Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis

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Summary

PTEN (also known as MMAC-1 or TEP-1) is one of the most frequently mutated tumor suppressors in human cancer. It is also essential for embryonic development. PTEN functions primarily as a lipid phosphatase to regulate crucial signal transduction pathways; a key target is phosphatidylinositol 3,4,5-trisphosphate. In addition, it displays weak tyrosine phosphatase activity, which may downmodulate signaling pathways that involve focal adhesion kinase (FAK) or Shc. Levels of PTEN are regulated in embryos and adult organisms, and gene-targeting studies demonstrate that it has a crucial role in normal development. Functions for PTEN have been identified in the regulation of many normal cell processes, including growth, adhesion, migration, invasion and apoptosis. PTEN appears to play particularly important roles in regulating anoikis (apoptosis of cells after loss of contact with extracellular matrix) and cell migration. Gene targeting and transient expression studies have provided insight into the specific signaling pathways that regulate these processes. Characterization of the diverse signaling networks modulated by PTEN, as well as the regulation of PTEN concentration, enzymatic activity, and coordination with other phosphatases, should provide intriguing new insight into the biology of normal and malignant cells.

Key words: Tumor suppressor, Migration, Adhesion, Cytoskeleton, Integrin, Anoikis

Introduction

Just when we thought we understood the major components of signal transduction, a novel protein was identified that opened our eyes to powerful new ways of regulating cell behavior. PTEN was discovered only in 1997 as a new tumor suppressor, and yet it is now known to play major roles not only in suppressing cancer but also in embryonic development, cell migration and apoptosis (reviews include Maehama and Dixon, 1999; Cantley and Neel, 1999; Besson et al., 1999; Tamura et al., 1999c; Ali et al., 1999; Di Cristofano and Pandolfi, 2000; Vazquez and Sellers, 2000; Bonneau and Longy, 2000; Simpson and Parsons, 2001). It seems surprising in retrospect that such an important regulatory protein could remain undiscovered until recently. One reason may be that PTEN is an unusual phosphatase whose primary functional target is a signaling phosphoinositide lipid. Yet when one considers the myriad of protein and lipid kinases that phosphorylate molecules regulating growth, metabolism and movement, it is obvious that counter-regulation by phosphatases must also be crucial.

Increasing numbers of biologically important phosphatases are being characterized (Li and Dixon, 2000; Tonks and Neel, 1996), but PTEN has been the focus of particularly intense interest because of its central role in suppressing malignancy. Although substantial progress has been made in understanding the role of PTEN in tumor suppression, much less is known about its role in normal embryonic development (except that PTEN-knockout mice die early in development) or about its regulation during normal tissue function.

Like any newly discovered protein, the specific molecular targets of PTEN were originally a mystery. Systematic approaches to defining the function of PTEN included exploration of potential functions in transient overexpression studies, searches for mutants having a subset of normal PTEN activities, generation of null mutations, and transient or stable reconstitution of wild-type and mutant PTEN in cells lacking the protein. For signaling molecules such as PTEN, there are caveats to each approach. For example, overexpression may swamp normally fine-tuned signaling pathways (see Noselli and Perrimon, 2000). In addition, transient restoration of PTEN (or other proteins) to tumor cells that lack PTEN might induce a stronger initial phenotype, which might disappear during steady-state, stable expression. Conversely, knockout cells might compensate for the absence of PTEN.

Nevertheless, the application of all of these approaches together has established that PTEN is part of a complex signaling system that affects a variety of important cell biological functions (Fig. 1; Fig. 2). In the course of these studies, however, varying results and interpretations have arisen. We suggest that these differences are not mere technical controversies, but instead indicate that regulation of PTEN function is highly sensitive to the cellular environment.

Here, we briefly review the discovery and characterization of PTEN, but focus primarily on the cell biological roles of PTEN. In particular, we emphasize its functions in apoptosis and in cell adhesion and migration. Since this review is necessarily limited in length, we apologize for any omissions. Further information on this topic can be found in other recent reviews of this rapidly growing field (Maehama and Dixon, 1999; Cantley and Neel, 1999; Besson et al., 1999; Tamura et al., 1999c; Ali et al., 1999; Di Cristofano and Pandolfi, 2000; Vazquez and Sellers, 2000; Bonneau and Longy, 2000; Simpson and Parsons, 2001).

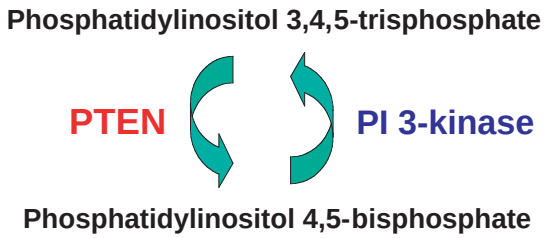

Fig. 1. Major enzymatic function of PTEN. The tumor suppressor PTEN opposes the action of phosphoinositide 3-kinase (PI 3-kinase) by dephosphorylating the signaling lipid phosphatidylinositol (3,4,5)-trisphosphate.

Structural analyses of PTEN

PTEN is a major and frequently mutated tumor suppressor protein. Its different names highlight various features: PTEN (**p**hosphatase and **ten**sin homolog deleted on chromosome **ten**; Li et al., 1997); MMAC1 (**m**utated in **m**ultiple **a**dvanced **c**ancers; Steck et al., 1997); and TEP1 (**T**GFβ-regulated and **e**pithelial-cell-enriched **p**hosphatase; Li and Sun, 1997). Contrary to original hopes of finding multiple family members, however, only one additional family member, the testis-specific PTEN 2, has been reported to date, although C-terminal alternative-splicing variants of PTEN also exist (Wu et al., 2001; Sharrard and Maitland, 2000).

Mutations in both alleles of the *PTEN* gene arise during cancer progression in a remarkable variety of cancers, including brain, prostate, breast and endometrial cancers, plus melanoma; frequencies of mutations in both alleles reach 50% for certain cancers in some studies. The *PTEN* gene is also mutated in inherited cancer syndromes such as Cowden syndrome, and studies of heterozygous *PTEN*-mutated mice further support its role as a tumor suppressor (reviewed by Simpson and Parsons, 2001).

It is noteworthy that the *PTEN* gene can be found mutated both very early in tumorigenesis (as in hereditary cancer syndromes) and also much later in advanced cancers. One explanation may be that defects in PTEN, whether they are inherited mutations, or arise through later somatic mutation or epigenetic reduction, can cooperate at multiple stages with the loss of other tumor suppressors and/or activation of oncogenes to promote malignancy. In fact, cooperation between mutations in PTEN and in p27KIP1 or Wnt-1 has recently been shown to promote oncogenesis (Di Cristofano et al., 2001; Li et al., 2001). In addition, however, we suggest that recently identified functions of PTEN in regulating cell interactions with the extracellular matrix (see below) may become particularly important in later stages of cancer progression. Alterations in cell adhesion to matrix proteins, migration and apoptosis after loss of contact with the matrix have each been implicated in tumor invasion and metastasis, and the loss of PTEN may provide a central mechanism for such changes.

The crystal structure of PTEN was determined promptly (Lee et al., 1999), although two flexible regions of unknown function had to be deleted for technical reasons. PTEN contains a phosphatase domain that has a structure resembling previously characterized protein tyrosine phosphatases but containing an enlarged active site that can account for its ability to bind PtdIns(3,4,5)P_3. Such enlargement of the active site, however, would be expected to entail diminished specific phosphotyrosine binding and protein phosphatase activity.

PTEN has a second major domain that binds phospholipids. This C2 domain appears to bind PTEN to the plasma membrane, and it might orient the catalytic domain appropriately for interactions with phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and other potential substrates (Lee et al., 1999). One puzzling structural feature of PTEN is a region that shares substantial sequence identity with tensin and auxillin (Li et al., 1997). Although this site could be linked to the effects of PTEN on cell adhesion and migration, its function remains unknown.

Several studies have identified intriguing regulatory features in the C-terminal tail of PTEN (Georgescu et al., 1999, Georgescu et al., 2000; Leslie et al., 2000; Vazquez et al., 2000). Mutagenesis studies demonstrate that phosphorylation of certain serine and threonine residues (S380, T382 and T383) can modulate both the enzymatic activity and the stability of PTEN. Dephosphorylation or deletion of the tail results in enhanced phosphatase activity and rapid degradation (Vazquez et al., 2000). A key enzyme regulating the phosphorylation of this C-terminal cluster of serine and threonine residues appears to be the protein kinase CK2 (known previously as casein kinase II), which modulates PTEN stability to proteasome-mediated degradation (Torres and Pulido, 2001). A PDZ domain in the tail might also play roles in altering the balance

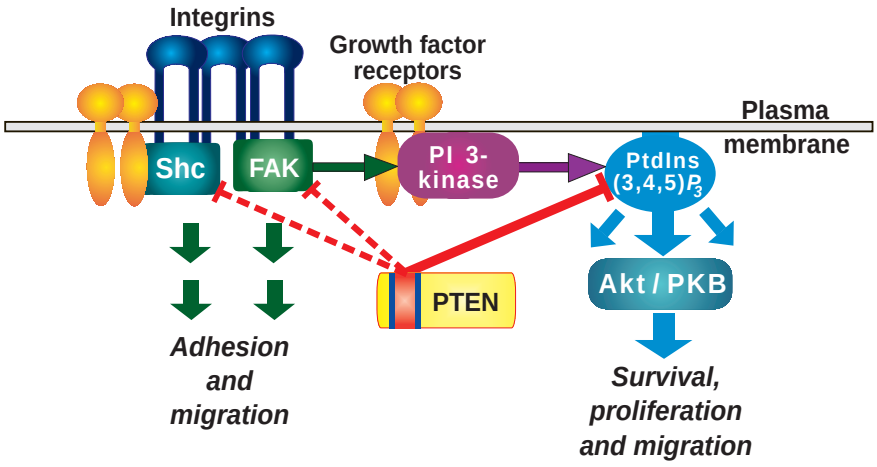

Fig. 2. Reported sites of action of PTEN. Extracellular interactions trigger signaling from integrins and growth factor receptors. The major function of PTEN appears to be downregulation of the PI 3-kinase product PtdIns(3,4,5)P_3, which regulates Akt and complex downstream pathways affecting cell growth, survival and migration. In addition, PTEN has weak protein tyrosine phosphatase activity, which may target focal adhesion kinase (FAK) and Shc, and thereby modulate other complex pathways. The phosphatase domain of PTEN (red) dephosphorylates and downregulates (red lines) substrate molecules.

of PTEN effects on potential downstream signaling targets such as Akt (also known as protein kinase B) versus some other system, such as Rac signaling (Leslie et al., 2000). Further studies should provide insight into the physiological modulation of PTEN activities by kinases, phosphatases, and other regulators acting on this C-terminal tail.

PTEN is developmentally regulated and necessary for embryonic development

Expression levels of PTEN protein are low in development until approximately day 11, when levels rise substantially in multiple tissues (Podsypanina et al., 1999), and the protein becomes widely distributed (Gimm et al., 2000). Soon after its discovery, several laboratories generated null mutations of the *PTEN* gene in mice to assess its function in vivo. The phenotypes differed considerably, presumably because of genetic differences between the mice used by each group. Nevertheless, all *PTEN*-knockout mice die before birth, demonstrating a requirement for PTEN in embryogenesis (Di Cristofano et al., 1998; Suzuki et al., 1998; Podsypanina et al., 1999). The phenotypes differed substantially: one study identified major defects in proper differentiation and organization of the ectoderm, mesoderm and endoderm (Di Cristofano et al., 1998) and observed death by embryonic day (E) 7.5; another found severe malformations of cephalic and caudal regions, which suggested an imbalance of growth and patterning, and observed death by E9.5 (Suzuki et al., 1998); a third study found severe defects by approximately E6.5, indicating that even the low level of PTEN protein present in early embryos is needed for successful embryonic development (Podsypanina et al., 1999). These differing results suggest a major role for context in PTEN functions. For example, PTEN may play important roles in germ layer organization or differentiation in one genetic background, but not in another; it may instead play crucial roles in regulating local apoptosis or proliferation in another setting. This theme of striking variability in biological or biochemical roles of PTEN under slightly different conditions recurs throughout the PTEN literature, suggesting that PTEN actions are influenced by tissue environment, cell type and/or cell culture conditions.

A homolog of PTEN exists in *Drosophila*, and, as in mammals, it plays roles in antagonizing phosphoinositide 3-kinase (PI 3-kinase), albeit in an insulin signal transduction pathway controlling cell size. Recent findings suggest that the *PTEN*-null phenotype in this organism does more than just affect the PI 3-kinase pathway: it also affects an independent pathway affecting proliferation (Huang et al., 1999; Gao et al., 2000).

Molecular targets of PTEN

PTEN phosphatase activity has been observed against both lipid and protein substrates. Overall, the primary physiological substrate of PTEN appears to be the signaling lipid PtdIns(3,4,5)P_3 (Fig. 1; Maehama and Dixon, 1998; Myers et al., 1998). PtdIns(3,4,5)P_3 is a major product of PI 3-kinase, which is activated by cell receptors including various tyrosine kinase growth factor receptors and integrins (Rameh and Cantley, 1999; Leevers et al., 1999). PTEN cleaves the 3′ phosphate from PtdIns(3,4,5)P_3 to generate PtdIns(4,5)P_2 (Maehama and Dixon, 1998), which lacks the activities of PtdIns(3,4,5)P_3 but has its own actions on cytoskeletal function. By antagonizing the action of PI 3-kinase, PTEN affects a number of cell biological processes (see below). In addition, it can dephosphorylate the signaling molecule inositol (1,3,4,5)-tetrakisphosphate (Maehama and Dixon, 1998), although the biological importance of this activity is not yet clear.

In vitro, PTEN can also remove phosphate residues from phosphotyrosine-containing peptides and proteins (Li and Sun, 1997; Myers et al., 1997; Tamura et al., 1998; Gu et al., 1998; Gu et al., 1999), although the relative importance of this enzymatic function in vivo compared with its lipid phosphatase activity has been controversial. Two cytoplasmic phosphoprotein substrates of PTEN are focal adhesion kinase (FAK) and the adapter protein Shc, whereas a number of other cellular tyrosine-phosphorylated proteins appear unaffected by PTEN (Tamura et al., 1998; Gu et al., 1999). FAK and Shc are central components of distinct signaling pathways (Fig. 2). The FAK signaling pathway is activated by integrins and other receptors and is linked to cell migration and other cellular activities. The Shc pathway is activated by receptors that include various tyrosine kinase receptors and integrins, and is part of a pathway that leads to activation of ERK MAP kinases. One study found a reduction of MAP kinase activation attributable to effects on Shc, whereas another found inhibition of MAP kinase activation through effects on IRS-1 (Gu et al., 1998; Weng et al., 2001). Although these in vitro effects have proven valuable for dissecting pathways that regulate cell migration (see below), analyses of cells from *PTEN*-knockout embryos fail to show changes in basal FAK phosphorylation or ERK activity (Stambolic et al., 1998; Liliental et al., 2000). By contrast, null mutations of the tyrosine phosphatases Shp-2 and PTP-PEST impair FAK dephosphorylation and alter focal contacts; the former also alters ERK activation (Yu et al., 1998; Angers-Loustau et al., 1999). These findings indicate that the major target of PTEN under steady-state conditions is PtdIns(3,4,5)P_3 and not FAK, although transient changes in PTEN levels might nevertheless still have physiological effects on FAK/Shc activity.

Interestingly, the G129E PTEN mutation abrogates most PTEN activity against PtdIns(3,4,5)P_3, but it retains activity against peptide and protein substrates (Myers et al., 1998); this mutation is found in some cancers. One puzzle about the tyrosine phosphatase activity of PTEN is that, even though effective activity is seen against substrates immobilized on nitrocellulose after SDS gel electrophoresis (Tamura et al., 1998), activity in living cells ranges from partial (e.g. 50% reduction in FAK tyrosine phosphorylation; Tamura et al., 1998) to undetectable (Maier et al., 1999). It has been pointed out that the amounts of enzyme needed for the in vitro tyrosine phosphatase activity are stoichiometric rather than catalytic (Cantley and Neel, 1999). For example, 15 μg/ml recombinant PTEN was needed to dephosphorylate FAK (Tamura et al., 1998). Nevertheless, this high concentration of PTEN is close to physiological levels, as determined by direct measurements of PTEN concentrations in fibroblasts, astrocytes and other cells (M.A., unpublished). Interestingly, even the lipid phosphatase activity of PTEN appears to be in limiting concentration: gene dosage effects are seen in heterozygous mice that have one defective and one normal *PTEN* allele,

which show a lymphoproliferative disorder in which Fas-mediated apoptosis is impaired because of the lower activity of PTEN in antagonizing PI 3-kinase and PtdIns(3,4,5)P_3 in heterozygotes (Di Cristofano et al., 1999).

Binding interactions and regulation of PTEN

The intracellular localization of PTEN varies depending on the cell type and the study, but it is generally relatively diffusely localized and does not associate preferentially with adhesion structures. Nevertheless, several binding interactions have been described. Wild-type PTEN does not bind effectively to phosphoproteins such as FAK. However, a strategy used for other phosphatases to generate a 'trapping mutant' of PTEN that can bind to a substrate without hydrolyzing it demonstrates that PTEN can interact with FAK and Shc (Tamura et al., 1998; Gu et al., 1999). In addition, novel interactions are being identified, including the binding of PTEN to the PDZ-interaction domains of the membrane-associated guanylate kinases MAGI2 and MAGI3. These are located in epithelial cell tight junctions and appear to cooperate with PTEN to regulate the activity of Akt (Wu et al., 2000a; Wu et al., 2000b).

Another contributor to the complexity of PTEN effects is likely to be the ratio of the strength of a stimulus to the level of PTEN. Specifically, even though PTEN can counteract insulin and PDGF signaling through PI 3-kinase to Akt under some conditions, it does not in others (e.g. compare Maehama and Dixon, 1998, Tamura et al., 1999a and references therein). The simplest explanation is that the magnitude of the growth factor receptor stimulus is important - if high, it may transiently overwhelm PTEN action.

Downstream changes in gene regulation and signaling after PTEN reconstitution (i.e. its restoration to cells lacking PTEN) appear to be complex. Microarray analysis indicates that PTEN overexpression in lung carcinoma cells leads to many changes in gene expression (Hong et al., 2000). In the signaling cascade downstream of Akt, forkhead family transcription factors appear to be key mediators of PTEN effects on G_1 phase cell cycle arrest and apoptosis (Nakamura et al., 2000). Besides targeting Akt, the PTEN substrate PtdIns(3,4,5)P_3 might regulate effectors that control ARF6, which regulates plasma membrane trafficking and cortical actin function (Cullen and Venkateswarlu, 1999; Vitale et al., 2000; Knorr et al., 2000; Jackson et al., 2000). These and other downstream effectors contribute to the complexities of PTEN activity.

PTEN levels themselves are negatively regulated by TGF-β (Li and Sun, 1997). They also vary substantially during the menstrual cycle in epithelial and stromal components of the endometrium (Mutter et al., 2000). In addition, frequent downregulation of PTEN expression in prostatic tumors and melanomas in which PTEN protein levels are reduced even in the absence of gene mutations has been described (Whang et al., 1998; Zhou et al., 2000). Analyses of PTEN function may be further complicated by widespread expression of a PTEN pseudogene in human but not mouse cells (Fujii et al., 1999; Kwabi-Addo et al., 2000); this pseudogene can even display phosphatase activity if expressed as protein.

PTEN in growth, apoptosis and anoikis

Because a tumor suppressor might be expected to suppress cell proliferation, several research groups have tested whether restoration of PTEN expression to cells that have mutated *PTEN* alleles suppresses growth. Transient expression using plasmid or adenoviral PTEN vectors suppresses proliferation. However, results are not always consistent, even in the same cell line. Most studies show suppression of proliferation due to arrest in G_1 phase of the cell cycle and corresponding increases in the levels of cell cycle inhibitors such as $p27^{KIP1}$ and decreased levels of retinoblastoma (Rb) protein phosphorylation (Furnari et al., 1998; Li and Sun, 1998; reviewed by Tamura et al., 1999c and Simpson and Parsons, 2001). However, one study finds this to be true only under reduced serum conditions but not in regular, 10% serum medium (Furnari et al., 1998), and G_1 arrest is also not always found (see Di Cristofano and Pandolfi, 2000). The G_1 phase cell cycle arrest is due to the lipid phosphatase activity of PTEN against PtdIns(3,4,5)P_3 (Ramaswamy et al., 1999). In fact, cell cycle effects of PTEN can be mimicked by SHIP-2, an enzyme that hydrolyzes another phosphate group on PtdIns(3,4,5)P_3 (Taylor et al., 2000). However, results using the G129E mutant provide evidence for an additional role for protein (versus lipid) phosphatase activity in G_1 cell cycle arrest (Hlobilkova et al., 2000).

It should be emphasized that PTEN has not been shown to be a physiological regulator of the normal cell cycle, since the above studies involved sudden restoration (and possibly overexpression) of an enzyme in cells adapted to proliferate in its absence. Moreover, PTEN cannot be a simple blocker of proliferation, since normal cells expressing PTEN can undergo rapid proliferation. In fact, one group found that rates of cell proliferation and levels of $p27^{KIP1}$ were normal in *PTEN*-null fibroblasts, despite being abnormal in *PTEN*-null embryonic stem cells (Liliental et al., 2000; Sun et al., 1999). Thus, even though sudden reconstitution of PTEN can suppress proliferation, the long-term role of this activity in cancer progression remains unclear.

The role of PTEN in apoptosis is clearer. Re-expression of PTEN in several carcinoma cell lines can induce apoptosis directly (Li et al., 1998), even though an apoptotic stimulus is often needed (Stambolic et al., 1998; reviewed by Tamura et al., 1999c; Simpson and Parsons, 2001). However, a particularly important role of PTEN is in the form of apoptosis termed anoikis. Anoikis is the induction of apoptosis in cells after loss of contact with the extracellular matrix (Frisch and Ruoslahti, 1997). This property may be a central feature of normal epithelial cell function (and perhaps certain other cell types) that prevents growth at abnormal sites, especially in suspension. This anchorage dependence of survival is defective in many transformed and malignant cells. Reconstitution of PTEN in cells that have *PTEN* mutations restores anoikis (Davies et al., 1998; Tamura et al., 1999a; Davies et al., 1999; Lu et al., 1999).

Anoikis has been linked to the signaling and scaffold protein FAK (Frisch et al., 1996). Levels of FAK are reported to be elevated in malignant cells (Owens et al., 1995), which may help these cells bypass anoikis. In fact, a cell line that becomes sensitive to anoikis after PTEN re-expression can acquire partial resistance when FAK is overexpressed along with PTEN (Tamura et al., 1999a).

PTEN modulates apoptosis by reducing levels of PtdIns(3,4,5)P_3. This signaling lipid regulates activation of Akt

through the kinases PDK-1 and PDK-2 (e.g. see Toker and Newton, 2000; Persad et al., 2000). Akt is well known as a central regulator of apoptosis. Re-expression of PTEN in various tumor cell lines decreases PtdIns(3,4,5)P_3 levels and reduces Akt activation (Stambolic et al., 1998; Haas-Kogan et al., 1998; Myers et al., 1998; Davies et al., 1998). The details of the pathway between PtdIns(3,4,5)P_3 and Akt remain to be confirmed, but a key intermediate appears to be integrin-linked kinase (ILK), which is reported to provide PDK-2 activity (Persad et al., 2000). The role of FAK in anoikis may involve at least in part its ability to increase levels of PtdIns(3,4,5)P_3 by enhancing PI 3-kinase activity (Tamura et al., 1999a). Dephosphorylation of FAK by PTEN would enhance the effects of PTEN on PtdIns(3,4,5)P_3, since FAK phosphorylation enhances PI 3-kinase activity (Reiske et al., 1999); the combined effects of reduced PI 3-kinase activity and direct reductions in PtdIns(3,4,5)P_3 levels by PTEN would block Akt activation and enhance apoptosis.

PTEN in cell adhesion, migration and invasion

PTEN reconstitution or overexpression inhibits cell migration (Tamura et al., 1998; Liliental et al., 2000). This inhibition can be accompanied by transient effects on cell adhesion and spreading: the number of focal contacts - specialized contacts mediating cell-substrate adhesion - is reduced, and the actin cytoskeleton is altered (Tamura et al., 1998), although the remaining focal contacts often appear to be larger. The mechanisms by which focal contacts can be modulated by PTEN include effects on the FAK-p130^{Cas} signaling pathway (Gu et al., 1999) and selective effects on focal contact constituents caused by changes in PtdIns(3,4,5)P_3 levels, as suggested by unrelated studies showing that PDGF can modulate focal contacts through PtdIns(3,4,5)P_3 (Greenwood et al., 2000).

PTEN suppresses migration of a variety of cell types, including primary human fibroblasts, non-transformed mouse fibroblasts, and tumor cells (Tamura et al., 1998; Tamura et al., 1999b). *PTEN*-null mouse fibroblasts also show enhanced rates of migration, which are reduced by reintroduction of PTEN (Liliental et al., 2000). PTEN also suppresses tumor cell invasion, as measured by in vitro assays of invasion across barriers of basement membrane extract (Tamura et al., 1999b). It reduces rates of migration through several mechanisms. One recently identified mechanism is through its effects on PtdIns(3,4,5)P_3 levels (see Fig. 3), which have downstream effects on Rac and Cdc42 signaling (Liliental et al., 2000; M.A., unpublished). However, tyrosine-phosphorylated FAK can bind to and activate PI 3-kinase (Reiske et al., 1999), and a contributing pathway affecting PtdIns(3,4,5)P_3 through FAK and PI 3-kinase has also been identified (Tamura et al., 1999a).

A second mechanism by which PTEN inhibits phosphotyrosine-based signaling pathways has proven useful for dissecting the signaling pathways that regulate cell migration (Fig. 3), although they do not prove that PTEN normally regulates these pathways. The regulation of cell migration can be divided into two components: (1) a directionally persistent migratory component promoted by the FAK-FAK-p130^{Cas} signaling pathway, which involves enhanced orientation of the actin cytoskeleton and increased focal contacts; and (2) a random-motility component promoted by Shc, MEK, and ERK MAP kinase signaling, which involves a modest increase in F-actin levels and random orientation of the actin cytoskeleton (Gu et al., 1999; see also Han et al., 2000; Klemke et al., 1997). Issues that remain to be resolved include whether the cytoskeletal changes are causal and how universal this system is for determining the overall rate and directional persistence of migration. Interestingly, at least one form of chemotaxis appears to use PtdIns(3,4,5)P_3 and Akt gradients for promoting directionality of migration in leukocytes (Wymann et al., 2000).

Conclusions and perspectives

PTEN is now known to be critically important both during embryonic development and in mature organisms as a tumor suppressor. Studies of its functions are providing novel insight into the regulation of apoptosis, migration, and tumor progression. PTEN appears to serve as a hub or switchpoint linking complex signaling pathways. Its disruption can lead to context-dependent, sometimes unpredictable or conflicting effects. Similar complexity has been described for p53, loss of which can be likened to disrupting a hub of the Internet (Vogelstein et al., 2000) or to the complex disruptions of air travel caused by problems at a single critical hub airport.

An additional source of potential complexity is the fact that PTEN is just one of several phosphatases that regulate cellular functions. Although knockout studies and the tumor suppressor literature identify crucial roles for PTEN, there may be partial but inadequate compensation by other phosphatases that have

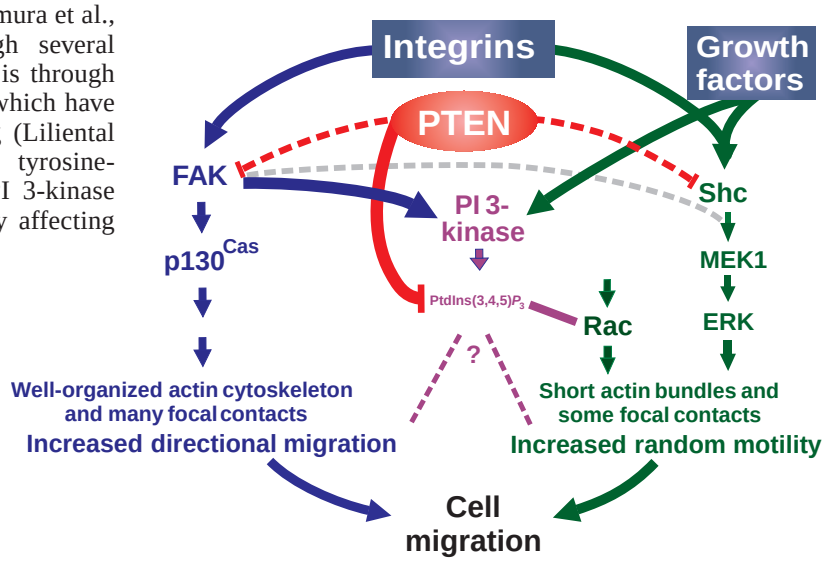

Fig. 3. Regulation of cell migration by integrins, growth factors and PTEN. The signaling networks regulating the speed and directional persistence of migration can be dissected by PTEN reconstitution and overexpression of individual signaling molecules and mutants affecting specific steps in signal transduction pathways. These signaling networks modulate the directionality and speed of cell migration, which combine to govern overall cell migration.

overlapping specificities. Conversely, transient overexpression of PTEN has helped to identify potential activities (e.g. when PTEN is induced), but there are also crucial basal functions of PTEN in PtdIns(3,4,5)P_3 homeostasis.

Now that the basic concepts of PTEN structure and function are firmly established, its possible functional regulation needs clarification. Do changes in its phosphorylation state regulate its activities? Why does it display tyrosine phosphatase activities under some conditions but often not under others? Does it have any other type of protein or lipid phosphatase activities? Why is it such an inefficient enzyme - for example, why do heterozygous mice have abnormal phenotypes? Are there problems of protein expression that contribute to human disease? For example, alterations in PTEN levels have been implicated in rheumatoid arthritis and tumor-induced angiogenesis (Pap et al., 2000; Wen et al., 2001); more examples are likely to be found. And, more generally, what is the full extent of intermolecular interactions involving PTEN, and how do its molecular targets vary under different physiological conditions (e.g. during development, the menstrual cycle and cancer progression)? These are only a few of the many questions still remaining to be explored in studies of this novel protein.

The explosive advances and exciting roles found for this recently discovered protein bode well for future studies of the many other novel proteins awaiting characterization after their identification in the human, mouse and other genome projects. However, the intriguing inconsistencies between the various in vitro and in vivo phenotypes found in studies of PTEN function by current approaches also suggest caution in assuming that one understands function on the basis of just one or two functional analyses in a single cell type or genetic background. Particularly informative future research could use gene-substitution methods to permit studies of regulated expression of genes such as *PTEN* in individual tissues over specific time courses and under different physiological conditions in order to help us understand its actions without resorting to overexpression. Such approaches should provide further insight into the roles of PTEN and other complex central regulators of cellular function.

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