New insights into PTEN

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Summary

The functions ascribed to PTEN have become more diverse since its discovery as a putative phosphatase mutated in many human tumors. Although it can dephosphorylate lipids and proteins, it also has functions independent of phosphatase activity in normal and pathological states. In addition, control of PTEN function is very complex. It is positively and negatively regulated at the transcriptional level, as well as post-translationally by phosphorylation, ubiquitylation, oxidation and acetylation. Although most of its tumor suppressor activity is likely to be caused by lipid dephosphorylation at the plasma membrane, PTEN also resides in the cytoplasm and nucleus, and its subcellular distribution is under strict control. Deregulation of PTEN function is implicated in other human diseases in addition to cancer, including diabetes and autism.

Key words: PI3-kinase activity, PTEN, Phosphatase, Cancer, Diabetes

Introduction

Phosphatase and tensin homolog (*PTEN*) was identified in 1997 as the relevant gene in a region of chromosome 10 that is often lost in late-stage human tumors, especially those of the brain, prostate and endometrium (Li et al., 1997; Steck et al., 1997). Soon after, it was discovered that PTEN demonstrates phosphatase activity against the phospholipid products of PI3-kinase activity, namely phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3], as well as other 3′ phosphorylated phosphoinositides, albeit to a lesser extent (Maehama et al., 2001). PTEN can also dephosphorylate protein substrates such as FAK (PTK2) (Tamura et al., 1999), as well as itself (Raftopoulou et al., 2004). The relative contributions of the lipid phosphatase activity and protein phosphatase activity, as well as additional phosphatase-independent functions, to the roles of PTEN in development and normal physiology, as well as in pathological states such as diabetes and cancer, are only just beginning to be unraveled. This Commentary focuses on recent insights into PTEN, in particular those that shed light on the regulation of its levels and activity, its role in human diseases, and novel downstream functions independent of PI3-kinase.

Transcriptional regulation of *PTEN*

Positive regulation of *PTEN* transcription

PTEN was initially assumed to be constitutively expressed, but several transcription factors have now been shown to bind directly to the *PTEN* promoter and regulate *PTEN* expression (Fig. 1). Following sequencing of the promoter, Virolle et al. noticed potential binding sites for early growth regulated transcription factor 1 (EGR1) (Virolle et al., 2001) and showed that *PTEN* is upregulated by EGR1 in response to radiation treatment (Virolle et al., 2001), and by IGF2 – as part of a negative-feedback loop (Moorehead et al., 2003). EGR1 also upregulates PTEN following treatment with the phosphatase inhibitor calyculin A, which might contribute to the apoptotic effects of this agent (Okamura et al., 2005). EGR1 levels strongly correlate with PTEN levels in a cohort of non-small cell lung cancer (NSCLC) tumors, and upregulation of these two proteins is associated with poor prognosis (Ferraro et al., 2005). An association between EGR1 and PTEN levels in thyroid cancers is not as clear (Di Loreto et al., 2005); PTEN is therefore likely to be regulated by other factors as well.

Indeed, Patel et al. have shown that peroxisome proliferator-activated receptor γ (PPARγ) also regulates *PTEN* transcription (Patel et al., 2001). Activation of PPARγ by its selective ligand, rosiglitazone – an anti-diabetic drug from the thiazolidinedione class – leads to binding of PPARγ to the *PTEN* promoter at two sites (PPRE1 and PPRE2) and to the consequent upregulation of *PTEN* in normal and cancer cells in humans. This is accompanied by a decrease both in phosphorylation of PKB (AKT) and in cell proliferation, leading the authors to speculate that PPARγ agonists might be beneficial for the treatment of cancers in which PTEN is still functional. For example, rosiglitazone increases *PTEN* expression and reduces migration in the hepatocarcinoma cell line BEL-7404 (Zhang, W. et al., 2006). Han and Roman showed growth suppression by rosiglitazone in NSCLC cells (Han and Roman, 2006). This effect has a PPARγ- and PTEN-dependent component but also a PPARγ-independent component via increased phosphorylation of AMP-activated protein kinase, which suppresses growth by inactivating the mammalian target of rapamycin (mTOR, also known as FRAP1).

p53 can also transcriptionally upregulate *PTEN* by binding to its promoter (Stambolic et al., 2001). A complex interplay exists between p53 and PTEN, which regulate the levels and activities of each other in several ways. For example, PTEN is required for p53-mediated apoptosis in immortalized mouse embryonic fibroblasts (MEFs). Indeed, $p53^{-/-}$ MEFs display only ~30% of the PTEN protein levels seen in $p53^{+/+}$ cells (Wang et al., 2005).

Another protein that modulates growth factor actions, human sprouty homolog 2 (SPRY2), has recently been shown to mediate its anti-proliferative effect by the regulation of PTEN. Over-expression of SPRY2 upregulates *PTEN* mRNA

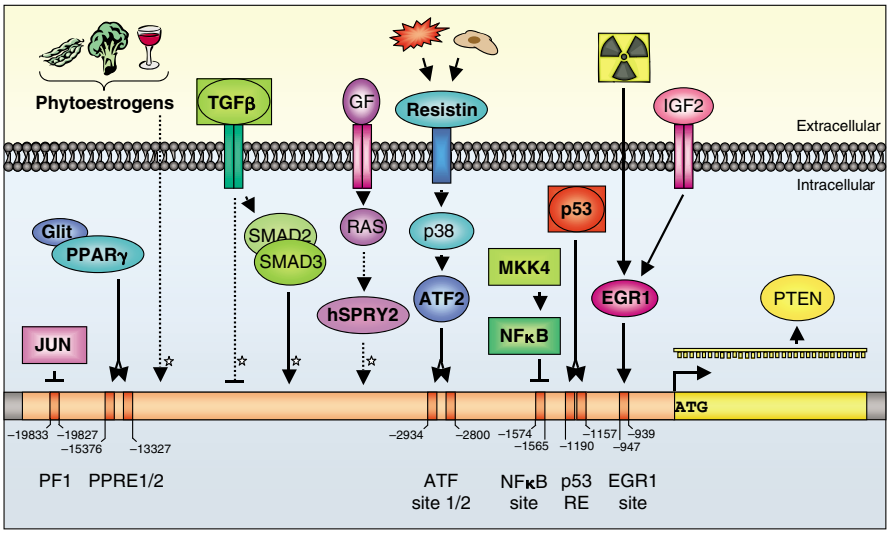

Fig. 1. Transcriptional regulation of *PTEN*. Illustrated are the pathways recently found to be involved in the regulation of *PTEN* transcription. Components of activating pathways are displayed as ovals and those of suppressing pathways as rectangles (note that some function as both activators and repressors). JUN was found to suppress *PTEN* transcription by binding to a variant activator 1 (AP-1) site, called PF1, roughly 19 kb upstream of the transcriptional start site (Hettinger et al., 2007). Activation of PPARγ by its selective ligands of the glitazone (Glit) group, used in the treatment of diabetes, upregulates *PTEN* expression by binding to two PPAR response elements (PPREs) (Patel et al., 2001). Upregulation of *PTEN* by glitazones has been observed by several other groups (Han and Roman, 2006; Lee et al., 2005; Zhang, W. et al., 2006). Phytoestrogens, such as genistein from soybeans, indole-3-carbinole from cruciferous vegetables like broccoli, and resveratrol found in red wine, lead to an increase in *PTEN* mRNA (Waite et al., 2005) and protein (Dave et al., 2005; Waite et al., 2005). In pancreatic cancer cells, TGFβ positively regulates *PTEN* transcription in a SMAD-dependent manner and negatively controls it in a SMAD-independent way (Chow et al., 2007). Furthermore, in a mesangial cell model for diabetic nephropathy, in which high glucose levels lead to a decrease in PTEN (protein) expression, this decrease was found to be mediated by suppressive effects of TGFβ (Mahimainathan et al., 2006). By contrast, binding of growth factor (GF) to its receptors in the cell membrane activates, via RAS, human SPRY2 (hSPRY2) to upregulate *PTEN* transcription (Edwin et al., 2006). Resistin, a peptide secreted by adipocytes and other cell types during inflammation, also positively regulates *PTEN* transcription. Resistin leads to activation of the p38 pathway and of ATF2, as well as to the binding of ATF2 to the *PTEN* promoter (shown in orange) to two ATF binding sites (Shen et al., 2006). Moreover, MKK4 inhibits *PTEN* transcription by activating NFκB, a transcriptional repressor that binds to the *PTEN* promoter ~1.5 kb upstream of the ATG (Xia et al., 2007). p53 regulates PTEN both positively at the transcriptional level and negatively at the protein-stability level: a functional p53 response element (RE) has been found in the *PTEN* promoter, and p53 induction leads to elevated *PTEN* mRNA and protein levels (Stambolic et al., 2001; Tang and Eng, 2006b). PTEN might autoregulate its own expression through stabilization of p53 protein independently of its phosphatase activity (Tang and Eng, 2006b). EGR1 binds to the *PTEN* promoter and upregulates its expression in response to radiation (Virolle et al., 2001) and IGF2 (Moorehead et al., 2003). (For pathways marked with a star, no specific *PTEN* promoter site has been identified as being involved in the regulation observed.) Solid lines represent demonstrated direct interaction of the respective protein with DNA; broken lines indicate indirect action.

and protein levels (but decreases phosphorylation of PTEN at S370 and S385, and its stability), and the ability of SPRY2 to restrain proliferation is abolished in PTEN-negative cells (Edwin et al., 2006).

Resistin, a cytokine involved in inflammation and insulin resistance, increases *PTEN* expression in human aortic vascular endothelial cells by activating p38 MAPK and activating transcription factor 2 (ATF2), which leads to decreased activation of PKB and of its target, endothelial nitric oxide synthase (eNOS, also known as NOS3). This provides a potential mechanism for resistin-mediated impairment of insulin signaling and of its role in cardiovascular diseases (Shen et al., 2006). Furthermore, phytoestrogens such as genistein (in soy), resveratrol (in red wine) and quercetin (in fruit and vegetables) also lead to increased PTEN expression, with a concomitant decrease in phospho-PKB levels and increase in levels of the CDK inhibitor p27 (Waite et al., 2005). Epidemiological data suggest that phytoestrogen consumption can protect against cancer (Park and Surh, 2004; Verheus et al., 2007). Upregulation of *PTEN* expression might therefore be one basis for these beneficial effects. Indeed, mammary glands of rats fed with soy protein or a genistein-supplemented diet display higher PTEN levels and a higher apoptotic index (Dave et al., 2005). The same is true for MCF-7 cells cultured in serum from such rats (Dave et al., 2005). Similarly, indole-3-carbinol, a phytochemical derived from cruciferous vegetables such as broccoli, upregulates *PTEN* expression in the cervical epithelium in a mouse model for cervical cancer (Qi et al., 2005).

Negative regulation of *PTEN* transcription

Most of the data so far have demonstrated positive regulation of *PTEN* transcription. Negative regulation of *PTEN* expression has also been shown, however (Fig. 1). Mitogen-activated protein kinase kinase-4 (MKK4) inhibits *PTEN* transcription by activating NFκB, which binds to a site in the *PTEN* promoter (Xia et al., 2007). Transforming growth factor (TGF)β also decreases *PTEN* transcription in pancreatic cancer cells (Chow et al., 2007) and in mesangial cells (Mahimainathan et al., 2006). Likewise, the proto-oncogenic

transcription factor JUN suppresses *PTEN* expression by binding to a variant AP-1 site in the *PTEN* promoter (this site is named PF1), and an inverse correlation between JUN and PTEN levels has been found in a panel of human tumor cell lines (Hettinger et al., 2007).

Interactions with other proteins and PTEN stability

PTEN levels are also modulated by changes in protein stability due to interactions with other proteins. Through its C-terminal PDZ-domain-binding motif, PTEN interacts with various proteins that contain PDZ motifs. Wu et al. showed that PTEN interacts with members of the membrane guanylate-kinase inverted (MAGI) family, such as MAGI2, which results in its stabilization (Wu, X. et al., 2000; Wu, Y. et al., 2000). More recently, the ubiquitously expressed cytoskeletal protein vinculin was shown to modulate this interaction. Vinculin-null cells have undetectable PTEN protein despite normal mRNA levels (Subauste et al., 2005). Subauste and colleagues have demonstrated that vinculin is required to maintain β-catenin–MAGI2 interactions at adherens junctions and thereby limit ubiquitin-mediated degradation of PTEN (Subauste et al., 2005). Recently, Valiente et al. showed that binding of PTEN to the microtubule-associated serine/threonine (MAST) kinases MAST3 and syntrophin-associated serine/threonine kinase (SAST, also known as MAST1) also stabilizes PTEN and facilitates its phosphorylation by these kinases (Valiente et al., 2005). PTEN-p53 interactions, by contrast, promote caspase-mediated degradation of PTEN (Tang and Eng, 2006b).

Regulation of PTEN by post-translational mechanisms

PTEN activity is heavily regulated by phosphorylation, acetylation, oxidation and control of its localization (Fig. 2). Below, we summarize some of the findings in this field; further details can be found in several excellent reviews published elsewhere (Baker, 2007; Gericke et al., 2006; Gil et al., 2007; Leslie, 2006; Vazquez and Devreotes, 2006).

Phosphorylation

PTEN is regulated by phosphorylation of a cluster of serine and threonine residues in its C-terminus (Fig. 2A). Phosphorylation of these residues is not strictly required for activity but stabilizes PTEN; alanine substitutions at these phosphorylation sites generally lead to higher catalytic activity but also to rapid degradation of PTEN in cells. Casein kinase 2 (CK2, also known as CSNK2) has previously been implicated in phosphorylation of some of these sites (Torres and Pulido, 2001). More recent evidence suggests that it might also prime some sites for phosphorylation by glycogen synthase kinase 3β (GSK3β). CK2 mainly phosphorylates S370 and S385, whereas GSK3β targets S362 and T366. The involvement of GSK3β could be part of a negative-feedback loop that regulates PTEN and PI3-kinase activity (Al-Khouri et al., 2005).

Neither CK2 nor GSK3β affects phosphorylation at S380 (Al-Khouri et al., 2005), but glioma tumor suppressor candidate region 2 (GLTSCR2, also known as PICT-1) was recently shown to interact with PTEN, promote its phosphorylation at S380 and upregulate its levels (Okahara et al., 2006; Yim et al., 2007).

Odriozola et al. have studied activity and localization of several C-terminal mutants of PTEN (Odriozola et al., 2007). They propose that the phosphorylated C-terminal tail (residues 380-385) interacts with the C2 and phosphatase domains in PTEN, serving as a pseudosubstrate and therefore causing auto-inhibition. According to this model, dephosphorylation of S385 followed by dephosphorylation of other residues in its vicinity leads to a more open conformation and unmasks the catalytic pocket, which leads to increased membrane affinity and increased PTEN activity (Fig. 2B). Additionally, there is evidence that RhoA-associated kinase (ROCK) phosphorylates S229, T232, T319 and T321 in the C2 domain to activate PTEN and target it to the membrane in chemoattractant-stimulated leukocytes (Li et al., 2005) (Fig. 2A). Similarly, the findings of Papakonstanti et al. place RhoA and ROCK upstream of PTEN (Papakonstanti et al., 2007). The mechanistic consequences underlying ROCK-mediated phosphorylation and activation of PTEN are not understood. Unexpectedly, the PI3-kinase catalytic subunit p110δ was found to inactivate PTEN through a pathway involving RhoA and ROCK, and increased tyrosine phosphorylation of PTEN, although the mechanism by which this occurs remains to be determined (Papakonstanti et al., 2007).

Acetylation

Acetylation is another mechanism that appears to regulate PTEN activity (Okumura et al., 2006) (Fig. 2A). The histone acetyltransferase p300/CBP-associated factor (PCAF) interacts with PTEN and acetylates lysines 125 and 128 in response to growth factors. These residues are located within the catalytic cleft of PTEN and are essential for PtdIns(3,4,5)P_3 specificity; consequently, PCAF functions as a negative regulator of PTEN.

Oxidation

PTEN activity can also be downregulated by reactive oxygen species (ROS), which oxidize the catalytic site cysteine residue, C124, to form an intramolecular disulfide bond with C71. Initially thought to be merely by-products of metabolism, ROS are now known to play a role in cell signaling. Using two neuroblastoma cell lines, one that can produce ROS and one that cannot, Seo et al. demonstrated that generation of ROS and subsequent inactivation of PTEN is required for insulin-mediated activation of PKB (Seo et al., 2005) (Fig. 2A).

Regulation of PTEN localization

Membrane recruitment

Localization of PTEN plays an important role in the regulation of its activity (Fig. 2B,C), as mentioned above. The best described function of PTEN is the dephosphorylation of PtdIns(3,4,5)P_3 at the plasma membrane. To access its target, PTEN needs to be recruited to the membrane. The C2 domain (Das et al., 2003), the phosphatase domain (Das et al., 2003) and the PtdIns(4,5)P_2-binding domain (PBD) (Downes et al., 2007; Vazquez and Devreotes, 2006) of PTEN are crucial for its binding to lipids. Additionally, PTEN is recruited to the membrane by interaction with proteins through its PDZ-binding motif (see above). Among these are MAGI1b, MAGI2, MAGI3, MAST3 and SAST. PTEN also interacts with NHERF1 (EBP50) and NHERF2 (Na^+/H^+ exchanger regulatory factor) adaptor proteins through its PDZ-domain-

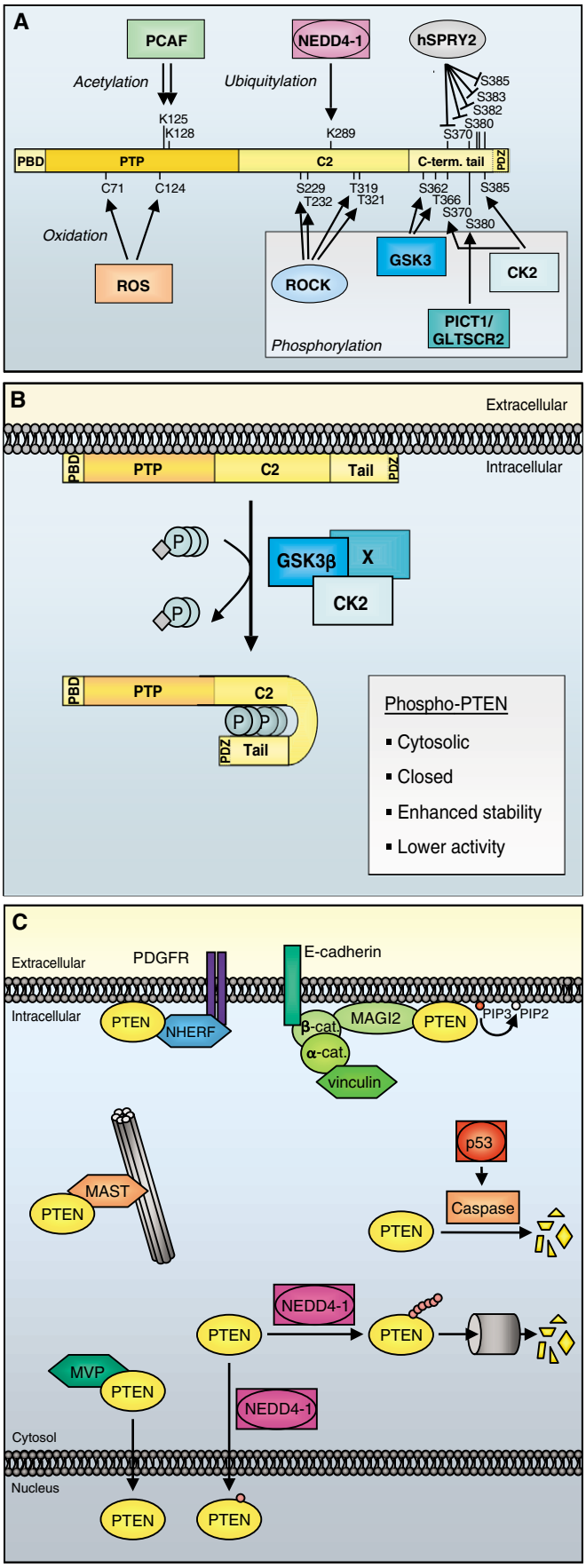

Fig. 2. Post-translational regulation of PTEN. Components of activating pathways are displayed as ovals and those of suppressing pathways as rectangles (note that some function as both activators and repressors). (A) PTEN activity, localization and stability are regulated by acetylation, ubiquitylation, oxidation and phosphorylation. In the presence of growth factors, the histone acetyltransferase PCAF acetylates PTEN at lysines 125 and 128 in the catalytic cleft. This acetylation negatively regulates PTEN activity, probably by interfering with its binding to its substrate, PtdIns(3,4,5)P_3 (Okamura et al., 2006). Ubiquitylation at K289 by NEDD4-1 affects both PTEN localization and stability (see C) (Trotman et al., 2007; Wang, L. et al., 2007). Human SPRY2 (hSPRY2) decreases phosphorylation of PTEN at several sites and thereby increases PTEN levels and activity (Edwin et al., 2006). CK2 phosphorylates PTEN at S370 and S385, and GSK3β phosphorylates S362 and T366. Phosphorylation at T366 is strongly increased by prior phosphorylation of the protein by CK2 (Al-Khouri et al., 2005). GLTSCR2 interacts with PTEN to promote phosphorylation at S380 and to positively regulate its levels (Okahara et al., 2006; Yim et al., 2007). Generally, phosphorylation in the C-terminal tail of PTEN is thought to enhance stability and to decrease membrane localization and activity. However, phosphorylation at T366 is linked to destabilization of PTEN (Maccario et al., 2007). ROCK activates PTEN and targets it to the plasma membrane, presumably by direct phosphorylation of S229, T232, T319 and T321 in its C2 domain (Li et al., 2005). (B) PTEN localization, activity and stability are controlled by phosphorylation. Phosphorylation of PTEN in the C-terminal tail by GSK3β, CK2 and possibly other kinases is generally thought to decrease membrane association and protein activity but to enhance protein stability. Phosphorylation of residues in the C-terminus of PTEN might cause the C-terminal tail to interact with the C2 domain, inducing a closed conformation and a predominantly cytosolic localization that inhibit PTEN activity (Odriozola et al., 2007). (C) PTEN localization and stability are controlled by interaction with other proteins. PTEN interacts with the NHERF1 and NHERF2 adaptor proteins by virtue of its PDZ-binding motif, and PTEN and NHERF proteins are found in a ternary complex with PDGFR. Activation of PI3-kinase after PDGFR stimulation is prolonged in *NHERF1*$^{-/-}$ MEFs and in *NHERF2* siRNA knockdown experiments, indicating that NHERF proteins normally recruit PTEN to PDGFR in order to restrict the activation of PI3-kinase (Takahashi et al., 2006). Interactions between MAGI2 and PTEN at the cell membrane prevent PTEN degradation. Vinculin is required to maintain β-catenin–MAGI2 interactions at adherens junctions and thereby limit ubiquitin-mediated degradation of PTEN (Subauste et al., 2005). Binding of PTEN to the MAST kinases MAST3 and SAST also stabilizes PTEN and facilitates its phosphorylation by these proteins (Valiente et al., 2005). Association of PTEN with MVP, a putative nucleocytoplasmatic transport protein, carries PTEN to the nucleus. This is dependent on two nuclear-localization-signal-like sequences but independent of PTEN phosphorylation and of its phosphatase activity (Chung et al., 2005). Monoubiquitylation of PTEN by NEDD4-1 increases nuclear localization (Trotman et al., 2007), whereas polyubiquitylation by NEDD4-1 promotes its degradation in the cytoplasm (Wang, X. et al., 2007). p53 also downregulates PTEN protein levels by promoting caspase-mediated degradation of PTEN (Tang and Eng, 2006a).

binding motif, and PTEN and NHERF proteins are found in a ternary complex with the platelet-derived growth factor receptor (PDGFR). Activation of PI3-kinase after PDGFR stimulation is prolonged in *NHERF1*$^{-/-}$ MEFs and when *NHERF2* is knocked down by RNAi. This indicates that NHERF proteins normally recruit PTEN to PDGFR to restrict the effects of PI3-kinase activation (Takahashi et al., 2006).

Exciting studies using single-molecule total internal reflection fluorescence (TIRF) microscopy in living cells have revealed that PTEN binds to the membrane for several milliseconds, which is sufficient to degrade several PtdIns(3,4,5)P_3 molecules. These also confirm that phosphorylation of the C-terminal tail constrains PTEN conformation, which limits its association with the membrane (Vazquez et al., 2006), supporting the earlier models (e.g. Li et al., 2005; Odriozola et al., 2007).

Nuclear/cytoplasmic shuttling

The existence of nuclear PtdIns(3,4,5)P_3 and PtdIns(4,5)P_2 point to a role for PTEN in the nucleus (Caramelli et al., 1996; Mazzotti et al., 1995), but this function is less clear than its well-established role at the plasma membrane. Whereas some researchers have detected PTEN exclusively in the cytoplasm (Gu et al., 1998), others have found it in both the cytoplasm and nucleus, and loss of nuclear PTEN has been associated with neoplasias and tumor formation (Gimm et al., 2000; Perren et al., 1999), indicating a tumor suppressor function of nuclear PTEN.

Employing different-sized GFP-PTEN fusion proteins, Liu et al. used fluorescence recovery after photobleaching (FRAP) to conclude that PTEN can passively diffuse through nuclear pores (Liu et al., 2005). By contrast, Chung et al. have described bipartite nuclear localization sequences in PTEN that are required for major vault protein (MVP)-mediated nuclear import (Chung et al., 2005). They have also detected different effects of cytoplasmic versus nuclear PTEN: cytoplasmic PTEN decreases phospho-PKB levels, upregulates $p27^{kip1}$ and is required for apoptosis, whereas nuclear PTEN downregulates cyclin D1 and phospho-MAPK and is crucial for cell cycle arrest (Chung and Eng, 2005; Chung et al., 2006). Gil et al. have also provided evidence for active transport of PTEN into the nucleus (Gil et al., 2006). Using a series of PTEN mutants, they suggest that multiple nuclear exclusion motifs (in the phosphatase domain, C2-domain and C-terminus) and NLS domains (in the N-terminus) control PTEN localization in a RAN-dependent manner.

The discovery of a Cowden-syndrome-associated lysine mutation in PTEN, K289E, led to further insight into the control of PTEN localization. This mutant retains catalytic activity but fails to accumulate in the nucleus owing to an import defect (Trotman et al., 2007). K289 and other lysines residing in PTEN are monoubiquitylated by the E3 ligase neural precursor cell expressed, developmentally downregulated 4-1 (NEDD4-1) in the cytoplasm, which permits nuclear import. Cytoplasmic monoubiquitylated PTEN can be ubiquitylated further by NEDD4-1 and degraded by the proteasome, can shuttle into the nucleus and back, or can be deubiquitylated in the nucleus, remain nuclear and thus be protected from cytoplasmic degradation (Wang, X. et al., 2007). This connection is illustrated by analyses of PTEN half-life: wild-type PTEN, both cytoplasmic and nuclear, has a $t_{1/2}$ of ~7.5 hours; forced cytoplasmic localization leads to a decrease in stability to $t_{1/2}$=4.5 hours; and forced nuclear accumulation increases this to 15 hours (Trotman et al., 2007).

Therefore, the traditional viewpoint that PTEN exerts all of its effects via dephosphorylation of PtdIns(3,4,5)P_3 at the plasma membrane needs to be re-evaluated in light of the overwhelming evidence for the existence and importance of nuclear PTEN. The exact targets and pathways that are affected by nuclear PTEN remain an exciting opportunity for future research.

Downstream consequences of PTEN that are independent of PI3-kinase

Many effects of PTEN are due to its ability to dephosphorylate PtdIns(3,4,5)P_3 and thereby antagonize PI3-kinase (Chow and Baker, 2006; Sansal and Sellers, 2004). However, recent evidence indicates that it has effects beyond its function as a phospholipid phosphatase, both in normal and in pathological signaling. For example, an important function of PTEN is likely to be its ability to restrain cell migration. This was shown to be independent of the lipid phosphatase activity of PTEN but dependent on its protein phosphatase activity. Surprisingly, the protein phosphatase activity is required only intramolecularly, and expression of the isolated C2 domain of PTEN, or a catalytically inactive T383A mutant, both inhibit cell migration as effectively as wild-type PTEN (Raftopoulou et al., 2004).

Another clue to the phosphatase-independent functions of PTEN came from studies of p53 regulation. In cells lacking PTEN, p53 levels are significantly reduced owing to decreased stability. Expression of wild-type or phosphatase-dead forms of PTEN increases p53 stability in an MDM2-independent manner (Freeman et al., 2003). This is because the C2 domain of PTEN binds to the C-terminus of p53, which increases its DNA binding and transcriptional activity (Freeman et al., 2003). A similar conclusion was reached by Li et al., who showed that PTEN promotes p53 acetylation through direct association with the histone acetyl transferase p300/CBP in response to DNA damage (Li et al., 2006). Li et al. further demonstrated that PTEN induces p53-acetylation-dependent tetramerization of p53, which promotes interaction with PTEN in a phosphatase-independent manner. This could account for the G1 arrest induced by nuclear-targeted PTEN but not G1 arrest induced by cytoplasmic-targeted PTEN. Liu et al. have already noted the PI3-kinase- and AKT-independent effects of nuclear-targeted PTEN, although they found PTEN phosphatase activity to be required for G1 arrest and for inhibition of soft agar growth (Liu et al., 2005).

PTEN and disease

Novel mechanisms underlying PTEN loss in tumors and diabetes in humans

Although mutation of one *PTEN* allele and loss of the second represents the most common mechanism underlying PTEN loss-of-function in human tumors, additional mechanisms have recently been discovered. Methylation of the *PTEN* promoter is a frequent occurrence in certain types of cancer, such as thyroid cancer (Alvarez-Nunez et al., 2006), melanoma (Mirmohammadsadegh et al., 2006), lung cancer (Marsit et al., 2005) and low grade/secondary glioblastoma mulitforme (GBM) tumors (Wiencke et al., 2007). There does not seem to be a straightforward correlation between *PTEN* promoter methylation and loss of *PTEN* expression in all cases analyzed, possibly owing to the small percentage of cells displaying *PTEN* methylation. Because *PTEN* methylation correlates with AKT phosphorylation in glial tumors, the most straightforward explanation is that the former causes the latter. However, recent results have shown that AKT activity can influence

methylation patterns (Cha et al., 2005), which suggests potential alternative mechanisms.

Additional mechanisms underlying PTEN alterations have also just been uncovered, including promoter mutations in GBM (Tunca et al., 2007), translocations in thyroid cancer (Puxeddu et al., 2005) and Cowden syndrome (CS)-like disease (Yue et al., 2005), and splicing mutations in Bannayan-Riley-Ruvalcaba syndromes (BRRS) (Suphapeetiporn et al., 2006), CS (Agrawal et al., 2005) and sporadic breast cancers (Agrawal and Eng, 2006). Furthermore, a micro-RNA that could target *PTEN* (miR-21) has been shown to be highly expressed in human cholangiocarcinoma cell lines (Meng et al., 2006) and hepatocellular carcinoma (Meng et al., 2007). Although modulation of miR-21 levels was shown to affect *PTEN* expression, note that miRs have been suggested to have many potential targets (Bentwich, 2005).

These recent data highlight the variety of genetic and epigenetic alterations that might underlie loss of PTEN in tumorigenesis and could be useful for identifying patients who might be candidates for PI3-kinase-targeted therapies. Interestingly, a germline polymorphism in the 5′ untranslated region (UTR) of the *PTEN* promoter was associated with patients with type 2 diabetes in a small study of a Japanese cohort (Ishihara et al., 2003). This polymorphism might increase *PTEN* expression and reduce PI3-kinase signaling in response to insulin (Ishihara et al., 2003), and illustrates the importance of PTEN activity in diseases other than cancer.

New animal models

Mice systemically lacking both copies of *PTEN* die early in embryogenesis. Heterozygous mice and chimeric mice lacking *PTEN* in some cells, by contrast, survive but spontaneously develop tumors in many organs (Bradley and Luo, 1998). Studies of heterozygous mice have revealed some unexpected genetic interactions, such as the dramatic decrease in tumors seen when they are crossed with PDK1 hypomorphic mice (Bayascas et al., 2005) and the dramatic increase when crossed with *TSC2*$^{+/-}$ mice (Manning et al., 2005; Ma et al., 2005). Interestingly, a recent study demonstrated that the onset and incidence of tumor formation in mice lacking one copy of *PTEN* is highly dependent on the genetic background, even when the deletions are identical (Freeman et al., 2006). Differences in genetic backgrounds might well be why at least three phenotypically distinct syndromes [CS, BRRS and Lehrmitte Duclos syndrome (LDS)] arise from germline *PTEN* mutations, some of which are also identical (reviewed in Zbuk and Eng, 2007).

Generation of conditional *PTEN*-knockout mice showed that loss of *PTEN* results in increased size and number of cells in almost all cases (reviewed in Stiles et al., 2004). However, these models have also revealed the role of *PTEN* in additional diseases. Specific deletion of *PTEN* either from muscle (using MCK-Cre) or adipocytes (using aP2-Cre), for example, does not cause either rhabdomyosarcomas or lipomas, respectively (as might be expected). These mice are healthy and fertile, and are instead protected from high-fat-diet- (Wijesekara et al., 2005) or streptozotocin (Kurlawalla-Martinez et al., 2005)-induced diabetes. Adipocyte-specific deletion of *PTEN* further results in increased energy expenditure and body temperature (Komazawa et al., 2004). Loss of *PTEN* specifically in pancreatic β-cells also protects from streptozotocin-induced diabetes, although these mice are significantly smaller than control littermates (Stiles et al., 2006). Finally, *PTEN*$^{+/-}$ mice are also protected from diabetes caused by knocking out insulin receptor substrate 2 (IRS2) (Kushner et al., 2005). Inhibition of PTEN in these tissues might thus represent an effective and non-toxic method for controlling type 2, diet-induced diabetes, a major world health problem.

Another non-tumor-related phenotype caused by *PTEN* deletion occurs following loss of *PTEN* in the neurons of the cerebral cortex and hippocampus. These mice exhibit a variety of abnormal social behaviors, increased response to sensory stimuli and decreased learning abilities (Kwon et al., 2006). Kwon et al. propose that this is reminiscent of autism spectrum disorders. Interestingly, a recent report found germline missense *PTEN* mutations in three out of 18 human subjects with autism spectrum disorders (Butler et al., 2005). Apart from macrocephaly, no other clinical features of CS, BRRS or LDS were seen in these patients, which further emphasizes the wide variety of phenotypes that result from PTEN alterations.

Attempts to associate PTEN alterations with additional neurological diseases, such as Alzheimers and Parkinsons disease, have been unsuccessful so far (Hamilton et al., 2006). Indirect evidence linking PTEN to Parkinsons disease (PD), however, does exist. PTEN-inducible kinase (*PINK1*, *PARK6*) was identified as one of 99 genes whose expression increases following expression of exogenous PTEN (Unoki and Nakamura, 2001). Germline mutations in *PINK1* were subsequently shown to be associated with a rare form of early onset PD (Valente et al., 2004). Another gene altered in PD patients is *DJ-1* (also known as *PARK7*). DJ-1 was independently isolated as a suppressor of PTEN function in a genetic screen in *Drosophila* (Kim et al., 2005). The exact mechanisms by which PINK1 and DJ-1 contribute to normal and pathogenic functions of PTEN remain to be determined.

PTEN and stem cells

Recently, an important role for PTEN in maintaining stem cells has emerged, which could affect how we interpret its tumor suppressor function and target it. Conditional deletion of PTEN in hematopoietic cells (Yilmaz et al., 2006; Zhang, J. et al., 2006) causes a myeloproliferative disorder and leukemia, which is consistent with its role as a tumor suppressor in many tissues. The number of hematopoietic stem cells initially expand, but ultimately decline, and cannot repopulate irradiated hosts when transplanted. Inhibition of PI3-kinase signaling by rapamycin simultaneously prevents leukemogenesis and restores the normal self-renewing capacity of the stem cell population. The inhibitory effect of PTEN loss on normal stem cell function might be tissue-specific, because deletion of PTEN in neural stem cells increases proliferation and the cells maintain their self-renewing capacity (Groszer et al., 2006). Deletion of PTEN in the basal stem cells of the prostate also causes increased proliferation, differentiation and prostatic intraepithelial neoplasia (Wang et al., 2006), which confirms the ability of PTEN loss in stem cell compartments to contribute to tumor formation. The effects of PI3-kinase-pathway inhibitors on tumor stem cells versus normal stem cells will clearly need to be studied before the safety profile of such drugs can be fully assessed.

Concluding remarks

The recent findings about PTEN regulation and function discussed above demonstrate that the pace of discovery is increasing, with the number of publications regarding this protein rising each year since its discovery. This has resulted in a greater understanding of the mechanisms underlying the regulation of PTEN and its roles in different human diseases, especially diabetes and cancer. Our comprehension of PTEN deregulation will hopefully translate into improved diagnosis, prognosis and treatment for patients. For example, PTEN levels can be used to stratify tumors that respond to new targeted therapies, including herceptin (Fujita et al., 2006), tarceva (Haas-Kogan et al., 2005) and tamoxifen (Shoman et al., 2005). Greater understanding of the different mechanisms underlying PTEN loss will undoubtedly refine the ability to more optimally treat patients. It will be exciting to see further studies examining the interaction of PTEN with other tumor suppressors or oncogenes. As new diabetes treatments are introduced, it will be interesting to see whether PTEN influences the response to these agents. Furthermore, as knowledge about PTEN post-translational regulation increases, more reagents should become available to examine under which conditions these occur. Another challenge for the future will be to understand the roles of nuclear versus cytoplasmic PTEN further, as well as phosphatase-dependent versus -independent functions and their biological relevance.

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