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The PTEN protein: cellular localisation and post-translational regulation

Nick R. Leslie, Nisha Kriplani, Miguel A. Hermida, Virginia Alvarez-Garcia and Helen M. Wise

Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot Watt University, EDINBURGH, EH14 4AS, UK

Correspondence email: n.r.leslie@hw.ac.uk Tel: 44 131 451 8157

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Abbreviations:

PTEN: Phosphatase and Tensin homolog deleted on chromosome 10

PI3K: Phosphoinositide 3-Kinase

PtdIns: Phosphatidylinositol

PtdSer: Phosphatidylserine

PIP_3: Phosphatidylinositol 3,4,5-trisphosphate

mTOR: Mammalian target of rapamycin

SPOP: Speckle type POZ protein

NEDD4: Neural precursor cell expressed, developmentally downregulated 4

MKRN1: Makorin Ring Finger Protein 1

SUMO: Small ubiquitin-like modifier

Abstract

The PTEN phosphatase dephosphorylates PIP_3, the lipid product of the class I PI 3-Kinases, and suppresses the growth and proliferation of many cell types. It has been heavily studied, in large part due to its status as a tumour suppressor, the loss of function of which is observed through diverse mechanisms in many tumour types. Here we present a concise review of our understanding of the PTEN protein and highlight recent advances, particularly in our understanding of its localisation and regulation by ubiquitination and SUMOylation.

Introduction

PTEN is a tumour suppressor protein which dephosphorylates the cellular lipid signal, PtdIns(3,4,5)P_3, acting in direct opposition to the class I phosphoinositide 3-kinases (PI3Ks) to influence cell processes including growth, proliferation and polarisation [1-3]. These cellular effects of PI3K and PIP_3 are mediated by a complex downstream signalling network, comprising numerous PIP_3 binding proteins which can be regulated in part by the abundance and localisation of the lipid, including for example the heavily studied AKT group of oncogenic protein kinases. The network is also often considered to include other components further downstream that are regulated in part by PI3K activity, such as the mTOR protein kinase which is a key regulator of cell growth and also an important drug target [2, 4]. PTEN is found in the cytosol and nucleus, appearing to transiently associate with the plasma membrane through non-substrate interactions with membrane lipids, to metabolise its principal substrate, but also binding to a large number of partner proteins [3, 5-7].

A remarkable feature of PTEN is its degree of amino acid sequence conservation, particularly within vertebrates. For example, the human and mouse PTEN proteins, each 403 amino acids long, differ only by one (highly conservative) serine to threonine change, located in the regulatory C-terminal tail, i.e. 99.75% identical. This is a far stronger conservation than most other protein orthologs, with analyses finding an average amino acid identity of around 85% for human and mouse pairs [8]. This high level of conservation implies that strong selection pressure limits even apparently minor divergence from the current structure and that almost any amino acid change impairs the function of the protein somewhat in terms of catalysis, regulation, stability etc. Analysis of human variation within the PTEN gene supports this conclusion. Data collated by the 1000 Genomes project (www.1000genomes.org) identify 38 single nucleotide variants as candidate SNPs in the PTEN coding sequence: around half are pathogenic, only 3 have been identified in more than 1 unrelated individual and none have an apparent allele frequency above 0.001.

A potentially related finding is that unlike many other tumour suppressor proteins, and in disagreement with the established paradigm, PTEN's functions as a tumour suppressor appear strongly dose dependent, with even modest reductions in expression leading to significant increases in tumour risk [9, 10]. This would seem to agree with the diversity of functions of PTEN. The human genome encodes many tens of proteins that appear to be independently regulated by PTEN's PIP_3 substrate, each mediating a different set of controlling influences of PIP_3 abundance/localisation on numerous downstream biological processes, with different potential influences on tumour formation in different cell lineages. Similarly downstream, individual targets of PIP_3, such as the AKT group of protein kinases have many protein substrates mediating different dose relationships between PIP_3 and AKT activity and multiple regulated processes. There are good AKT substrates that are stoichiometrically phosphorylated by even low AKT activity and other weaker substrates that require much greater kinase activity to drive their phosphorylation.

The cellular localisation of PTEN

In addition to a cytosolic pool of PTEN, many cell types display a nuclear pool of PTEN which is in some cases dominant, e.g. in follicular thyroid and pancreatic islet cells, [11-13]. Little is known with confidence about why PTEN is found in the nucleus, although the exit of PTEN from the nucleus caused by ionising radiation, and its potential regulation by SUMOylation (see below) shows the potential for dynamic regulation of this cytoplasmic/nuclear balance [14].

It has been known for some time that cytosolic PTEN is able to transiently associate with the plasma membrane through the interaction of basic residues in its phosphatase and C2 domains with acidic lipids, including PtdIns(4,5)P_2 and PtdSer [7]. Accordingly, the N-

terminus of PTEN includes a basic motif that appears to interact with some specificity with PtdIns(4,5)P_2 and may play a more complex role than simply membrane binding [15]. More recently, a novel screen for PTEN membrane binding has allowed the more complete determination of the many amino acids involved in the PTEN-membrane interaction [16]. This novel method has also facilitated the optimisation of tumour suppressor function [16].

In addition to this ability to interact with the plasma membrane to access its substrate, PTEN activity appears to be modulated by protein-protein interactions. Despite a long list of proposed PTEN binding proteins, consistent data for its focal localisation only exists in a limited number of cell types. In polarised epithelial cells including those of the retinal pigment epithelium, neoplastic prostate, the epiblast in the developing chick embryo and fly photoreceptors, an enrichment of PTEN at the apical membrane, perhaps specifically at cell-cell junctions has been observed, in agreement with extensive biochemical data showing that PTEN can interact with components of adherens junctions including PAR3 and the MAGI proteins and has been shown to control epithelial junction stability [7, 17, 18]. PTEN has also been shown to be enriched on the cell membrane at the trailing edge of *Dictyostelium* cells during chemotaxis [19, 20] and relatedly at the back of neutrophils during chemotaxis, although in this latter case, PTEN is less obviously membrane associated [21, 22]. Notably, in these polarised epithelial cells, and dictyostelia and neutrophils during chemotaxis, the PTEN substrate lipid PIP_3 has been shown to be enriched with a converse localisation to PTEN, ie enriched in the basolateral and leading edge membranes respectively [7].

Most recently, using Structured Illumination Microscopy (SIM), a super-resolution technique, a PTEN pool associated with endosomes and distributed along microtubules was identified [23]. The many studies showing that plasma membrane located PIP_3 pools, even localised with the resolution possible using electron microscopy [24], are reduced by PTEN activity strongly suggest that much of the PIP_3 dephosphorylation mediated by PTEN occurs at the plasma membrane. Additionally, the data supporting the, probably transient, localisation of PTEN itself to the plasma membrane is reasonably strong [25-27]. However, the consequences of PTEN association with PI3P rich vesicles associated with microtubules may be very significant, both in terms of where PTEN acts on its substrate(s) and from a regulatory point of view.

Further complexity was added to the question of where PTEN is found with the discovery that in addition to the 403 amino acid PTEN protein, which appears to be ubiquitously expressed in healthy cells, many cells also produce a longer 576 amino acid protein with the inclusion a 173 amino acid N-terminal extension [28]. This longer form, called PTEN-Long or PTEN-L appears to be secreted and enter other cells to influence PI3K signalling intercellularly, which implies new potential mechanisms of intercellular tumour suppression and influences ideas about the initial consequences of mutations in nascent tumour cells [28]. PTEN-L has also been independently discovered and proposed to play a role in regulating mitochondrial function [29]. Further studies should reveal how many of the functions previously attributed to PTEN are actually selectively mediated by the longer form, but it is notable in this regard that there is no evident longer PTEN form outside vertebrate lineages and even amongst vertebrates, the sequence of the N-terminal extension is far less conserved that the core 403amino acid PTEN protein.

Post-translational regulation of PTEN: modification of PTEN with Ubiquitin and SUMO.

Diverse regulation of PTEN function is applied by post-translational modifications, including phosphorylation, oxidation, nitrosylation and acetylation distributed throughout the protein and has been reviewed elsewhere [7, 30, 31]. However, most recently, several new insights have emerged that are starting to build a better picture of the significance and complexity of PTEN regulation which occurs through the posttranslational ligation of the protein modifiers, Ubiquitin and SUMO.

Ubiquitin is a small (76 amino acids, 8.5kD) protein which is used to regulate many (and probably nearly all) other cellular proteins through reversible post-translational covalent ligation to lysine residues on its targets [32]. As with many proteins, initial studies of PTEN ubiquitination provided strong evidence that the regulated polyubiquitination of PTEN leads to its degradation by the proteasome complex [33-35]. However, later work implies much greater regulatory complexity, with evidence that monoubiquitination can influence the accumulation of PTEN in the nucleus, evidence that multiple sites of ubiquitination exist on PTEN [13], for many different enzymes adding [36-42] and removing [43-45] ubiquitin units and that ubiquitination can directly regulate catalytic activity as well as localisation and stability [46]. One feature that has emerged from several of these studies is evidence that PTEN activity can be reduced in some tumours indirectly through effects on its ubiquitination mediated by mutation or altered expression of ubiquitin ligases and proteases [38, 42, 44, 45]. However, this is not always the case, as, for example, although SPOP has been proposed to ubiquitinate PTEN [39], SPOP mutations in prostate cancers appear to cause loss of its function but do not correlate with PTEN expression [47]. Another feature of PTEN regulation by ubiquitination is that it can be affected by signalling downstream of PI3K/PTEN in what appear to be feedback mechanisms. Negative feedback stabilisation of PTEN by downstream signalling has been described in neurons and HeLa cells, perhaps linked to the E3 ubiquitin ligase NEDD4 [48, 49]. However, experiments mostly in cervical carcinoma cells have also showed that long term exposure to growth factors induced positive feedback destabilisation of PTEN and that this was mediated downstream of AKT by the E3 Ub ligase MKRN1 [38].

Addition of ubiquitin can be in the form of single ubiquitin monomers at single or multiple sites on a target or in the form of polyubiquitin chains, the latter potentially linked through any of the 7 lysine residues on ubiquitin or its N-terminus and there are many classes of ubiquitin-binding protein domains which mediate regulated protein-protein interactions through ubiquitin dependent binding [32]. In almost all cases, the details of the regulation of PTEN by ubiquitination in these contexts remain to be determined. It is also emerging that PTEN can be regulated by the addition of other ubiquitin-like molecules, in particular SUMO family members, which are functionally distinct and somewhat larger, around 100 amino acids/12kD. Several research groups have reported PTEN SUMOylation, showing this on lysine residues at positions 254 and 266 in the C2 domain of PTEN [14, 50, 51]. This work indicates that 266 SUMOylation may influence the nuclear-cytoplasmic shuttling of PTEN [14, 50] and that nuclear SUMOylated PTEN plays a role in the cellular DNA damage response [14] potentially starting to explain previous observations that loss of PTEN leads to genome instability [52].

Future perspectives

Our understanding of PTEN regulation is still very limited. Although several post-translational modifications of PTEN have bene identified, in almost all cases, the role that these modifications play in cellular regulatory systems is unclear. One reason for this is our limited picture of PI3K signalling. Much of the current data demonstrating that any observed regulatory effect on PTEN has functional consequences has in the past relied on the analysis of AKT phosphorylation in cell lysates, due to the ease and robust nature of this approach. However, it seems likely that many modifications of PTEN that only affect a localised fraction of the total cellular PTEN pool may not influence total cellular AKT phosphorylation, for example a change to PTEN which affects the localised PIP_3 pools observed in cells undergoing chemotaxis or during the establishment of stable cell polarity. To provide real insight into these mechanisms, in many cases, better insight into PI3K signalling (or if significant, other PI3K independent systems affected by PTEN) and further functional assays for PTEN are required. Given the recognised importance of PTEN function in maintaining human health, and the availability of drugs targeting the PI3K/PTEN pathway,

such deeper understanding should provide potential opportunities for improving the treatment and management of disease.

As discussed, other specific gaps in our understanding of PTEN include details of its functions and/or regulation occurring in the nucleus, the existence and identity of authentic protein substrates (perhaps other than itself) and even whether it is possible to efficiently inhibit or activate PTEN therapeutically.

This slowly developing deeper insight into PI3K/PTEN driven oncogenesis specifically relates to intensive ongoing efforts to provide predictive biomarkers for the identification of patients who will respond to inhibitors of individual nodes in the PI3K/PTEN/mTOR signalling network. Many early clinical trials with these targeted therapies had very poor response rates, but often included some patients who did responded well. An example of this patient selection paradigm is the apparent correlation between loss of function of the TSC complex and sensitivity to the rapamycin group of mTOR inhibitors in bladder and liver cancers [53, 54]. Whether there is more success in this area will greatly influence the long term usefulness of these agents in the clinic.

Figure Legend

Figure 1. The PTEN protein.

A. The 403 amino acid (aa) PTEN protein is represented, divided into its N-terminal protein tyrosine phosphatase (Phos) and more C-terminal C2 domains. Additionally the positions are shown of the N-terminal PtdIns(4,5)P_2 binding motif marked in light green and the extreme C-terminal PDZ-binding sequence in darker green. A small number of the identified sites of post-translational modification by phosphorylation (P), Ubiquitin, (Ub) and SUMO are indicated. **B**. and **C**. show the sequence conservation within 6 vertebrate species PTEN orthologs, showing (**B**) the regions of the protein with greatest conservation and (**C**) the percentage amino acid identity in each pairwise comparison.

Figure 2. The PTEN protein.

The PTEN protein is represented as in Figure 1, highlighting components that influence membrane locaiisation(upper panel) and nuclear localisation (lower panel).

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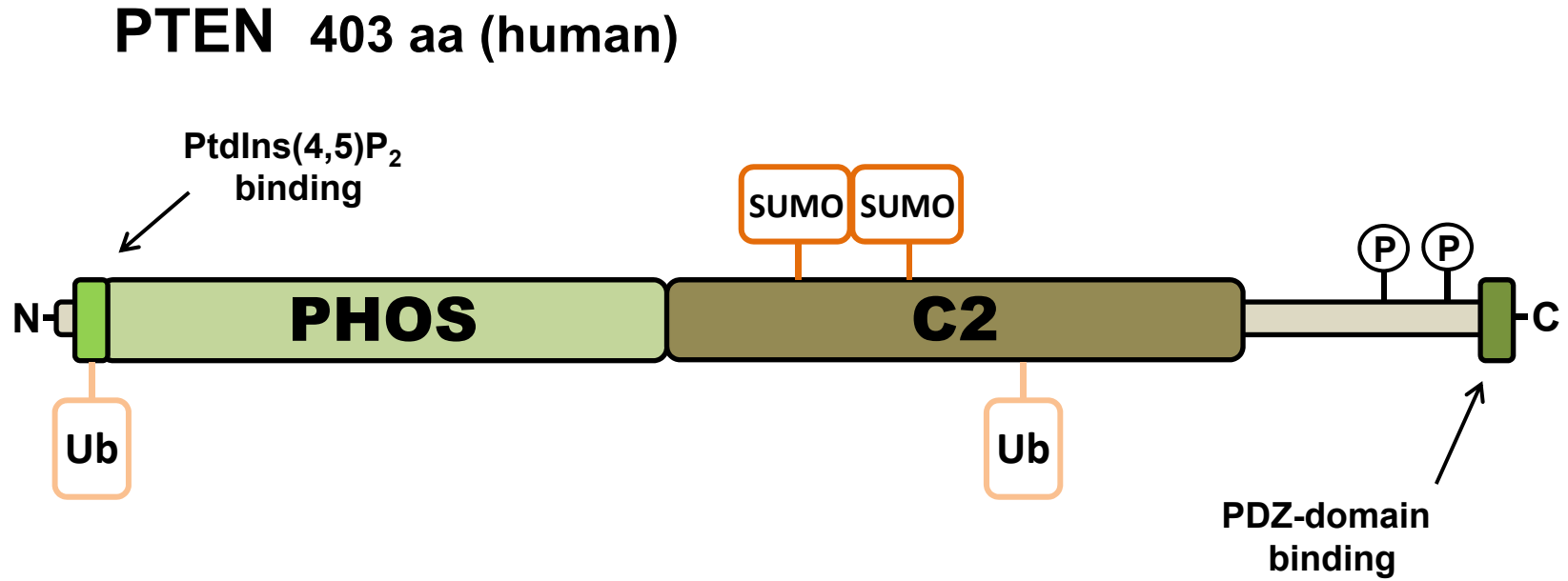
FIG. 1

A

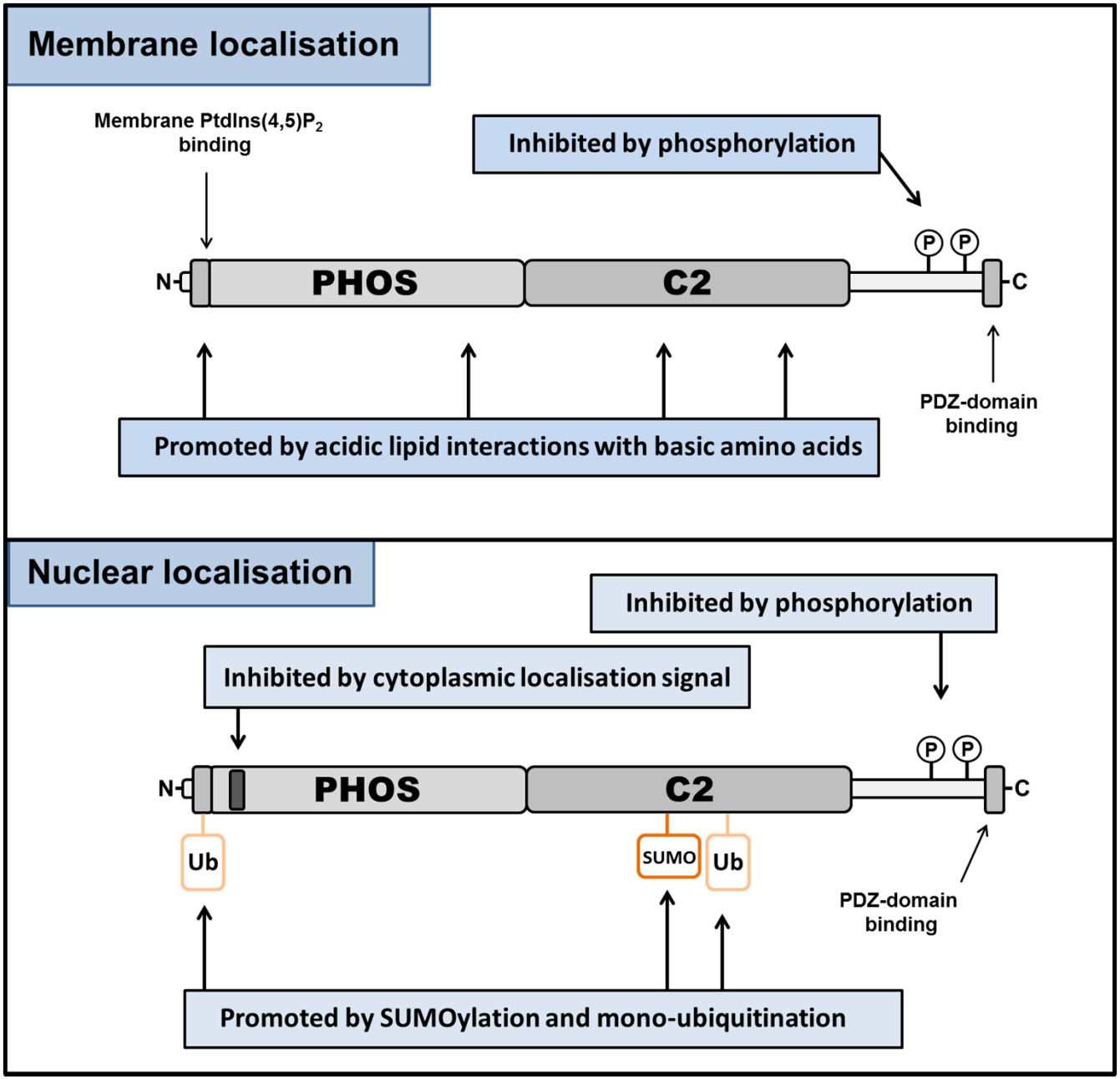


B

C

	Human	Mouse	Chicken	Frog	Zebrafish	Pufferfish
Human	100	99.75	95.02	88.78	87.19	87.84
Mouse		100	94.78	88.53	86.93	87.59
Chicken			100	90.02	87.69	87.34
Frog				100	85.89	83.58
Zebrafish					100	88.97
Pufferfish						100

FIG. 2


Membrane localisation
Membrane PtdIns(4,5)P2 binding
Inhibited by phosphorylation
N
PHOS
C2
C
P
P
PDZ-domain binding
Promoted by acidic lipid interactions with basic amino acids
Nuclear localisation
Inhibited by phosphorylation
Inhibited by cytoplasmic localisation signal
N
PHOS
C2
C
P
P
Ub
SUMO
Ub
PDZ-domain binding
Promoted by SUMOylation and mono-ubiquitination