Metabolic switching of PI3K-dependent lipid signals

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Abstract

The lipid phosphatase, PTEN (phosphatase and tensin homologue deleted on chromosome 10), is the product of a major tumour suppressor gene that antagonizes PI3K (phosphoinositide 3-kinase) signalling by dephosphorylating the 3-position of the inositol ring of PtdIns(3,4,5)P3. PtdIns(3,4,5)P3 is also metabolized by removal of the 5-phosphate catalysed by a distinct family of enzymes exemplified by SHIP1 [SH2 (Src homology 2)-containing inositol phosphatase-1] and SHIP2. Mouse knockout studies, however, suggest that PTEN and SHIP2 have profoundly different biological functions. One important reason for this is likely to be that SHIP2 exists in a relatively inactive state until cells are exposed to growth factors or other stimuli. Hence, regulation of SHIP2 is geared towards stimulus dependent antagonism of PI3K signalling. PTEN, on the other hand, appears to be active in unstimulated cells and functions to maintain basal PtdIns(3,4,5)P3 levels below the critical signalling threshold. We suggest that concomitant inhibition of cysteine-dependent phosphatases, such as PTEN, with activation of SHIP2 functions as a metabolic switch to regulate independently the relative levels of PtdIns(3,4,5)P3 and PtdIns(3,4)P2.

Introduction

Myo-inositol is an extremely versatile component of multiple cytosolic and membrane-bound signalling molecules. For example, more than 30 distinct inositol phosphate species and eight inositol glycerophospholipids have been identified in cell extracts [1]. These molecules differ with respect to the number and distribution of monoester phosphate groups around the inositol ring and they are interconverted by kinases and phosphatases with varying degrees of substrate and positional specificity. Since many, if not all, of these molecules have potent signalling roles, cells have evolved mechanisms to control independently the concentration and spatial distribution of each signal despite the metabolic relationships between related species.

An example of this problem that we are studying is the synthesis and subsequent metabolism of PtdIns(3,4,5)P3. PtdIns(3,4,5)P3 is a potent second messenger that mediates many of the pleiotropic responses to growth factors and insulin [2]. It is synthesized by Class I PI3Ks (phosphoinositide 3-kinases) acting on PtdIns(4,5)P2, which is itself an important signalling molecule with roles in membrane trafficking, control of ion channels and regulation of the actin cytoskeleton [3]. PtdIns(3,4,5)P3 is in turn metabolized by two classes of phosphatases. The tumour suppressor, PTEN (phosphatase and tensin homologue deleted on chromosome 10), selectively removes the 3-phosphate to regenerate PtdIns(4,5)P2 [4,5], while 5-phosphatases such as SHIP1 [SH2 (Src homology 2)-containing inositol phosphatase-1] and SHIP2 selectively remove the 5-phosphate to generate a further lipid signal, namely PtdIns(3,4)P2 [6]. Studies using knockout mice have established that PTEN and SHIP2, both of which have broad tissue distributions, nevertheless have distinct biological functions. Homozygous PTEN knockout is early embryonic lethal in mice and, although heterozygotes survive, they are prone to the development of multiple sporadic tumours [7,8]. Initially it was thought that SHIP2 knockout caused early postnatal lethality due to hypoglycaemia caused by increased insulin-sensitivity [9]. This conclusion is, however, compromised by the finding that the mice inadvertently also lack a functional Pbox2a gene. A second SHIP2 knockout that does not suffer from this problem has an even more interesting phenotype being highly resistant to the effects of a high-fat diet [6]. Hence mechanisms that regulate the distribution of PtdIns(3,4,5)P3 metabolism between these distinct routes are likely to have profound physiological and pathological implications.

PtdIns(3,4)P2 is generated mainly via 5-phosphatase(s) acting on PtdIns(3,4,5)P3 as described above. Hence its production is generally blocked by PI3K inhibitors. This lipid binds to several PH domain (pleckstrin homology domain)-containing proteins that also bind PtdIns(3,4,5)P3, but addition binds at least two known proteins specifically [TAPP1 (tandem PH-domain-containing protein 1) and lamellipodin] [10,11]. Moreover, a major route for its own metabolism is via inositol polyphosphate 4-phosphatases that generate PtdIns3P, itself an important signal molecule [12–14]. An essential prerequisite for the independent control

Key words: lipid signal, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), phosphatidylinositol bisphosphate metabolism, phosphatidylinositol trisphosphate metabolism, reactive oxygen species, Src homology 2-containing inositol phosphatase-2 (SHIP2).

Abbreviations used: CDPase, cysteine-dependent phosphatase; GST, glutathione S-transferase; PKB, protein kinase B; PTEN, phosphatase and tensin homologue deleted on chromosome 10; ROS, reactive oxygen species; Src, Src homology 2; SH2, SH2-containing inositol phosphatase-2; TAPP1, tandem PH-domain-containing protein 1.

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Activation of growth factor receptors stimulates the conversion of PtdIns(4,5)P$_2$ into PtdIns(3,4,5)P$_3$ by Class I PI3Ks. The latter can either be inactivated by PTEN, which regenerates PtdIns(4,5)P$_2$, or converted into another signalling lipid, PtdIns(3,4)P$_2$. The relative levels of PtdIns(3,4,5)P$_3$ and PtdIns(3,4)P$_2$ are controlled by constitutively active PTEN that can be acutely inhibited and constrained SHIP, or alternative 5-phosphatases, that are activated by growth factors. Independent metabolic and spatial control of these lipid species is necessary because each serves a distinct set of purposes as illustrated (reviewed in [3]).

PTEN is constitutively active and subject to acute and chronic negative regulation

The gene encoding PTEN is one of the most commonly mutated genes in human cancer, especially in glioblastoma and endometrial carcinoma. PTEN functions to antagonize Class I PI3K-dependent signalling and has a key role in maintaining unstimulated levels of PtdIns(3,4,5)P$_3$ below its signalling threshold [5]. Hence loss of PTEN allows basal PtdIns(3,4,5)P$_3$ to rise and signal constitutively in tumours. We addressed the question of whether loss of PTEN activity is purely pathological or if inhibition of PTEN activity occurs as a normal physiological control mechanism.

The first reported example of acute negative regulation of PTEN activity was in cells responding to an oxidative stress [15]. PTEN is a member of the CDPase (cysteine-dependent phosphatase) superfamily, the members of which are characterized by a reactive cysteine residue. In PTEN this cysteine residue undergoes reversible oxidation in the presence of ROS (reactive oxygen species), forming a disulfide bond with a neighbouring cysteine residue and rendering the enzyme inactive [16]. We were able to show that cellular PTEN was reversibly oxidized in response to H$_2$O$_2$ and that this was required for oxidative stress to stimulate the PI3K pathway as indicated by the phosphorylation and activation of PKB (protein kinase B). PTEN was also partially oxidized in a macrophage cell line following stimulation of Toll-like receptors with lipopolysaccharide and this appeared to account for anti-oxidant reversible activation of PKB in these cells.
Other groups subsequently demonstrated reversible oxidation of PTEN in response to generation of endogenous ROS after stimulation of cells with PDGF (platelet-derived growth factor) or insulin [17,18] respectively. In this context, inhibition of PTEN may represent a powerful feed forward mechanism in which ROS generated by activated growth factor receptors augments PI3K signalling by preventing metabolism of PtdIns(3,4,5)\(_\text{P}_3\). If such a mechanism operates at a local level it might lead to the generation of the steep gradients of PtdIns(3,4,5)\(_\text{P}_3\) that are needed to drive highly polarized cell responses such as directed cell movement.

More recently, in collaboration with Mike Ashford and his colleagues, we demonstrated a second mode of acute inhibitory regulation of PTEN by the leptin-stimulated phosphorylation of PTEN at its cluster of C-terminal serine and threonine residues [19]. These sites can be phosphorylated by protein kinase CK2 (formerly known as casein kinase 2) \textit{in vitro} and their phosphorylation in cells is decreased by protein kinase CK2 inhibitors. Based on the use of phosphosite-specific antibodies it has generally been assumed that cellular PTEN exists in a predominantly phosphorylated and hence inactivated state. However, this proved not to be the case in hypothalamic cell lines in which PTEN phosphorylation could be increased 10–20-fold after stimulation with leptin for 15 min. By this means, leptin inhibited PTEN activity and this enhanced PI3K-dependent signalling. Phosphorylation induced by leptin also inhibited the protein phosphatase activity of PTEN and this proved necessary for leptin to stimulate F-actin depolymerization. The latter explains why leptin and insulin signalling differ despite both being able to activate the PI3K pathway.

Although, as noted above, PTEN mutations are common in cancer, loss of PTEN expression appears to occur even more commonly in the absence of evidence for bi-allelic mutation [5]. In addition PTEN is one of the few tumour suppressors where reduced expression (such as in mice heterozygous for the wild-type PTEN gene or in humans with familial PTEN mutations such as sufferers from Cowden’s disease) appears to enhance tumour development. Hence it will be important to understand mechanisms that operate to regulate the expression of PTEN protein. In this regard, the stability of PTEN seems to be regulated through mechanisms involving phosphorylation of the C-terminal cluster described above. In this case phosphorylated PTEN appears to be relatively metabolically stable compared with the dephosphorylated protein [20]. The C-terminal tail also appears to bind a protein, termed PICT1 (protein interacting with C-terminal tail 1), which stabilizes PTEN so that RNA interference-induced loss of PICT1 simulates PTEN turnover and reduces the latter’s steady-state expression level [21].

**SHIP proteins have suppressed activities that can be overcome by cellular stimulation**

We have consistently observed in a range of cell types that basal levels of PtdIns(3,4)\(_\text{P}_2\) are extremely low and that the relative levels of PtdIns(3,4,5)\(_\text{P}_3\) and PtdIns(3,4)\(_\text{P}_2\) vary substantially over time, in different cells and in response to different stimuli. This suggests that cells have evolved the ability to regulate these two signals independently despite their metabolic relationship. We are trying to determine the extent to which regulation of SHIP proteins may account for these differences. Other 5-phosphatases that may play a part in this process but that are not discussed further here include SKIP (skeletal-muscle- and kidney-enriched inositol phosphatase) and PIPP (proline-rich inositol polyphosphatase) [22]. Since SHIP itself is largely restricted to haemopoietic cells, this discussion focuses mainly on what is known about the more widely distributed SHIP2 protein.

SHIP2 is a large, 150 kDa protein with an inositol polyphosphate phosphatase domain, PRD (proline-rich domain), SH2 domain and sterile \(a\) male and NPXY motifs [23]. The latter appears to be the target for tyrosine phosphorylation stimulated by several growth factors [24], although the kinases responsible remain ill-defined as do the binding partners of tyrosine-phosphorylated SHIP2. Many studies also report translocation of SHIP2 in response to stimulation, for example to the submembraneous cytoskeleton in activated platelets [25], and this often correlates with tyrosine phosphorylation and/or requires a functional SH2 domain. Strikingly, there are no reports of any change in the specific activity of SHIP2 in response to cell stimulation, which implies that proximity to substrate-rich membranes is a key determinant of activity \textit{in vivo}. In our preliminary studies, however, in which we used vanadate analogues and/or growth factors to stimulate tyrosine phosphorylation of SHIP2, we found large increases in the specific activity of the enzyme which accompanied translocation. These results form the basis of our proposal that SHIP2 is held in a relatively inactive state until stimulated to generate a large increase in the rate of turnover of PtdIns(3,4,5)\(_\text{P}_3\). If this effect coincides with inhibition of PTEN (see above) it follows that there will be a large change in the distribution of PtdIns(3,4,5)\(_\text{P}_3\) metabolism from PtdIns(4,5)\(_\text{P}_2\) to PtdIns(3,4)\(_\text{P}_2\).

**Inositol polyphosphate 4-phosphatases, like PTEN, are members of the CDPase superfamily**

Based on their reported substrate specificities the inositol polyphosphate 4-phosphatases (Types I and II) are candidate PtdIns(3,4)\(_\text{P}_2\) phosphatases that convert the latter into PtdIns3P. Indeed embryonic fibroblasts from Weeble mice that lack the Type I 4-phosphatase [26] contain elevated levels of PtdIns(3,4)\(_\text{P}_2\) and concomitantly decreased PtdIns3P [12,27]. Current evidence suggests that the 4-phosphatase(s) convert PtdIns(3,4)\(_\text{P}_2\) into PtdIns3P at both the plasma membrane and in endosomes. In the latter case the sequential conversion of PtdIns(3,4,5)\(_\text{P}_3\) into PtdIns3P is apparently co-ordinated by the GTPase, Rab5, which binds both the 4-phosphatase and a 5-phosphatase [27]. The ability of the 4-phosphatase to generate PtdIns3P in endosomes appears to be functionally important as Weeble mouse embryonic

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fibroblasts had dilated early endosomes [12]. Like PTEN, the inositol polyphosphate 4-phosphatases possess a CDPase catalytic domain and a C2 domain that targets the enzyme to substrate-containing membranes. Because they have a reactive cysteine residue within their active sites, these enzymes are likely to be susceptible to inhibition by ROS. This may explain our previous observation that extraordinarily high levels of PtdIns(3,4,5)P3 accumulate in cells treated with H2O2 through the concerted effects of inhibiting both PTEN and the 4-phosphatases.

**Spatial regulation of inositol lipid signals**

While the above discussion emphasizes the regulation of phosphatase activities as a mechanism that determines the relative amounts of distinct lipid signals, the spatial organization of these signalling events is clearly critical to biological function. Indeed, phospholipids are ideally suited to functions requiring spatial restriction being for the most part confined to the membranes in which they are synthesized. In order to study the spatial distribution of inositol phospholipids at an ultrastructural level of resolution, we made use of PH domain probes with rigorously defined specificities for particular phosphoinositide species. We developed an on-section labelling approach using GST (glutathione S-transferase)-tagged PH domains that could be detected and quantified in the electron microscope using Protein A–gold-labelled anti-GST. Using the PH domain of PLCδ1 (phospholipase Cδ) as a specific probe for PtdIns(4,5)P2, we found that this lipid is located primarily in the plasma membrane, with smaller pools in the Golgi, endosomes and endoplasmic reticulum and with a substantial non-membrane-associated pool in the nuclear matrix [28]. Substantial experiments using the PH domain of GRP1 (general receptor for phosphoinositides-1) to detect PtdIns(3,4,5)P3 suggest this lipid is also predominantly located in the plasma membrane and, although it can be detected in endosomes, its concentration in that compartment is comparably very low. This suggests that internalized growth factor receptors no longer signal efficiently to Class I PI3Ks and/or that PtdIns(3,4,5)P3 is rapidly metabolized in the endosomal compartment. Since we found relatively high levels of PtdIns(3,4)P2 in endosomes (using the PH domain of TAPP1 as a specific probe), it seems likely that PtdIns(3,4,5)P3 is subject to metabolism by 5-phosphatases in this compartment and, by this means, the distinctive phosphoinositide content of endosomes can be maintained [29]. In fact PtdIns3P is the characteristic phosphoinositide of endosomes that is generated partly by metabolism of PtdIns(3,4)P2 by 4-phosphatases, as described above and also by Class III PI3K that phosphorylates PtdIns directly to PtdIns3P [3]. In this way, it seems, signalling lipids generated in the plasma membrane are rapidly metabolized upon internalization to generate an endosomal compartment with its distinctive phosphoinositide composition. This is important because PtdIns3P, either directly or via the synthesis of PtdIns(3,5)P2, binds and regulates many proteins that have key roles in endosomal function.

**Conclusions**

PtdIns(3,4,5)P3 and PtdIns(3,4)P2 are two important lipid signals that have overlapping, but distinctive roles. Independent control of their signalling functions is achieved by having two routes of metabolism of PtdIns(3,4,5)P3. In-activation is achieved by PTEN, which is a constitutively active enzyme that can be switched off acutely or down-regulated, thus amplifying PI3K pathway activity. SHIPs and possibly other enzymes convert PtdIns(3,4,5)P3 into PtdIns(3,4)P2, but need to be specifically switched on for this to occur. PtdIns(3,4)P2 is itself metabolized to PtdIns3P and the regulation of this step is just beginning to be explored. This metabolic switch from PTEN to SHIP-catalysed metabolism may be augmented by spatial segregation of lipid signals with PtdIns(3,4,5)P3 being mainly in the plasma membrane, PtdIns3P being characteristic of endosomes and PtdIns(3,4)P2 being present in both of these compartments.

**References**


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