53 levels have to be induced by DNA-damaging agents. Results obtained by electrophoretic mobility shift analysis left the question of a PTEN-p53 complex on DNA still open, while chromatin immunoprecipitation experiments using a p53 antibody and p21-specific primers confirmed the notion that transfection of PTEN independently of its enzymatic activity may result in an increase of p53 levels, hence potentiating its function. To assess the importance of Pten-p53 interactions in vivo, the authors made use of a compound mutant mouse model by crossing *Pten* and *p53* knockout mutant mice. They found that in a *p53* heterozygous background, the status of *Pten* was crucial since loss of just one allele of *Pten* dramatically accelerated tumor onset to the rate observed in *p53* null mice. Although mutations of the remaining *p53* and *Pten* alleles were not formally excluded, these preliminary results are in agreement with the author's observations in murine cell lines. In summary, compelling experimental evidence unraveling an intimate functional crosstalk between PTEN and p53 has been accrued in the past two years. These findings are in agreement with a recent observation made in human breast cancers, demonstrating that *PTEN* and *p53* somatic mutations are mutually exclusive and do not occur in the same compartment (stroma versus epithelium) or in the same sample (Kurose et al., 2002).

The study by Wu and colleagues raises many important questions. It is not presently clear how much a direct interaction with PTEN contributes to p53 stabilization, nor is it known how PTEN binding would lead to p53 stabilization. It would also be interesting to determine whether p53 modifications such as phosphorylation or ubiquitination affect it or if, in turn, PTEN binding regulates these posttranslational modifications. If PTEN interacts with p53 in the cytoplasm (Figure 1A, pathway 3) and whether this regulates p53 nuclear import need also to be assessed. This is of particular relevance in view of the recent identification of proteins such as PARC that actively anchor p53 in the cytoplasm in the absence of cellular stress (Nikolaev et al., 2003). It also remains to be determined to what extent p53-mediated apoptosis depends on a direct PTEN-p53 interaction. To this end, it will be important to study whether cancer-associated mutations of *PTEN* or *p53* specifically interfere with their interaction, which would firmly establish the relevance of the novel finding to human disease.

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Selected reading

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