one of the major limitations associated with single-tip instruments that operate in serial fashion—namely, prohibitive processing times. In addition, these instruments can now operate over substrate areas on the centimeter scale typically associated with lower resolution conventional lithographic methods. Scanning probe lithographies are also being developed for soft (organic) materials, paving the way to devices that use small collections of, or even individual, organic molecules as electronic components. One of the most powerful and promising lithographic methods of this type is dip pen nanolithography (DPN), which uses an AFM as a pen to transport molecule-based inks directly to a substrate (*9*). The direct-write capabilities of DPN and its compatibility with soft materials set it apart from virtually all other scanning probe lithographies, allowing one to pattern multiple chemical components onto a substrate with a current spatial resolution limit of 5 nm.

Collectively, these scanning probe advances allow researchers to fabricate extraordinary structures on a time scale that could enable their transition from research curiosities to commercial processing tools. But absent from this nanotechnology tool kit are analogs to many tools in our macroscopic kits. For example, single, or even multiple-tip scanning probe instruments cannot be used to tweeze or grab nanoscale objects. This is why the advance reported by Kim and Lieber is important. To demonstrate the concept of tweezing on the nanoscopic scale (see the figure) they first patterned a glass pipette probe with two electrodes on opposite sides of the probe and then attached a carbon nanotube to each of the electrodes. Kim and Lieber took advantage of the mechanical toughness and electrical conductivity of the carbon nanotubes to miniaturize a previously demonstrated microscopic phenomenon (*10*), namely that application of an appropriate bias voltage across two tweezer arms can effect a mechanical change in the two arms that results in their pinching together. Removal of the applied voltage relaxes the tweezers but does not open them because of van der Waals interactions between the carbon nanotubes. Application of the appropriate bias voltage reopens the tweezers. The previously demonstrated micrometer-scale tweezers operated at large actuating voltages (45 to 150 V depending on arm composition and size). Besides being substantially smaller and offering the potential for further miniaturization through the use of smaller bundles of single-walled nanotubes, the nanotweezers operate at substantially lower actuating voltages (8.5 V).

Lieber and Kim have demonstrated that their tweezers can be used to grab and manipulate mesoscopic clusters and spheres and even gallium arsenide nanowires. In addition, because the tweezer arms are also individually addressable electrodes, there is the tantalizing prospect of transforming this technology into a two-probe STM device, which could enable electrical measurements over features separated by relatively short distances (*11*). The ability of the nanotweezers to grasp and physically extract a nanoscale component from a complex mixture and then make an electrical measurement across it could make them a valuable analytical tool for studying the intrinsic electrical properties of the components of heterogeneous materials and nanoscale devices. Many other applications for the nanotweezers are within the realm of possibility, including its use in manipulating biological structures on surfaces or even within cells.

Further miniaturization of the device is desirable such that even smaller structures can be picked up and manipulated. Suppression of nonspecific adsorption on the tweezer arms would also substantially improve the concept. Nevertheless, this is a powerful demonstration and an important step toward the development of a useful new tool for the nanotechnologist.

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PERSPECTIVES: CANCER

Structural Assets of a Tumor Suppressor

Nicholas K. Tonks and Michael P. Myers

Inactivation of tumor suppressor genes is one way in which cancer cells circumvent normal growth control. Mutations in the stretch of DNA at q23 on chromosome 10 are among the most commonly observed in human cancer and are particularly prevalent in glioblastomas, advanced prostate cancers, and endometrial carcinomas. Early in 1997, two groups reported the discovery of a new tumor suppressor gene at this locus, which they called *PTEN* (Phosphatase and Tensin homolog deleted on chromosome Ten) or *MMAC* (Mutated in Multiple Advanced Cancers) (*1*, *2*). Mutations in *PTEN* are also recognized as the cause of cancer predisposition syndromes, such as Cowden disease (characterized by an increased risk of breast cancer). Since this discovery, rapid progress has been made in characterizing the PTEN protein and its role in regulating the signaling events that control cell proliferation and survival. The latest chapter in this story, published by Pavletich's group in a recent issue of *Cell* (*3*), describes the crystal structure of the PTEN protein and sheds new light on how this phosphatase is regulated.

PTEN contains the signature motif that defines the protein tyrosine phosphatase (PTP) family of enzymes, which remove phosphate groups from other proteins. It was originally described as a dual-specificity phosphatase, that is, it recognizes proteins phosphorylated on tyrosine and on serine/threonine residues. Curiously, PTEN also displays a very unusual specificity for highly acidic substrates (*4*). Insights into the identity of its physiological substrates came with the observation that PTEN has the capacity to dephosphorylate (remove phosphate groups from) inositol phospholipids such as PIP_3 (phosphatidylinositol-3,4,5, P_3). These molecules are crucial secondary messengers in signaling pathways that control proliferation and survival (*5*). A substantial body of data from studies involving disruption of PTEN in mice and in the worm *Caenorhabditis elegans*, together with expression of this phosphatase in tumor cells bearing mutant PTEN alleles, now support the conclusion that PTEN is a lipid phosphatase in vivo (*6*).

The structure of PTEN described by Pavletich's group (*3*) reveals the same overall fold as observed for both phosphotyrosine-specific and dual-specificity phosphatases. However, its active-site cleft is twice as wide as, but of similar depth to, the phosphotyrosine-specific enzymes and both wider and deeper than that of the dual-specificity phosphatases. In addition, the signature motif of PTEN displays a uniquely basic character. Although they do not present the structure of PTEN bound to

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substrate, Pavletich and co-workers propose a model for this interaction. In their model, the active site is sufficiently wide to accommodate the bulky, polyphosphorylated sugar moiety of PIP_3, and the basic residues in the signature motif coordinate the additional phosphate groups on the sugar ring while orienting the phosphate on the 3 position for selective removal.

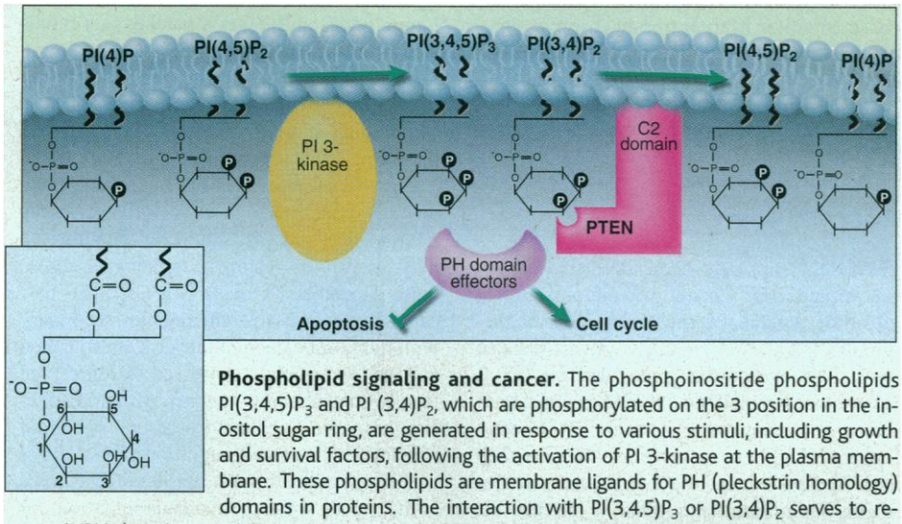
Although the preponderance of data demonstrate that PTEN dephosphorylates inositol phospholipids in vivo, it remains controversial as to whether it is also a protein phosphatase. There have been suggestions that one of PTEN's primary substrates may be focal adhesion kinase (FAK) (*7*). This protein tyrosine kinase is a critical component of the signaling events that are initiated by integrins (the cell surface receptors that bind to the extracellular matrix) and govern cell migration. Characterization of disease-derived mutations in PTEN has clarified this issue. The G129E mutation (substitution of glycine at position 129 by glutamic acid), which is found in Cowden disease and endometrial cancer, disrupts the tumor suppressor capabilities of PTEN. The lipid phosphatase activity of the PTEN-G129E mutant protein is ablated, whereas its protein phosphatase activity is maintained (*8*). Additional mutants that share this characteristic have now been identified (*9*). The properties of these mutants indicate that it is the lipid phosphatase activity of PTEN that is critical for its tumor suppressor function.

The Pavletich structure reveals that residue G129 is at the bottom of the active site in the region in which the cleft is extended relative to other PTPs. However, a second mutation at this residue, G129R (substitution of glycine at position 129 by arginine), ablates both lipid and protein phosphatase activities, suggesting that the effect cannot be explained simply by partial occlusion of the active site. Although the protein phosphatase activity of PTEN may not be critical for tumor suppressor function, it may be of physiological importance. Parallels with phosphatidyl inositol 3-kinase (PI 3-kinase)—an oncogenic enzyme that catalyzes the opposing reaction to PTEN by phosphorylating the 3 position in the sugar ring of phosphatidylinositol lipids—are striking (*10*). PI 3-kinase also possesses protein kinase activity, and when it phosphorylates itself (autophosphorylation) its lipid kinase activity is decreased. Perhaps the major protein substrate for PTEN is PTEN itself, with dephosphorylation of the phosphatase serving as a mechanism to regulate its ability to dephosphorylate lipids.

By dephosphorylating PIP_3, PTEN has the potential to exert a profound effect upon a wide array of physiological functions. This has focused attention on mechanisms by which the activity of PTEN is controlled. A critical element of this control is regulation of PTEN's access to phospholipid substrates in the plasma membrane. Tumor-associated mutations in PTEN are found not only in the amino-terminal, 179-residue phosphatase domain, but also in a 166-residue segment of the protein's carboxyl terminus. Pavletich and colleagues show that this carboxyl-terminal segment adopts a β-sandwich structure that displays features of a C2 domain (a motif that mediates recruitment of several signaling proteins to the membrane). The C2 domain of PTEN lacks critical acidic residues that bind calcium ions in classical C2 domains, and so it appears to be more similar to such domains in calcium-independent isoforms of protein kinase C. The PTEN C2 domain binds to phospholipid membranes, and the residues responsible for membrane association are important for tumor suppressor function. In addition, the phosphatase and C2 domains interact over a large surface area, with most of the residues that make interdomain hydrogen bonds subject to mutation in tumors. Thus, the integrity of the interaction between the phosphatase and its C2 domain seems to be important for the tumor suppressor function of PTEN.

It is possible that the C2 domain contributes not only to targeting PTEN to the membrane but also to optimizing its orientation with respect to the PIP_3 substrate. Attention has also been drawn to the extreme end of PTEN's carboxyl terminus, which contains a consensus binding motif for proteins with PDZ domains. Many groups have isolated PDZ domain–containing proteins that bind to PTEN. These proteins show features of scaffolding molecules and have been implicated in the assembly of multiprotein signaling complexes within the cell. Although they may also be involved in membrane targeting and regulation of activity, their precise function remains unclear as the extreme carboxyl terminus of PTEN appears to be dispensable for its tumor suppressor function (*11*). Unfortunately, this portion of PTEN is missing from the Pavletich structure.

Although the structure has provided insights into PTEN regulation and function, much remains to be discovered. The precise structural definition of the mechanism underlying selective ablation of the lipid phosphatase activity of PTEN in certain tumor-derived mutations and the mechanism by which PTEN recognizes both lipid and protein substrates will require further investigation. The integration of structural and biological approaches to the study of PTEN should continue to enhance our understanding of cellular signaling mechanisms and their disruption in cancer. Hopefully, this will yield insights into improved therapeutic strategies.



Phospholipid signaling and cancer. The phosphoinositide phospholipids $PI(3,4,5)P_3$ and $PI(3,4)P_2$, which are phosphorylated on the 3 position in the inositol sugar ring, are generated in response to various stimuli, including growth and survival factors, following the activation of PI 3-kinase at the plasma membrane. These phospholipids are membrane ligands for PH (pleckstrin homology) domains in proteins. The interaction with $PI(3,4,5)P_3$ or $PI(3,4)P_2$ serves to recruit PH domain-containing proteins, such as the protein kinase PKB/Akt, to the plasma membrane where they become activated and transduce signals that promote cell survival and proliferation. Importantly, these phosphoinositide-dependent signals are kept in check by the action of the tumor suppressor phosphatase PTEN, which dephosphorylates the 3 position in the inositol ring. The crystal structure of PTEN reveals the presence of a C2 domain at the COOH-terminus of the protein.

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