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Mutant PTEN in Cancer: Worse Than Nothing

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Tumor suppressors block the development of cancer and are often lost during tumor development. Papa et al. show that partial loss of normal PTEN tumor suppressor function can be compounded by additional disruption caused by the expression of inactive mutant PTEN protein. This has significant implications for patients with *PTEN* gene mutations.

If half of the cooks in a crowded kitchen just wander around not doing any work, the working cooks would almost certainly work better without them. Similarly, in biology, there are examples of mutant proteins that interfere with the function of their normal functional counterparts within the same cells. In cancer biology, perhaps the best recognized example of this phenomenon is the p53 tumor suppressor, in which partially inactive mutant proteins aggravate tumor phenotypes both through interference with normal p53 when both proteins are present and also through mechanisms independent of p53 (Muller and Vousden, 2013). In this issue of *Cell*, a new study of another key tumor-suppressor protein establishes this paradigm further in cancer biology (Papa et al., 2014).

An important step in the development of most cancers is the functional disruption of proteins that have actions that normally inhibit tumor development, termed tumor suppressors. PTEN is a tumor suppressor that is frequently lost, either partially or fully, from many sporadic tumor types. A range of mechanisms causes these losses of function, including missense and truncation mutations and deletions in and of the *PTEN* gene, as well as reduced expression of active PTEN mediated by promoter methylation, the effects of miRNAs, and the suppression of PTEN enzyme activity (Leslie and Foti, 2011). Loss of both copies of the *PTEN* gene seems to lead to death during embryonic development, but humans and other vertebrates can survive carrying one active and one mutant *PTEN* gene. Importantly, *PTEN* mutation carriers display a diverse range of pathologies, including tumor susceptibility, developmental abnormalities, and autism.

The PTEN tumor suppressor appears to act in a dose-dependent manner, and in many tumors, loss of function appears only partial (Alimonti et al., 2010; Carracedo et al., 2011). Accordingly, it is not uncommon to find evidence that cells express both normal and inactive mutant PTEN proteins, both in sporadic tumors and throughout the body of individuals who inherit one functional and one mutant gene. The status of PTEN as a tumor suppressor was established in part through the study of several lines of transgenic mice engineered to carry one wild-type copy of *Pten* and one null allele that expresses no active protein. These heterozygous mice develop a diverse range of tumors, with substantial overlap with the tumor spectrum observed in human *PTEN* mutation carriers and often retaining some normal Pten expression (Freeman et al., 2006; Knobbe et al.,

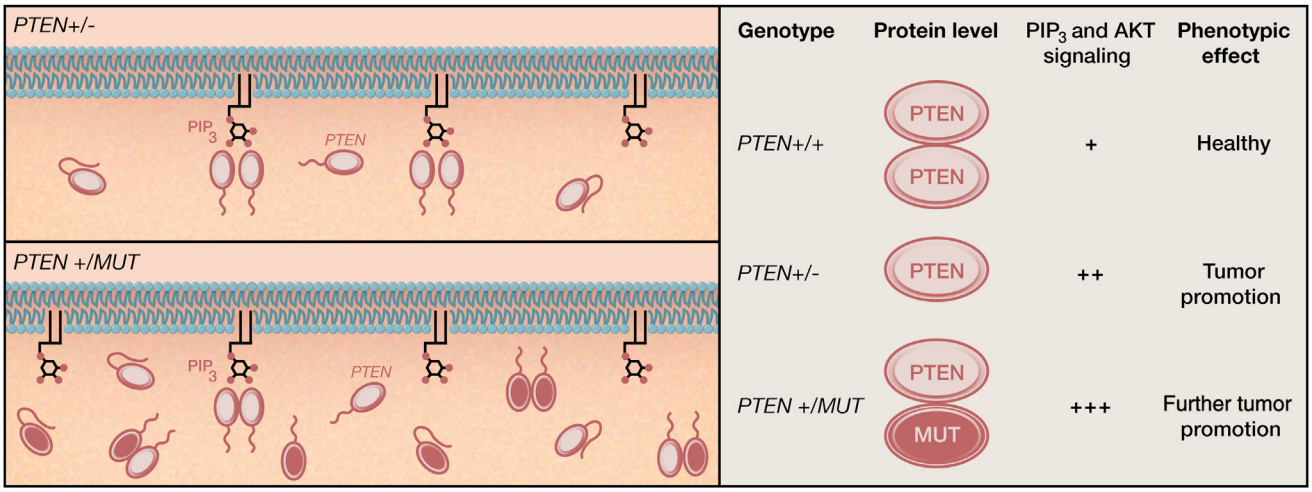

Figure 1. The Activity of Dimeric PTEN

A model for the action of dimeric PTEN to metabolize PIP_3 at the plasma membrane and interference by inactive mutant (MUT) PTEN (red) forming weakly active mixed dimers with the wild-type enzyme. Several factors remain to be determined definitively, including the fraction of cellular monomeric and dimeric PTEN and their contributions to overall activity.

2008). Papa et al. (2014) compare tumor suppression in heterozygous mice carrying one normal *Pten* gene copy alongside either a null deletion allele (often written $Pten^{+/-}$) or a mutant allele encoding a mutant PTEN protein lacking lipid phosphatase activity. Two different mutant PTEN enzymes that have been previously identified in tumors are studied in this way: PTEN C124S, which lacks all catalytic activity due to replacement of the active site cysteine nucleophile, and PTEN G129E, which lacks lipid phosphatase activity while retaining activity against model protein substrates. Heterozygous mice expressing either of these stable mutant proteins (often written $Pten^{+/C124S}$ and $Pten^{+/G129E}$) develop similar but more severe tumor phenotypes than mice simply lacking one functional gene copy, which is consistent with previous observations of $Pten^{+/G129E}$ mice (Wang et al., 2010). Phenotypes included the development in around a third of $Pten^{+/C214S}$ and $Pten^{+/G129E}$ mice of large invasive mammary adenocarcinoma and also a similar frequency of cerebellar hypertrophy. This latter phenotype was not observed in $Pten^{+/-}$ heterozygous mice but is reminiscent of the human condition Lhermitte-Duclos disease, known to be associated with *PTEN* mutations. This analysis provides strong support for the hypothesis that the diversity of phenotypes observed in human *PTEN* mutation carriers may be related to whether or not they express a stable inactive mutant protein.

Studies to determine the mechanisms by which mutant proteins affect tumor phenotype first confirmed that heterozygous knockin mice expressed levels of Pten protein similar to normal mice carrying two functional copies of *Pten* rather than the reduced level caused by having one null copy. Functionally, PTEN has lipid phosphatase activity that suppresses the class I PI 3-kinase/AKT signaling network by dephosphorylating phosphatidylinositol 3,4,5-trisphosphate (PIP_3). Analysis of the activation of the protein kinase Akt confirms that loss of one normal *Pten* gene copy causes a modest increase in Akt phosphorylation relative to normal cells. Importantly, a further modest increase in phosphorylation is observed both in tissues and primary cells derived from the heterozygous knockin mice carrying one active and one inactive *Pten* copy. This implies that the mutant Pten proteins are able to interfere with the ability of the normal enzyme to metabolize its PIP_3 lipid substrate and is also consistent with a significant role for the Akt kinases in promoting the observed tumorigenesis. The authors confirm their model by an elegant analysis of the two *PTEN* missense mutations most frequently identified in tumors, R130G and R130Q. Both mutant proteins are stable and act in a similar dominant-negative fashion as C124S and G129E. The high frequency of these mutations allows a valid analysis of the phosphorylation of AKT in publicly available data from 19 tumors harboring these mutations and in which a strong elevation is observed relative to tumors simply lacking one *PTEN* allele.

To address the question of how the inactive PTEN mutants interfere with the action of the coexpressed normal PTEN enzyme, the authors made the important observation that a population of the PTEN protein in cultured cells is present as a dimer. They then follow up the possibility that active PTEN protein function may be blocked by heterodimerization with inactive mutant protein. Although the binding of the phosphorylated C-terminal tail of PTEN to its phosphatase and C2 domains has been well established, this has generally been seen to be an intramolecular autoinhibitory interaction (Odriozola et al., 2007; Rahdar et al., 2009). In contrast, the observations of Papa et al. (2014) seem to favor the dimerization of unphosphorylated PTEN in a catalytically active complex (Figure 1). To confidently determine the fractions of cellular PTEN in monomeric and dimeric forms in different tissues, as well as the precise form and potential regulation of dimeric PTEN by phosphorylation and other posttranslational modifications such as ubiquitination, oxidation and

acetylation will take further work. However, this work raises the possibility that most cellular PTEN is phosphorylated, monomeric and autoinhibited, with a small proportion being unphosphorylated dimeric and active. On the other hand, other potential dominant-negative mechanisms by which inactive mutant PTEN may interfere with the functioning of the normal protein can be envisaged, such as competition as a monomer for binding to proteins involved in targeting the enzyme optimally to its lipid substrate or in posttranslational activation. The significance of the dominant-negative effects on tumor phenotype makes these important questions to answer. A goal must be that, in the future, the matching of treatments to patients may reflect knowledge of the cellular effects of defined classes of *PTEN* mutations.

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Protein Accounting in the Cellular Economy

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Knowing the copy number of cellular proteins is critical for understanding cell physiology. By being able to measure the absolute synthesis rates of the majority of cellular proteins, Li et al. gain insights into key aspects of translation regulation and fundamental principles of cellular strategies to adjust protein synthesis according to the functional needs.

Accurate accounting is the basis of an efficient economy. In order to understand the rules, trends, and directions of healthy economic growth, one must be able to track the precise amounts of individual products generated, the demand for these goods, and the strategies for allocating the resources for their production. In the cell, proteins are the main commodities. They control the majority of cellular activity, but their production is very expensive. Knowledge of how much of each protein is made is therefore central to understanding the organization, growth, and proliferation of the cell.

As basic as knowing the copy number of individual proteins in the cell may seem, it is a difficult aim to achieve. Although whole-cell proteomics and other genome-wide techniques provide useful insights into changes in gene expression under various physiological conditions, estimating the absolute amounts of even limited number of proteins is far more challenging. In this issue of *Cell*, Li et al. (2014) have succeeded in analyzing the translation output of more than 3,000 *E. coli* genes and quantify production of more than 95% of the proteins synthesized in fast-growing cells.

The revolutionary ribosome profiling technique developed by Weissman and colleagues several years ago provides a genome-wide view of translation of individual genes (Ingolia et al., 2009; Li et al., 2012). The method is based on next-generation sequencing of the mRNA fragments protected by ribosomes. Each "footprint" represents one translating ribosome, which will most likely generate one protein molecule encoded in the respective gene (Figure 1A). Deep sequencing of the ribosomal footprints hence provides a snapshot of cellular protein synthesis and allows the estimation of the fraction of ribosomes engaged in translation of individual mRNAs and, thus, the relative rate of expression of a given gene. Normalizing this by the total protein synthesized during the cell cycle renders the absolute protein synthesis