REVIEW ARTICLE

PTEN function: how normal cells control it and tumour cells lose it

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The PTEN (phosphatase and tensin homologue deleted on chromosome 10) tumour suppressor is a PI (phosphoinositide) 3-phosphatase that can inhibit cellular proliferation, survival and growth by inactivating PI 3-kinase-dependent signalling. It also suppresses cellular motility through mechanisms that may be partially independent of phosphatase activity. PTEN is one of the most commonly lost tumour suppressors in human cancer, and its deregulation is also implicated in several other diseases. Here we discuss recent developments in our understanding of how the cellular activity of PTEN is regulated, and the closely related question of how this activity is lost in tumours. Cellular PTEN function appears to be regulated by controlling both the expression of the enzyme and also its activity through mechanisms including oxidation and phosphorylation-based control of non-substrate membrane binding. Therefore mutation of PTEN in tumours disrupts not only the catalytic function of PTEN, but also its regulatory aspects. However, although mutation of PTEN is uncommon in many human tumour types, loss of PTEN expression seems to be more frequent. It is currently unclear how these tumours lose PTEN expression in the absence of mutation, and while some data implicate other potential tumour suppressors and oncogenes in this process, this area seems likely to be a key focus of future research.

Key words: phosphatase, phosphoinositide, protein kinase B (PKB)/Akt, PTEN (phosphatase and tensin homologue deleted on chromosome 10), tumour suppressor.

INTRODUCTION

The PTEN (phosphatase and tensin homologue deleted on chromosome 10) lipid phosphatase is a ubiquitous regulator of the cellular PI (phosphoinositide) 3-kinase signalling pathway (Figure 1). This signalling pathway is characterized by the regulated activation of class I PI 3-kinase enzymes, producing the second messenger PtdIns(3,4,5)P_3 (phosphatidylinositol 3,4,5-trisphosphate) from its relatively abundant precursor PtdIns(4,5)P_2 [1,2]. In turn, PtdIns(3,4,5)P_3 mediates downstream signalling through a range of effector proteins, including the proto-oncogene product PKB (protein kinase B)/Akt, that are able to recognize this lipid and bind it selectively [3–5]. PTEN antagonizes PI 3-kinase signalling by dephosphorylating the 3-position of the inositol ring of PtdIns(3,4,5)P_3 and thus inactivating downstream signalling [6–9] (Figure 1). Although other 3-phosphorylated inositol lipids and phosphates have been proposed as substrates for PTEN, current evidence indicates that PtdIns(3,4,5)P_3 may well be its only important physiological substrate of this type [10–12]. PTEN also has a weak protein phosphatase activity, and several target proteins have been proposed [13–15], including PTEN itself (see below) and the platelet-derived growth factor receptor, which may form a complex with PTEN [15]. Resolving whether these proteins are physiological substrates for the phosphatase is an important area for future work.

Signalling mediated by PtdIns(3,4,5)P_3 has been studied intensively, as it plays an important role in regulating many physiologically and pathologically significant processes, such as cellular proliferation, survival, growth and motility, and because levels of this lipid increase rapidly upon cellular exposure to a wide range of stimuli, including many growth factors. Particular attention has focused on PtdIns(3,4,5)P_3-dependent signalling in the field of cancer research, as deregulation of this signalling system appears to be an almost universal characteristic of human tumours, and plays at least some part in almost all of the cellular behaviours that characterize the tumour phenotype [3,16]. A recent review defined six characteristics that are usually necessary for tumour formation: evasion of apoptosis, self sufficiency in growth signals, insensitivity to anti-growth signals, sustained angiogenesis, tissue invasion and metastasis, and limitless replicative potential [17]. It is notable that PtdIns(3,4,5)P_3 signalling appears to be involved in the regulation of at least the first five of these six.

One reason for the importance of PTEN in cellular signalling and its tumour suppressor status in a wide range of tumour types is lack of functional redundancy. Although other PtdIns(3,4,5)P_3 phosphatases have been identified, there are no widely expressed PTEN homologues, and it appears that in many (or most) cell types, PTEN activity has a strong influence on both basal and stimulated PtdIns(3,4,5)P_3 levels [18,19]. Although the human genome contains at least seven gene-like sequences with significant identity to PTEN, including the PI 3-phosphatase TPIP (TPTE and PTEN homologous inositol lipid phosphatase), and also TPTE (transmembrane phosphatase with tensin homology), which appears to lack this activity, expressed sequence tags derived from many of the other gene copies appear to contain frameshift mutations or lack requisite parts of the phosphatase and C2 domains,

Abbreviations used: MAGI, membrane-associated guanylate kinase with inverted domain structure; NEP, neutral endopeptidase; PDZ, PSD-95/Dlg/ZO-1; PI, phosphoinositide; PICT1, protein interacting with C-terminal tail 1; PKB, protein kinase B; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PTP, protein tyrosine phosphatase; SH2, Src homology 2; SHIP, SH2-containing inositol phosphatase; TPIP, TPTE and PTEN homologous inositol lipid phosphatase; TPTE, transmembrane phosphatase with tensin homology.

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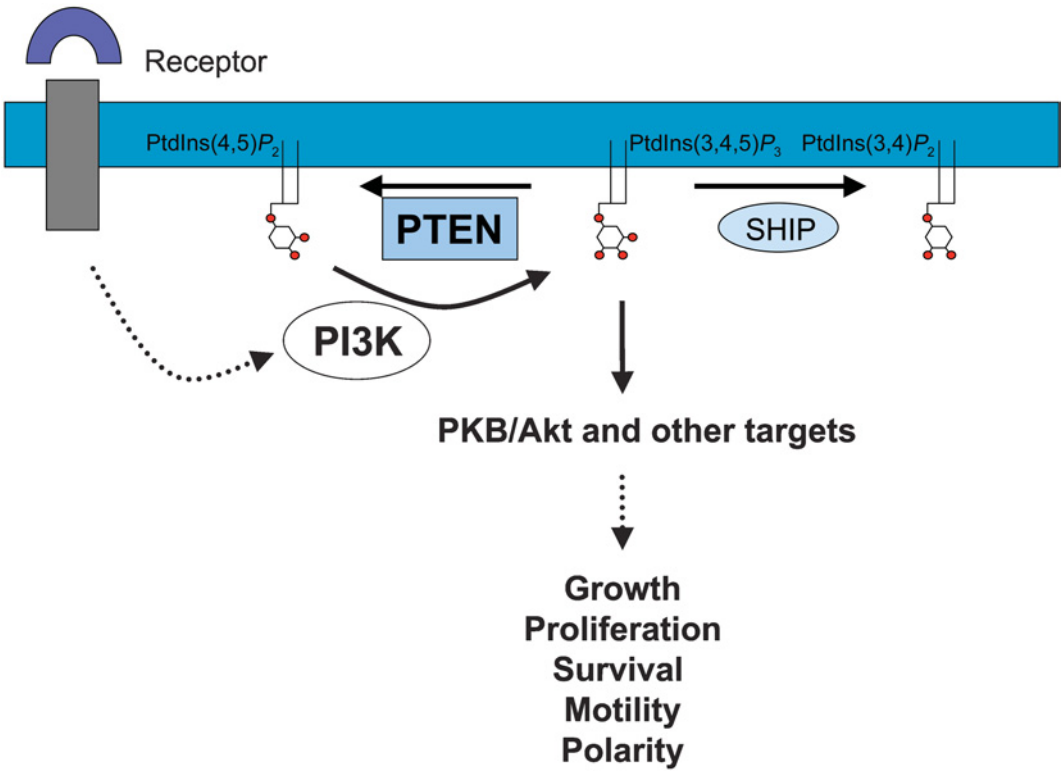

Figure 1 Model for the inhibition of PI 3-kinase signalling by PTEN and SHIP at the plasma membrane

The lipid second messenger PtdIns(3,4,5)P_3 is produced from the abundant cellular PI PtdIns(4,5)P_2 by the action of class I PI 3-kinase (PI3K) enzymes, which phosphorylate the 3-position of the inositol ring. PI 3-kinases are activated by diverse stimuli, including many that act through transmembrane growth factor receptors. PtdIns(3,4,5)P_3 is metabolized by two classes of phosphatases, exemplified by the PI 3-phosphatase, PTEN, which converts PtdIns(3,4,5)P_3 back into PtdIns(4,5)P_2, and the SHIP 5-phosphatases that convert PtdIns(3,4,5)P_3 into PtdIns(3,4)P_2. PtdIns(3,4,5)P_3 mediates effects on downstream signalling and cellular behaviour through protein targets, such as PKB/Akt, that are able to recognize this lipid and bind it selectively.

indicating that these may be pseudogenes [20,21]. Because the tissue distribution of TPIP and TPTE is limited, and since current evidence indicates that neither protein fulfils the function of PTEN in the regulation of PI 3-kinase-dependent signalling, it appears that these proteins do not represent widespread functional homologues of PTEN. Other unrelated enzymes that metabolize PtdIns(3,4,5)P_3 by dephosphorylation of the 5-position of the inositol ring have also been identified, most notably the SHIP [SH2 (Src homology 2)-containing inositol phosphatase] proteins (1 and 2) and possibly SKIP (skeletal muscle- and kidney-enriched inositol phosphatase), and these proteins do seem to be able to regulate PtdIns(3,4,5)P_3-dependent signalling [22–24]. However, the SHIP 5-phosphatases do not appear to regulate basal PtdIns(3,4,5)P_3 levels, since, in the absence of stimulation, cells lacking SHIP do not have the elevated basal levels of PtdIns(3,4,5)P_3 or PKB/Akt activity characteristic of PTEN-null cells [18,19,25–30]. Rather, loss of SHIP has been shown to enhance the duration and magnitude of stimulated rises in the levels of PtdIns(3,4,5)P_3 or PKB activity [25–28]. Thus it is probably the long-term elevation of lipid levels and activation of signalling caused by PTEN loss that is important for tumour development. This reflects findings indicating that the basal level of PtdIns(3,4,5)P_3 in unstimulated cells can play important permissive roles in cell signalling [31–33], a feature that is undoubtedly shared with many other signalling pathways.

This review will focus on the PTEN protein, and its physiological regulation and pathological deregulation. Other recent reviews have addressed broader aspects of the role of PI 3-kinase and PTEN signalling, including the signalling pathways upstream of PtdIns(3,4,5)P_3 and PI 3-kinase, and also downstream PtdIns(3,4,5)P_3-dependent signalling and physiology [3,34–36].

THE PTEN PROTEIN

PTEN (Figure 2) is a 403-amino-acid protein, and a member of the large PTP (protein tyrosine phosphatase) family. Determination of the PTEN crystal structure revealed that the N-terminal phosphatase domain is followed by a tightly associated C-terminal C2 domain [37]. These two domains together form a minimal catalytic unit, and comprise almost the entire protein, excluding only a very short N-terminal tail and a longer 50-amino-acid C-terminal tail. It is significant that the phosphatase/C2 domain core of PTEN, lacking the C-terminal tail, is sufficient for the metabolism of PtdIns(3,4,5)P_3 in cells, indicating that this not only is a competent enzyme, but is able to access its lipid substrate in the plasma membrane [37,38]. The termini, however, seem to play important roles in the regulation and/or targeting of enzyme activity. The N-terminal sequence contains a polybasic motif, extending somewhat into the start of the phosphatase domain, with some similarity to sequences found in proteins such as the ERM (ezrin, radixin and moesin) proteins and kinesin that have been proposed to bind PtdIns(4,5)P_2 [7,39,40]. The role of this motif in electrostatic membrane recruitment is discussed below. In contrast, the longer C-terminal tail contains a cluster of serine and threonine residues that become phosphorylated in many cells, a putative PEST sequence and, at the extreme C-terminus of the

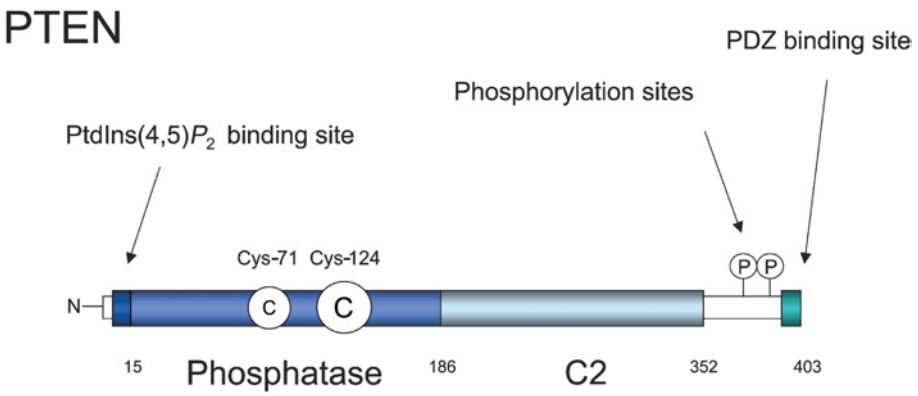

Figure 2 The PTEN protein

The 403-amino-acid PTEN protein is represented. The N-terminal phosphatase domain (amino acids 7–185) and the C2 domain (186–351) are both required for enzymic activity. The catalytic cysteine residue (Cys-124) is represented by a large encircled letter C, and Cys-71 by a smaller letter c. These residues form a reversible disulphide bond when the enzyme becomes oxidized. The N-terminal PtdIns(4,5)P_2 binding motif is shown at the N-terminal end of the phosphatase domain, although it is uncertain whether in cells this motif responds to PtdIns(4,5)P_2, or perhaps another more abundant acidic lipid such as phosphatidylserine. The extreme C-terminal PDZ binding sequence is also shown, and although it is represented as a small region, the extent of further sequences required for optimal specificity and affinity of binding is not known. The phosphorylation sites in the C-terminal tail are represented by a circled letter P.

protein, a binding site for a group of PDZ (PSD-95/Dlg/ZO-1) domain-containing proteins.

A recent study, of great potential importance, has shown that expression of the C2 domain of PTEN alone, in the absence of the phosphatase domain, is able to suppress cellular motility [41]. While these data do not show unequivocally that normal expression of the wild-type protein shares this effect, and the mechanism is unknown, the inactivity of other C2 domains and evidence for the regulation of this non-phosphatase function within PTEN supports the idea that this function is likely to be physiologically significant. Although the importance of PTEN lipid phosphatase activity for tumour suppression in some tumours and in Cowden disease is firmly established, it seems that PTEN may regulate cellular motility through two distinct mechanisms, and that both could play a role in the suppression of metastasis by PTEN. That study also provided evidence that the weak protein phosphatase activity of PTEN acts to dephosphorylate its C-terminal phosphorylation sites.

REGULATION OF PTEN FUNCTION IN NORMAL CELLS

In contrast with many other signalling enzymes, PTEN lacks obvious regulatory domains, and appears to have relatively high constitutive phosphatase activity, both *in vitro* and in cells [6,7,10,18,30]. For example, the other well characterized PtdIns(3,4,5)P_3 phosphatases, the SHIP 5-phosphatases, contain an SH2 domain, a poly-proline region and phosphotyrosine motifs, and appear to be tightly regulated, with low basal cellular activity. Thus a range of PTEN-null cells have been found to have greatly elevated basal PtdIns(3,4,5)P_3 levels, in contrast with cells lacking SHIP, which instead demonstrate a greater magnitude and duration of stimulated rises in the levels of this lipid [18,19,27,42].

Studies of PTEN regulation have taken some time to progress, reflecting the nature of these regulatory mechanisms. For example, regulation through oxidation of PTEN is reversible and is not conserved in normal lysis buffers containing reducing agents, and although phosphorylation of the enzyme appears to play a critical regulatory role in cells, it does not markedly affect the activity of the enzyme in simple *in vitro* assays. However, more recently, there has been relatively rapid progress in understanding how and under what circumstances PTEN phosphatase activity is regulated.

REGULATION OF THE PTEN PROTEIN: PHOSPHORYLATION, MEMBRANE RECRUITMENT AND OXIDATION

Phosphorylation of PTEN

Recent studies have given us a picture of PTEN regulation in which phosphorylation plays several important roles, mainly through induction of a conformational switch. Most cellular PTEN appears to be phosphorylated upon a cluster of serine and threonine residues (Ser-370, Ser-380, Thr-382, Thr-383 and Ser-385) in a highly acidic stretch of the C-terminal tail [43–48]. In this form, PTEN is also probably monomeric and cytosolic [43,44,46]. It seems likely that the identification of these phosphorylation sites was simplified by the fact that they appear to be phosphorylated constitutively, and it seems quite possible that other sites remain to be identified that only become phosphorylated in a small proportion of the cellular PTEN protein. Evidence has also been presented proposing the regulation of PTEN through tyrosine phosphorylation [49,50]. However, since physiological conditions that affect this phosphorylation have yet to be identified, the significance of this is currently unclear.

Using PTEN mutants in which the putative phosphorylation sites were changed to alanine residues, it has been inferred that dephosphorylation makes PTEN highly susceptible to proteolysis, increases its affinity for anionic lipids and enhances its localization to the plasma membrane. Also, despite the limited stability and expression levels of these proteins, non-phosphorylatable PTEN mutants have greatly enhanced biological activity in cells, presumably due to co-localization with their membrane-incorporated substrate [43,51]. It has been proposed that the dephosphorylation of these C-terminal residues causes a conformational opening up of the protein that mediates many of these effects of phosphorylation [46,51]. Although direct evidence for this is lacking, this seems a plausible hypothesis, since dephosphorylated protein is more sensitive to protease digestion *in vitro* [46], interacts much more efficiently with some protein binding partners [46], and also associates more effectively with cellular membranes [51]. It seems likely that the opening up of the PTEN structure reveals

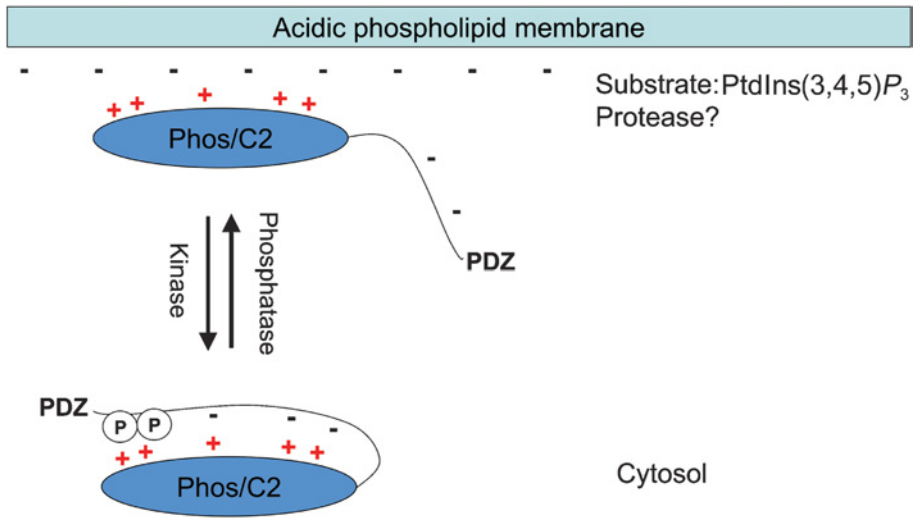

Figure 3 Model for regulation of PTEN activity by phosphorylation

A model for the regulation of PTEN activity has been developed, largely from the work of Vazquez et al. [43,46] and Das et al. [51]. Phosphorylated residues in the C-terminal tail of PTEN interact with basic regions of the phosphatase (Phos) and C2 domains, causing the long unstructured C-terminal tail to mask the basic membrane-binding surface of PTEN. This causes PTEN to remain in the cytosol, away from its membrane-located substrate, PtdIns(3,4,5)P_3. Dephosphorylation of these residues releases the C-terminal tail, allowing the electrostatic interaction of the phosphatase and C2 domains of PTEN with acidic membranes. This also enhances protein–protein interactions mediated by the C-terminal tail, particularly through the PDZ binding site. It is important to note that dephosphorylation of PTEN also enhances its proteolytic degradation.

the basic regions in PTEN that can mediate its binding to acidic membranes, and that in the phosphorylated protein these basic regions are hidden, perhaps through direct interaction with the highly acidic phosphorylated C-terminus. An illustration of this model is shown in Figure 3.

It has been proposed that the PTEN C-terminus is phosphorylated by the protein kinase CK2, which is considered to be constitutively active. In support of a role for CK2, this kinase phosphorylates PTEN very efficiently upon several C-terminal residues *in vitro*, and inhibitors of CK2 greatly decrease PTEN phosphorylation in cells [44,47]. However, since these inhibitors are weak and rather non-specific, this point remains to be demonstrated beyond reasonable doubt [52]. Most cellular PTEN appears to be maintained in a phosphorylated, inactive state, and its transient activation can be mediated through dephosphorylation. Therefore, identifying the phosphatases and pathways responsible for dephosphorylating PTEN *in vivo* is of significant interest.

Evidence that phosphorylation of PTEN is at least partially decreased by PI 3-kinase inhibitors supports the involvement of a PI 3-kinase-dependent kinase or a PI 3-kinase-inhibited phosphatase in a potential feedback control mechanism. One exciting additional possibility is that PTEN may initiate this regulatory switch itself, through autodephosphorylation specifically of Thr-383, by its own weak protein phosphatase activity [41,48]. This idea is supported by the finding that phosphatase-inactive PTEN mutants are found to be more highly phosphorylated upon C-terminal residues than the wild-type enzyme, and that the PI 3-kinase inhibitor wortmannin only partially decreased this phosphorylation [48]. In this model, autodephosphorylation reveals a phosphatase-independent capability of the PTEN C2 domain to inhibit cell motility. This property is present in the C2 domain expressed independently and in a full-length PTEN mutant (G129E) that retains protein phosphatase (but not lipid phosphatase) activity, but not in a totally phosphatase-dead mutant. However, mutation of a single C-terminal phosphorylation site, Thr-383, recovered the activity of phosphatase-dead PTEN. This would seem to indicate that the protein phosphatase activity of PTEN is required to dephosphorylate Thr-383, in order to relieve the inhibition of function caused by the phosphorylated C-terminus. Hopefully, it will be possible in the future to build upon this work, to form a picture of how the phosphorylation of PTEN upon the identified residues, and possibly others, is regulated.

PTEN shows a preference for substrates in acidic membranes

Several recent reports have shown that PTEN has greater activity towards substrates incorporated into lipid surfaces that have an acidic character, such as the inner leaflet of the cellular plasma membrane. PTEN shows enhanced activity *in vitro* against substrates presented in vesicles that include non-substrate acidic lipids, compared with uncharged vesicles [10]. Using surface plasmon resonance analysis, the transient interaction between PTEN and lipid surfaces was analysed directly, showing that this interaction was stronger with a more anionic surface, and that optimal binding required several basic regions of PTEN within the phosphatase and C2 domains [51]. Significantly, these findings were mirrored in the membrane binding of these proteins in cells [51]. Further analysis has shown that a polybasic motif at the PTEN N-terminus plays an important role in both the binding to anionic vesicles and the enhancement of activity, such that a mutation in this motif identified in a human glioblastoma (K13E) specifically impairs this binding and activation, and removes the ability of the enzyme to regulate cellular proliferation in culture [39].

The activation of PTEN by anionic lipids has also been observed using short-chain soluble lipids [53], and the authors proposed a conformational/allosteric activation mechanism. However, since such short-chain lipids strongly inhibit activity against the truly monomeric soluble substrate InsP_4, we feel that the data are better explained by a charge-based recruitment of PTEN to lipid micelles/aggregates. Together, all these data support a model in which PTEN is preferentially recruited to, and active against, lipids within membranes that contain relatively high levels of

anionic lipids, such as PtdIns(4,5)P_2 and phosphatidylserine. It is of some significance that data have recently been presented indicating that PTEN is active only against substrates located within the acidic plasma membrane, but not within internal membranes such as the less charged endoplasmic reticulum [54].

Localization to the cytosol, plasma membrane and nucleus

The localization of PTEN is somewhat controversial, due in part to the protein being localized in different places in different cell types. However, several lines of evidence indicate that the protein is largely cytosolic, that a small variable proportion is plasma membrane-associated, and that the proportion of plasma membrane-associated protein is tightly regulated and functionally significant. Many studies using expressed tagged PTEN have shown a uniform cytosolic localization (e.g. [55]), as have immunohistochemical studies of a variety of tissues [56–60]. These studies often also show significant nuclear staining of certain cell types, and in some cases predominantly nuclear staining [56–58,60]. Nuclear PTEN has rarely been seen in cultured cells, with the notable exceptions of differentiated PC12 cells [61] and primary vascular smooth muscle cells [62], where this localization was confirmed by both immunocytochemistry and subcellular fractionation. Very little is known regarding the functional consequences and mechanisms of localization of PTEN in the nucleus. More recently, some [63,64], but not all [62], immunofluorescence studies addressing the localization of the endogenous protein in cultured cells have identified a strong plasma membrane signal in addition to a diffuse cytosolic one. This is important because of recent evidence that many exogenously expressed tagged PTEN proteins are partially impaired in their membrane recruitment [39,65,66]. Therefore it seems likely that a significant fraction of endogenous PTEN is membrane associated, with some variations from cell to cell. Clearly, more studies on endogenous PTEN are required to confirm these conclusions. It is worthy of note that the recruitment of some molecules of PTEN on to the plasma membrane seems likely to be mediated through regulated electrostatic interactions with the acidic inner leaflet of the plasma membrane (see above), but probably not other endomembrane compartments such as the endoplasmic reticulum [39,51,54].

An important aspect of the localization of PTEN is the idea that it can be tightly regulated during chemotaxis in cells of the slime mould *Dictyostelium discoideum*, and possibly human neutrophils. PTEN relocalizes during chemotaxis to the trailing edge of these cells, and this may play a role in restricting the accumulation of PtdIns(3,4,5)P_3 at the leading edge of the cells during polarized cell movement [63,67]. Whether PTEN localizes to the rear of human cells during chemotaxis is still an important and controversial point, however [63,68,69]. Since human and *Dictyostelium* PTEN proteins are very different, this would represent a novel, highly conserved mechanism of PTEN regulation that would be expected to play an important role in the non-uniform accumulation of PtdIns(3,4,5)P_3 that appears to be an important event in many polarized cells. This implicates an interaction of PTEN with components specifically localized at the trailing edge of the cell, but the identity of such a component is currently unknown.

Membrane recruitment through protein–protein interaction

The factors regulating membrane recruitment through the electrostatic interaction of PTEN with membrane lipids are described above. However, PTEN also interacts with several membrane-located proteins. Most of these interactions involve the C-terminal tail of PTEN, beyond the C2 domain, and it is well established that, although this tail is not required for PTEN activity, either *in vitro* or in cells [37,43,70,71], it plays important regulatory roles. Thus the extreme C-terminus of PTEN is a binding site *in vitro* for PDZ domains within several proteins, including the MAGI (membrane-associated guanylate kinase with inverted domain structure) proteins 1, 2 and 3, DLG (discs large), MAST205 (microtubule-associated serine/threonine kinase of 205 kDa) and MUPP1 (multiple PDZ domain-containing protein 1) [65,72,73], and an interaction in cells has been shown for MAGI 2. Although the PDZ-dependent targeting of PTEN appears to be important in the cellular regulation of membrane ruffling, cell spreading and, at least in some studies, PKB/Akt [65,66,74], a clear picture has not yet emerged of which PDZ protein(s) are involved, or whether targeting of PTEN affects a specific pool of PtdIns(3,4,5)P_3 in a particular cellular location. It is conceivable that PDZ domain binding simply increases the effective concentration of PTEN at the plasma membrane or, alternatively, that PTEN is co-localized through such a mechanism with regulatory proteins such as protein kinases, phosphatases or antioxidant proteins.

A recent study has identified an interaction between the intracellular domain of plasma membrane-localized NEP (neutral endopeptidase) and a short stretch of the C-terminal tail of PTEN that includes the identified cluster of phosphorylation sites, although the interaction did not appear to require phosphorylation [64]. It appears that the interaction of PTEN with NEP is able to recruit the phosphatase to the membrane, and that the level of NEP may regulate downstream PKB/Akt through this mechanism, independently of its proteolytic activity [64].

Regulation through oxidation

PTEN is a member of the PTP enzyme family, which relies on a highly reactive active-site cysteine residue for catalysis. It has been recognized that this reactivity also makes PTP active-site cysteine residues sensitive to oxidation, and evidence is now accumulating that such oxidative inactivation may be an important physiological mechanism for the regulation of the activity of this diverse and important enzyme family. It has been shown recently that PTEN is highly sensitive to oxidation, with a disulphide bond being formed between the active-site cysteine Cys-124 and another cysteine, Cys-71, that lies very close to it in the PTEN crystal structure [75]. Studies have now shown that PTEN can become oxidized both during experimental oxidative stress and by endogenously produced reactive oxygen species, and that this oxidation of PTEN plays a non-redundant role in the redox regulation of PI 3-kinase-dependent signalling [76]. Although data are currently lacking, the regulation of PTEN by endogenous reactive oxygen species, its interaction with thioredoxin and the evolutionary conservation of Cys-71 in PTEN proteins from humans to *Drosophila* and *Dictyostelium*, despite this residue not being required for enzyme activity, all suggest that the regulation of this enzyme by redox processes is likely to be significant [75–77].

REGULATION OF PTEN EXPRESSION: TRANSCRIPTION AND PROTEIN STABILITY

Experimental evidence from mice and the study of a group of human inherited conditions has shown that PTEN heterozygosity is accompanied by phenotypic effects, some of which appear to result from haplo-insufficiency rather than loss of the remaining wild-type allele. Human Cowden disease patients, heterozygous for PTEN, display several developmental abnormalities and numerous benign polyps that appear to result from reduced expression of the PTEN protein. This and many other pieces of evidence

indicate the importance of correct regulation of the expression of PTEN.

Several circumstances have now been identified in which PTEN expression is transcriptionally regulated. The first was the identification of the *PTEN* gene as a target of the PPARγ (peroxisome proliferator-activated receptor γ) agonist rosiglitazone, currently in widespread use as an insulin-sensitizing agent in the treatment of diabetes [78]. The study identified two PPARγ binding sites in the PTEN promoter, and showed a dramatic up-regulation in PTEN expression in several cell types in response to rosiglitazone, and a corresponding inactivation of downstream signalling that did not occur in cells with reduced PPARγ expression [78]. This seems a paradoxical property for an insulin-sensitizing agent, and is the subject of further study examining whether it is a possible cause or a consequence of the insulin-sensitizing effects of the drug.

Subsequently, the transcription factors p53 and egr-1 have been shown to bind the PTEN promoter and regulate its expression, and it appears that both may play a role in the up-regulation of PTEN expression caused by irradiation [79,80]. Additionally, egr-1 appears to be required for the up-regulation of PTEN expression induced by insulin-like growth factor-II in the breast [81], and nuclear factor-κB to be required for the suppression of PTEN expression induced by tumour necrosis factor, although this latter effect appeared to be independent of p65 DNA binding [82]. Studies have also addressed the mechanism of constitutive expression of PTEN [83], and its down-regulation by transforming growth factor β [55] and up-regulation during granulocytic/monocytic [84] and neuronal [61] differentiation. However, despite these important studies, we have only a crude picture of the factors acting upon the PTEN promoter, or of how the regulation of PTEN expression fits in with other cellular signalling systems.

Significant evidence has also established that the abundance of PTEN is regulated through effects on the stability of the protein. This was originally identified in studies of the phosphorylation of PTEN in its C-terminal tail region [43,44] showing that the phosphorylated protein is relatively stable. Dephosphorylation of these residues leads to a functional activation of the protein (see above), but also to dramatic destabilization. Little is known about the precise regulation or mechanism of PTEN proteolysis, but several studies have implicated proteosomal degradation, since in some circumstances PTEN stability appears to be increased by proteasome inhibitors [44,45,85], and the detection of ubiquitinated forms of PTEN has been reported [45]. During apoptosis, it appears that PTEN protein becomes cleaved at several C-terminal sites, probably by caspase 3, sensitizing the protein to further degradation, and blocking interaction with PDZ domain-containing scaffold proteins [86]. However, the significance of these findings is currently unclear.

An exciting new study has identified a novel protein capable of regulating PTEN stability, which may also play a role in tumour development [87]. In a screen for proteins interacting with the C-terminal tail of PTEN, Maehama and colleagues identified a novel protein named PICT1 (protein interacting with C-terminal tail 1), which apparently localized to the nucleolus. When levels of PICT1 were lowered experimentally by RNA interference, this also led to a marked decrease in PTEN levels. Since the PICT1-interacting region of PTEN is very close to the C-terminal phosphorylation sites, it is interesting to speculate upon a common mechanism shared by the effects of PICT1 expression and PTEN phosphorylation on PTEN protein stability, but evidence for this is currently lacking. This research group then went on to show that PICT1 expression was lowered in a subset of human tumour samples, raising the exciting possibility that PICT1 might also act as a tumour suppressor.

DEREGULATION OF PTEN FUNCTION IN DISEASE

As discussed above, the PI 3-kinase signalling pathway plays an important role in the regulation of a wide range of significant biological processes. Thus it is unsurprising that deregulation of this signalling pathway appears to play a causal role in several different disease states, and indeed the pathway has been identified as a target for therapeutic intervention, with much recent activity surrounding attempts to develop isoform-specific inhibitors of PI 3-kinase enzymes for use in the treatment of several different conditions [88–90]. With regard to PTEN specifically, alterations in the expression and activity of the phosphatase have been proposed to play a causal role in the development of several conditions other than cancer, including rheumatoid arthritis, chronic obstructive pulmonary disease and pulmonary fibrosis [91–93]. However, understanding of the role of PTEN in these conditions is currently at an early stage, and here we will focus on the well established role of PTEN deregulation in cancer. Clearly the same mechanisms that are used by cells in the normal regulation of PTEN are found to be deregulated pathologically, but a better understanding specifically of this pathological deregulation should assist in the development of therapeutics targeting PTEN and the PI 3-kinase pathway.

The *PTEN* gene was originally identified due to its mutation in several types of sporadic tumour and tumour cell lines [94,95]. Evidence for mutation of the *PTEN* coding sequence in many diverse tumour types [96], the greatly elevated susceptibility of *PTEN* heterozygous mice to a range of tumour types [97–99] and several tissue-specific mouse knockout experiments [100–103] strongly support the status of PTEN as an important tumour suppressor for many types of cancer. In addition, soon after the identification of PTEN, it was discovered that inherited mutations in *PTEN* can cause several human disorders, including Cowden disease, Bannayan–Riley–Ruvalcaba syndrome and *Proteus* syndrome [104,105]. Cowden disease in particular is accompanied by an increased cancer risk, specifically of breast and thyroid tumours. It is currently unclear, and of some interest, how several rather different autosomal dominant human disorders can be caused by apparently equivalent mutations in the same gene [106]. Of the 150 or so mutations in PTEN that have been identified in patients suffering from these inherited disorders, none have been described in the C-terminal tail, in common with endometrial tumours, but in stark contrast with data from some other tumour types, such as glioblastoma. Since the C-terminal tail is dispensable for catalytic activity, this suggests that complete loss of PTEN activity is required for the development of these inherited syndromes, and possibly of endometrial tumours, whereas the partial loss of function caused by C-terminal mutation may be sufficient to assist the progression of other tumours such as glioblastomas. As described above, strong evidence indicates that loss of PTEN drives tumour development through deregulation of PI 3-kinase signalling and, in turn, processes including cell growth, proliferation and survival. It is also likely that PI 3-kinase-independent tumour suppressor pathways may exist [41].

The classical view of tumour suppressor genes has been that they are recessive, such that both copies must be lost in order to drive tumorigenesis. However, this view is being challenged by the identification of tumour suppressors for which partial loss has significant effects [107]. It now seems very likely that only partial loss of PTEN gives a selective advantage to tumour cells, and this finding has great implications for the mechanisms of PTEN loss in tumour development. *PTEN* haplo-insufficiency in both humans and mice confers significant phenotypes, despite retention of a wild-type allele, and can clearly promote tumour development [105,106,108,109]. In agreement with these findings, very many

Table 1 Approximate frequencies of mutation, loss of heterozygosity and loss of expression of *PTEN* in different tumour types, derived from the averages of data from various studies

Much of the data for mutation and loss of heterozygosity (LOH) comes from reviews by Ali et al. [96] and Eng [106], and most of the references refer to immunohistochemical studies of PTEN expression. For data regarding loss of expression, where possible data refer to expression levels undetectable in the relevant study, this does not include tumours with apparently reduced expression, and hence is likely to represent an underestimation of the significance of PTEN loss.

	Frequency (%)			
Tumour type	Mutation	LOH	Loss of expression	References
Breast	4	36	32	[59,114]
Lung	3	47	19	[116,127]
Prostate	13	33	27	[115,128,129]
Colon	11	22	17	[57,130,131]
Endometrial	42	50	35	[132–136]
Glioblastoma	20	76	54	[56,137]

tumours are found to retain an intact copy of *PTEN*, and partial loss of protein expression appears to be common (Table 1 and [106,110]).

Mechanisms of PTEN inactivation in tumours

PTEN was initially identified as a putative tumour suppressor through its mutation in a variety of tumour types [94,95]. Subsequently, mutation of the *PTEN* gene has been analysed in many studies of different tumour types [96,106]. These studies have revealed that *PTEN* mutations occur in a very wide range of sporadic tumour types, but at high frequencies (>15%) only in the rather uncommon endometrial carcinoma and glioblastoma multiforme, for which most estimates are approx. 30–50% and 15–30% respectively [96,106]. In these conditions, the frequency of loss of heterozygosity at the *PTEN* locus appears to be higher, with estimates being approx. 40–55% for endometrial carcinoma and 50–70% for glioblastoma (see Table 1).

Mutation of the *PTEN* coding sequence

Several mechanisms have come to light by which different classes of mutation of the *PTEN* gene promote tumour development. Most mutations occur within the coding sequence of the gene, and most of these inactivate the phosphatase activity of the encoded enzyme. It is known that truncation or frameshift mutations within the phosphatase/C2 domain catalytic unit destroy phosphatase activity. Also, an extensive analysis of the effects of tumour-derived point mutations on the phosphatase activity of PTEN has revealed that the great majority of such mutations abolish (81%) or greatly decrease (10%) phosphatase activity [111]. The great majority of PTEN mutations in sporadic tumours, and possibly all mutations in *PTEN*-associated inherited conditions, fall into these classes that interfere directly with enzymic activity. However, the work of Han et al. [111], and other studies, have shown that a small fraction of tumour-derived mutants retain strong phosphatase activity *in vitro*, indicating that these mutations interfere with other aspects of PTEN's function that are required for the enzyme to act efficiently as a tumour suppressor in cells.

Since the approx. 50-amino-acid C-terminal tail of PTEN is not required for catalytic activity, mutations within this region do not appear to affect enzymic activity directly, and although many mutations have been identified in this region of the protein in tumours, the predicted frequency of mutation is lower than for other regions of PTEN that are required for catalytic activity. As noted above, the C-terminal tail of PTEN contains phosphorylation sites, a binding site for a group of PDZ domain-containing proteins, and possibly other sequences mediating protein–protein interactions. Thus mutations in this region have been shown to affect protein stability and targeting, and while some C-terminal mutants have probably lost the ability to regulate global cellular PtdIns(3,4,5)P_3 levels and consequently PKB/Akt, other mutants retain this capability. These findings indicate that, while most PTEN mutations cause total loss of PTEN function, many (if not all) mutations in the C-terminal tail cause only a partial loss of function [66,70,71,111]. This may be of great importance in identifying pathways deregulated in particular tumour types. It is worthy of note, and highly statistically significant, that while many mutations in the C-terminal tail of PTEN have been identified in glioblastomas, they have never been identified in a similar number of endometrial carcinomas, or in families with Cowden disease [6,96,112].

Although smaller, the extreme N-terminus of PTEN appears similar in this analysis [39]. As described above, the N-terminus is required for the binding of PTEN to lipid surfaces and for biological activity, as indicated by the regulation of PKB/Akt and cellular proliferation in culture [39,51]. To our knowledge, only two mutations in the N-terminal motif have been identified in tumours. Other non-catalytic functions have not yet been shown to be mutated in tumours. Although artificial mutation of loops within the C2 domain has been shown to decrease non-substrate lipid binding and biological function, pathological mutations of this nature have yet to be identified, and missense point mutations are much less common within the C2 domain than within the phosphatase domain.

Loss of expression of the PTEN protein: genetics and epigenetics

Although the *PTEN* gene was originally identified through its mutation in several types of sporadic tumour, substantial evidence now indicates that loss of expression of the protein, through as yet largely unidentified mechanisms, may be of far greater significance in terms of patient numbers. Using conventional mutation detection techniques that have been highly successful with other tumour suppressors and with *PTEN* in tumours such as glioblastoma and endometrial carcinoma, mutation of the *PTEN* gene has not been detected at high frequency in common tumour types such as lung, breast, prostate or colon cancer (see Table 1 and [96,106]). However, it is potentially important that more recent immunohistochemical and other studies have presented data indicating that expression of the PTEN protein may be lost from many of these common tumours in addition to glial and endometrial tumours [57,113–116]. Some of these studies present particularly strong data, as they combine immunohistochemical and mutational analyses, identifying small numbers of tumours with characterized mutations, which give convincingly negative staining alongside adjacent normal PTEN-expressing tissues. These data also identify large numbers of tumours with no detectable mutation of the *PTEN* gene, but also with no detectable expression of the protein [57,59]. Significantly, similar data regarding loss of PTEN expression in tumours have been obtained using Western blotting and real-time reverse transcription–PCR [117,118].

In some of these tumour types (particularly breast and lung tumours), although mutation of the *PTEN* gene is rare, loss of heterozygosity at the *PTEN* locus is very common (occurring in approx. 31% and 47% of tumours respectively [106]). Analysis combining genetics with well controlled immunohistochemistry suggests that, in many tumours, one *PTEN* allele is deleted, and although these tumours retain one copy of *PTEN* with no detectable defect, expression of PTEN is undetectable by immunohistochemistry, presumably being lost through unknown epigenetic

mechanisms [57,59]. Although these and several other studies also describe tumours with decreased PTEN expression relative to surrounding tissues and other similar tumour cells, the lack of a clear reference level, despite the apparent ubiquitous expression of PTEN, makes the significance of this finding difficult to judge.

These potentially highly important studies raise the question of how expression of the apparently ubiquitous PTEN protein might be lost from these tumours. Several possible mechanisms for the deregulation of PTEN expression in tumours have been identified, although we have a rather poor understanding of these processes. It seems likely that other cellular changes that occur during tumour development, such as mutation of known tumour suppressors and oncogenes, for example loss of p53 [79,119] or of the recently described PICT1 [87], also result in loss of PTEN expression. One potential mechanism of loss of PTEN expression could be through mutation of the PTEN promoter. In a study looking for mutations of the *PTEN* gene in patients with Cowden disease or Bannayan–Riley–Ruvalcaba syndrome in which mutations had not been identified by standard methods, Zhou et al. [120] identified nine Cowden disease patients with mutations in the PTEN promoter. Since no mutations were identified in 186 normal patients, these mutations are probably pathogenic.

There is convincing evidence that the expression of tumour suppressor genes can be greatly decreased in some tumours by promoter methylation [121], and some reports have proposed that this may be the case for PTEN [116,122]. However, recent studies that included analysis of the *PTEN* pseudogene on chromosome 9 have called this conclusion into question. These studies indicated that the promoter of the *PTEN* pseudogene, and not of *PTEN*, is methylated in a variety of tumours and tumour-derived cell lines [123,124]. Since several studies have used techniques that would not distinguish between these possibilities, these data would argue against common methylation and silencing of the PTEN promoter. However, the issue remains controversial, and it may be that promoter methylation is important in some tumour types [125,126].

It should be clear from these points that we currently have no clear picture of the mechanisms by which PTEN expression may be lost in the absence of coding sequence mutation. However, the potential importance of these mechanisms in tumour formation is significant, and the pace of development has been rapid in the last few years.

FUTURE PERSPECTIVES

Since the discovery of PTEN as a lipid phosphatase and regulator of PI 3-kinase signalling, much has been achieved in defining its role in cellular physiology and identifying how the activity of the phosphatase is regulated. It is now clear that the deregulation of PTEN through mechanisms targeting both catalytic/structural and regulatory aspects of the protein is important in the development of many cancers and possibly other diseases. The recent identification of a function of PTEN mediated independently of phosphatase activity by the C2 domain is of great potential importance. It not only indicates that the weak protein phosphatase activity of PTEN may have a real role in self-regulating the activity of the protein, but also raises the possibility that some effects, previously believed to involve antagonism of PI 3-kinase signalling, may be mediated through this novel, but ill defined, mechanism. With the realization that loss of PTEN expression in the absence of mutation may be far more common than mutation of the *PTEN* gene in most cancers, future research must focus on processes regulating the transcription of the *PTEN* gene and/or turnover of the PTEN protein.

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