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INVITED EDITORIAL
PTEN: Sometimes Taking It Off Can Be Better than Putting It On

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One of the markers commonly used to identify tumor-suppressor genes is the frequent loss of heterozygosity (LOH) at a particular locus in tumor samples, indicating that at least one of the alleles has been deleted. LOH at chromosome 10q23 has been detected in ~75% of glioblastomas and in ~60% of advanced–prostate cancer samples (reviewed in Li et al. 1997; Steck et al. 1997), suggesting the presence of a tumor suppressor at this locus. In fact, reintroduction of this region of chromosome 10 into glioblastoma cell lines resulted in a loss of tumorigenicity in nude mice (Pershouse et al. 1993; Steck et al. 1995). Recently, a candidate tumor-suppressor gene, termed "PTEN" ("*p*hosphatase and *ten*sin homologue deleted from chromosome 10") or "MMAC1" ("*m*utated in *m*ultiple *a*dvanced *c*ancers"), has been isolated from chromosome 10q23 (Li et al. 1997; Steck et al. 1997). Importantly, in the majority of glioblastoma samples in which only one allele had been deleted, the other allele contained point mutations or small deletions, suggesting that disruption of both alleles is necessary for the formation of glioblastomas. Although the sample sizes used currently are small, LOH and/or point mutations have been found in PTEN in a growing number of tumor samples, including ~50% of endometrial cancers (Tashiro et al. 1997), suggesting that disruption of PTEN is necessary for the development of specific forms of cancer.

Strong evidence that PTEN is a bona fide tumor suppressor comes from a recent flurry of papers that have identified germ-line mutations of PTEN in three related, inheritable, neoplastic disorders: Cowden disease, Lhermitte-Duclos disease, and Bannayan-Zonana syndrome (Liaw et al. 1997; Marsh et al. 1997; Nelen et al. 1997). All these disorders share similar pathological traits, such as the formation of multiple benign tumors (mostly hamartomas) and an increased incidence of malignant cancers (Eng et al. 1994). Lhermitte-Duclos disease has additional pathologies, such as mental retardation and macrocephaly, whereas Bannayan-Zonana syndrome also includes lipomatosis and speckled penis and has an earlier onset of the disease (Grolin et al. 1992). The nonneoplastic pathologies seen in Cowden disease, Lhermitte-Duclos disease, and Bannayan-Zonana syndrome also indicate that PTEN functions during normal development.

One of the interesting aspects of PTEN is that it shares homology with the family of protein tyrosine phosphatases (PTPs) as well as with the cytoskeletal protein tensin (Li et al. 1997; Steck et al. 1997). Biochemical analysis of PTEN has revealed that it is a member of the family of dual-specificity protein phosphatases (DSPs) that dephosphorylate serine, threonine, and tyrosine residues (Myers et al. 1997). Significantly, naturally occurring point mutations isolated from tumor samples, as well as from tissue explants from patients with Bannayan-Zonana syndrome, resulted in pronounced inhibition of phosphatase activity, indicating that this activity is necessary in order for PTEN to function as a tumor suppressor (Myers et al. 1997).

The identification of PTEN as the tumor suppressor residing on 10q23 has generated a great deal of interest. PTEN represents a tumor suppressor with a defined enzymatic function, and it represents the first PTP to be implicated as a bona fide tumor suppressor. A large number of protein tyrosine kinases (PTKs) have been implicated as oncogenes, and disruption of the signaling components downstream of PTKs has been associated with the transformed phenotype. Since the initial purification and cloning of the PTP family, PTPs have been implicated in the inhibition of cell growth, and it has become apparent that PTPs that are the natural antagonists of the growth-promoting PTKs have been identified. In fact, treatment of normal rat kidney cells with an inhibitor of cellular PTPs resulted in transformation (Klarlund 1985), demonstrating the importance of this class of enzymes in maintaining control of cell proliferation. In addition to proliferation, PTPs have been implicated in a variety of nonneoplastic diseases, including diabetes (Begum et al. 1991) and myotubular myopathy (Laporte et al. 1996), and are essential virulence factors for a

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number of microbrial pathogens (Andersson et al. 1996). Furthermore, PTPs have been implicated in the regulation of immune-cell function (Neel and Tonks 1997).

Structure and Function of PTPs

PTEN is a member of the PTP family of enzymes. The entire family is characterized by the presence of a catalytic signature motif, HCXXGXXRS/T. In both the PTPs and the DSPs this motif is located within a conserved secondary structure consisting of seven α-helices and 10 β-sheets (Barford et al. 1995). The diversity of the PTP family arises mostly from the sequences that flank this conserved catalytic core and includes extracellular ligand-binding domains and motifs for protein-protein interaction that specifies subcellular localization or motifs for the regulation of enzymatic activity (Zhang et al. 1995). Subcellular targeting also may contribute to substrate specificity, by restricting the potential cellular targets. Conventional wisdom suggests that PTPs are promiscuous, but recent evidence has clearly demonstrated that the PTPs exhibit exceptional substrate specificity in a cellular context (Flint et al. 1997). The presence of transmembrane receptor–like PTPs (RPTPs) suggests that, as with the regulation of the receptor tyrosine kinases, these RPTPs may be regulated by the binding of ligands. In addition, many of the extracellular domains have homology to cell-adhesion molecules, indicating a role for these PTPs in the regulation of biological responses to the extracellular matrix or to cell-cell contact. It is important to note that PTPs can exert both positive and negative regulatory functions and in some cases have been shown to be positive regulators of cell proliferation. For example, two closely related PTPs, SHP-1 and SHP-2 (*src-h*omology domain 2–containing *p*hosphatase), have opposing effects on cell signaling. SHP-1 acts to control cytokine signaling by down-regulating the Janus family of PTKs, whereas SHP-2 is necessary for the growth factor–dependent activation of Ras (reviewed in Neel and Tonks 1997).

The PTPs and DSPs appear to utilize the same catalytic mechanism. Dephosphorylation is initiated by the active-site cysteine (located in the signature motif), which functions as a nucleophile to attack the phosphorus atom of the substrate, forming a thiol-phosphate intermediate. Another conserved residue, an aspartic acid located ~32 residues N-terminal to the catalytic cysteine, promotes the release of the dephosphorylated substrate from the enzyme-substrate complex. The active enzyme is regenerated by a water molecule, which attacks the thiol-phosphate bond, liberating the phosphoryl group from the catalytic cysteine (reviewed in Barford et al. 1995). This reaction can proceed very quickly, with PTP1B undergoing ~2,000 catalytic cycles/min (Flint et al. 1997), and the *Yersinia* PTP (YOP) can undergo an astonishing 90,000 catalytic cycles/min! Determination of the crystal structures of PTPs and DSPs has revealed the structural basis for the differences in amino acid specificity. In the PTPs, this catalytic cysteine residue is located at the base of a deep cleft (Barford et al. 1994*a*). The depth of this cleft is equivalent to the length of a phosphotyrosine residue. The much shorter phosphoseryl and phosphothreonyl residues are unable to reach the base of the cleft, where the nucleophilic attack by the invariant cysteine occurs, and therefore cannot be dephosphorylated. On the other hand, the catalytic cysteine in the DSPs is in a much shallower cleft that can accommodate all three phosphorylated hydroxyl amino acids (Yuvaniyama et al. 1996).

Substrate Specificity of PTEN

PTEN possesses intrinsic phosphatase activity and is a member of the dual-specificity family of PTPs (Myers et al. 1997). PTEN prefers extremely acidic substrates, exhibiting almost 50 times more activity toward poly-acidic substrates than toward more traditional substrates (Myers et al. 1997). The extreme selectivity of PTEN toward acidic substrates in vitro suggests that the physiological substrates also will be acidic. It is possible that the acidic character may be manifested by proteins that are phosphorylated on multiple sites. Although it is tempting to propose that the physiological substrates of PTEN will be phosphorylated on tyrosine residues, it is important to realize that PTEN may recognize proteins that are exclusively phosphorylated on serine and threonine residues. In this context, it should be noted that the DSP KAP-1 dephosphorylates cdk2 at Thr160 (Poon and Hunter 1995). Although the substrates of PTEN in vivo are likely to be proteinaceous, it has been shown that members of this class of enzymes can dephosphorylate a number of nonproteinaceous phosphoesters (Howell et al. 1996), including the 5′ phosphate from RNA (Takagi et al. 1997). However, RNA is unlikely to be a target of PTEN, since the capping of RNA occurs in the nucleus, and immunoflourescence analysis has indicated a largely cytoplasmic localization of PTEN (Li and Sun 1997; M. P. Myers, unpublished data). Nevertheless, one should consider acidic nonproteinaceous substrates, such as phosphoinositides, as potential targets for PTEN.

Effect of Point Mutations on PTEN Activity

Many naturally occurring point mutations in PTEN have been uncovered from tumor samples, tumor cell lines, and germ-line mutations that result in neoplastic diseases (Li et al. 1997; Liaw et al. 1997; Marsh et al. 1997; Nelen et al. 1997; Rhei et al. 1997; Steck et al. 1997; Tashiro et al. 1997). A number of these point

mutations have already been shown to be detrimental to the enzymatic activity of PTEN (Myers et al. 1997). It is possible, on the basis of the conserved secondary structure and catalytic mechanism of the PTPs, to predict how these point mutations disrupt PTEN activity. Point mutations have been found in three major clusters in PTEN (fig. 1). These clusters are also areas that are highly conserved between PTEN and tensin. One cluster surrounds the catalytic motif. Mutations in this region of PTEN are predicted to disrupt enzymatic activity, either by disrupting the catalytic cysteine directly or by altering the orientation of this cysteine in the catalytic cleft, thereby affecting its ability to attack the phosphorus atom (Barford et al. 1994*b*). The second cluster surrounds the conserved aspartic acid residue required for the release of the substrate from the PTP. The third cluster, near the last conserved structural feature of the PTP fold, an α-helix, also disrupts PTEN activity, which is consistent with the proposed role of these residues in coordinating a water molecule that hydrolyzes the thiol-phosphate bond, activating the enzyme for another round of catalysis (Barford et al. 1995; Myers et al. 1997). In addition, a number of point mutations have been found to be spaced throughout the N-terminus of PTEN and are believed to abolish PTEN enzymatic activity, by disrupting the overall secondary structure rather than by specifically disrupting the domains necessary for catalysis (Myers et al. 1997). In addition, two point mutations were tested that did not result in a loss in PTEN enzymatic activity. However, it has not been determined whether these mutations adversely affect the ability of PTEN to function inside cells, perhaps by altering interactions with important regulatory molecules, by altering the ability of PTEN to recognize its physiological substrates, or by adversely affecting the stability of the protein or the message.

It is currently unclear why germ-line mutations in PTEN give rise to at least three related, yet distinctive, disorders. Significantly, samples isolated from a Cowden disease patient indicated that the wild-type allele is present in nonaffected tissue but is lost in tumor samples, indicating that the development of tumors is dependent on the secondary mutation of the inherited wild-type allele (Liaw et al. 1997). Although the sample size is small, there does appear to be a correlation between the severity of the disorder and the enzymatic activity of the mutated allele (Myers et al. 1997), suggesting that some of these mutations may result in partial-loss-of-function alleles. Similarly, mutations in the RET protein tyrosine kinase have been shown to give rise to at least four different inheritable disorders (van Heyningen 1994). However, other genetic loci that interact with PTEN also may account for the range of phenotypes. Uncovering these other loci will provide important insights into PTEN function, since they are likely to encode members

MTAIIKEIVSRNKRRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDV
VRFLDSKHKNHYKIYNLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPF
CEDLDQWLSEDDNHVAAIHCKAGKGRTGVMICAYLLHRGKFLKAQEALDF
YGEVRTRDKKGVTIPSQRRYVYYYSYLLKNHLDYRPVALLFHKMMFETIPM
FSGGTCNPQFVVCQLKVKIYSSNSGPTRREDKFMYFEFPQPLPVCGDIKVEFF
HKQNKMLKKDKMFHFWVNTFFIPGPEETSEKVENGSLCDQEIDSICSIERAD
NDKEYLVLTLTKNDLDKANKDKANRYFSPNFKVKLYFTKTVEEPSNPEASS
STSVTPDVSDNEPDHYRYSDTTDSDPENEPFDEDQHTQITKV

Figure 1 Locations of point mutations in PTEN. The locations of missense mutations in PTEN are shown in boldface. Underlining designates areas with a high frequency of mutation.

of the same signaling pathway regulated by PTEN, such as the protein kinase(s) that works in opposition to PTEN.

Future Directions

Many lines of evidence support the classification of PTEN as a tumor suppressor, especially the finding that germ-line mutations in PTEN result in neoplastic disorders. In addition, PTEN has been found to be mutated in the majority of glioblastomas and advanced prostate cancers and in roughly one-half of endometrial cancers (Li et al. 1997; Steck et al. 1997; Tashiro et al. 1997). However, disruptions in PTEN occur only rarely in breast cancer and in other gynecological cancers (Tashiro et al. 1997), suggesting that PTEN plays a significant role in the development of only a subset of cancers. Significantly, polymorphisms in PTEN appear to be rare, and mutations that have been identified in PTEN have only been found in neoplastic disorders, strengthening the link between PTEN and neoplastic diseases (Li et al. 1997; Liaw et al. 1997; Marsh et al. 1997; Nelen et al. 1997). Determination of the prevalence of PTEN mutations in different kinds of cancer will be necessary to strengthen the statistical link between disruption of PTEN and the formation of specific tumor types.

Analysis of tumor samples has resulted in a number of insights into the potential function of PTEN. Disruption of PTEN appears to occur late during tumorigenesis, indicating that PTEN is probably not the so-called transforming event necessary to initiate malignant cell growth. In the case of glioblastoma, LOH at chromosome 10 usually is associated with an amplification and rearrangement of the epidermal growth factor (EGF) receptor (von Deimling et al. 1992), suggesting that amplification and activation of the EGF receptor was the transforming event, whereas disruption of PTEN may be required at a later stage of tumor progression, perhaps for regulation of a cell-cycle checkpoint that is several

steps downstream of the transforming event. However, given the advanced stages at which LOH at chromosome 10q23 occurs in glioblastomas and in prostate cancer, it seems likely that PTEN may function to modulate other cellular processes, such as angiogenesis, that are best detected in tumorigenicity rather than in transformation assays. The homology between PTEN and the cytoskeletal protein tensin suggests that the targets of PTEN may be cytoskeletal. Indeed, a growing body of evidence indicates an important role for tyrosine phosphorylation in the regulation of cytoskeletal function and the importance of cytoskeletal changes during oncogenesis (Cobb and Parsons 1993; Chrzanowska-Wodnicka and Burridge 1994).

The reversible nature of protein phosphorylation indicates that PTEN will function as a regulatory molecule that acts in opposition to a kinase. Fundamental insights into the biological function of PTEN will be revealed following the identification of its physiological substrates. The identity of these substrates will not only indicate which cellular signaling pathways are normally regulated by PTEN but also may lead to the identification of the potentially oncogenic kinase(s) that is antagonized by PTEN and eventually may lead to the characterization of the entire signaling pathway. There are precedents to illustrate that PTPs function to attenuate signals that normally promote such fundamental responses as growth and proliferation. By removing phosphate, these PTPs act to maintain the balance between an appropriate cellular response to a stimulus and an aberrant, hyperresponsive state that promotes the disease phenotype. Determination of the mechanism by which the opposing forces of PTEN and its antithetic protein kinase function in regulating cellular signaling will provide insights into the maintenance of normal cell physiology and how this is subverted in cancer.

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