

# Nuclear PTEN: a tale of many tails

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The PTEN tumour suppressor is one of the more commonly inactivated proteins in human cancer and a key regulator of the PI3K/Akt survival pathway. Its direct involvement in human disease, as well as its important role in many cellular processes, including cell development and differentiation, cell growth, apoptosis, cell motility, cell size, stem cell survival, and longevity, has made PTEN the focus of attention of many researchers and clinicians. The major biological function of PTEN resides in its phosphatase activity towards the lipid second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3), antagonizing the activity of the PI3K oncoproteins, and the association of PTEN to lipids at the plasma membrane is required to exert this function.<sup>1–3</sup> In addition, the presence of PTEN in the nucleus of many different cell types, and the finding of unexpected PTEN functions in the nucleus has revealed that the regulation of its nuclear/cytoplasmic distribution may also be a key mechanism to drive PTEN functions.<sup>4–6</sup> Here, we discuss the current models to explain the regulation of PTEN nuclear accumulation, and the molecular linkage between the targeting of PTEN to the nucleus and to lipid membranes. The distinct pathways that mediate nuclear PTEN functions in the control of cell growth and apoptosis are also discussed.

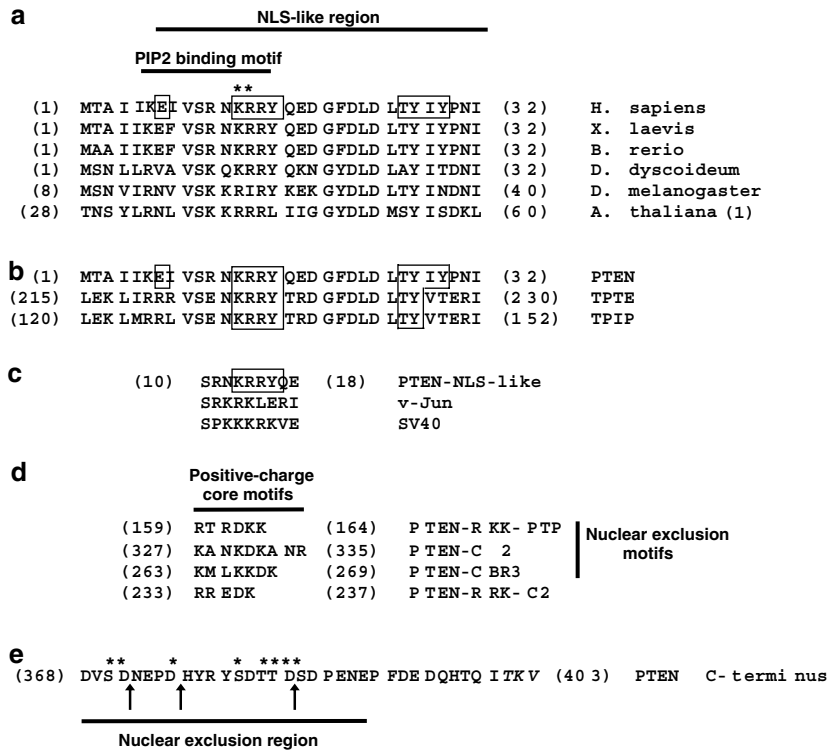
## Molecular Mechanisms of PTEN Nuclear Accumulation

The tumour suppressor PTEN protein is present in the nucleus of different cell types, including cell lines and tissue cells.<sup>7–10</sup> However, PTEN amino-acid sequence lacks obvious canonical nuclear localization signal sequences (NLS) or nuclear export sequences (NES) that could account for the targeting of the protein in or out of the nucleus, making elusive the molecular basis of PTEN nuclear/cytoplasmic distribution. Recent findings, however, suggest that PTEN entry and accumulation in the nucleus may be controlled by a variety of mechanisms. Chung *et al.*<sup>11</sup> have reported that, in the MCF-7 breast carcinoma cell line, PTEN may be transported into the nucleus using several putative NLS-like sequences located in the PTP and the C2 domains of the protein. Mutating such NLS-like sequences individually did not affect PTEN nuclear/cytoplasmic distribution. However, combined mutations of the NLS-like sequences caused defective PTEN nuclear accumulation. Based on the differential interaction of the major vault protein (MVP) with wild-type PTEN and with the NLS-like mutants, as well as on the nuclear localization pattern of MVP, the authors propose a model of PTEN nuclear import mediated by MVP. Interestingly, PTEN was found in this study to interact with importin  $\alpha$  and  $\beta$  proteins, although such

interaction was also observed in the NLS-like mutants that displayed defective nuclear accumulation. A second model of PTEN nuclear entry has been proposed by Liu *et al.*<sup>12</sup> Using PTEN fusion proteins of variable size, the authors of this study noticed that large PTEN fusion proteins (> 100 kDa) did not show nuclear localization, whereas PTEN alone (47 kDa) or a GFP-PTEN fusion protein (74 kDa) were present in both the cytoplasm and the nucleus. Also, nuclear localization of GFP-PTEN was not altered in tsBN2 cells, which show defective Ran-dependent nuclear transport at a nonpermissive temperature. These observations, together with the slow recovering of fluorescence observed on nuclear photobleaching experiments using GFP-PTEN, led Liu *et al.*<sup>12</sup> to propose a model in which PTEN may enter the nucleus by Ran-independent passive diffusion through nuclear pores. Remarkably, mutations on a putative NLS-like sequence at the PTEN N-terminus (residues Lys<sup>13</sup> and Arg;<sup>14</sup> see also below), diminished the nuclear localization of GFP-PTEN in this study. However, the N-terminal region of PTEN, when fused to chicken PK, did not facilitate the nuclear accumulation of this protein. Also of interest, Liu *et al.*<sup>12</sup> found a fast mobility of GFP-PTEN into the nucleus, whereas in the cytoplasm the movement of GFP-PTEN was much more restricted, suggesting the tethering of PTEN to cytoplasmic structures which could prevent nuclear translocation. A third model to explain the nuclear accumulation of PTEN has been proposed more recently by us. This model relies on the coexistence of both nuclear-entry-positive (NLS-like) and nuclear-entry-negative (nuclear exclusion motifs) intrinsic regulatory regions in the PTEN molecule (Figure 1).<sup>13</sup> Mutations at the nuclear exclusion motifs, which were identified at the PTEN C-terminal tail (residues 368–390) as well as at the PTP (RKK-PTP motif, residues 159–164) and C2 (C $\alpha$ 2 motif, residues 327–335; and CBR3 motif, residues 263–269) domains, triggered a strong PTEN nuclear accumulation. The integrity of the PTEN N-terminal tail was found to be required for such nuclear accumulation, and a functional NLS-like domain (residues 1–32) was defined at the PTEN N-terminus. Mutating key residues at the PTEN NLS-like region, as well as tagging of the PTEN N-terminus, blocked the nuclear accumulation of PTEN upon mutation of the nuclear exclusion motifs. Furthermore, the PTEN NLS-like domain was sufficient to trigger the nuclear accumulation of an otherwise cytoplasmic GST-GFP fusion protein. Coexpression of nuclear PTEN with nuclear importin  $\alpha$ 1 and importin  $\alpha$ 5 proteins lacking their importin beta-binding regions, or with the GTPase-deficient, dominant-negative RanQ69L, resulted in the relocalization of PTEN to the

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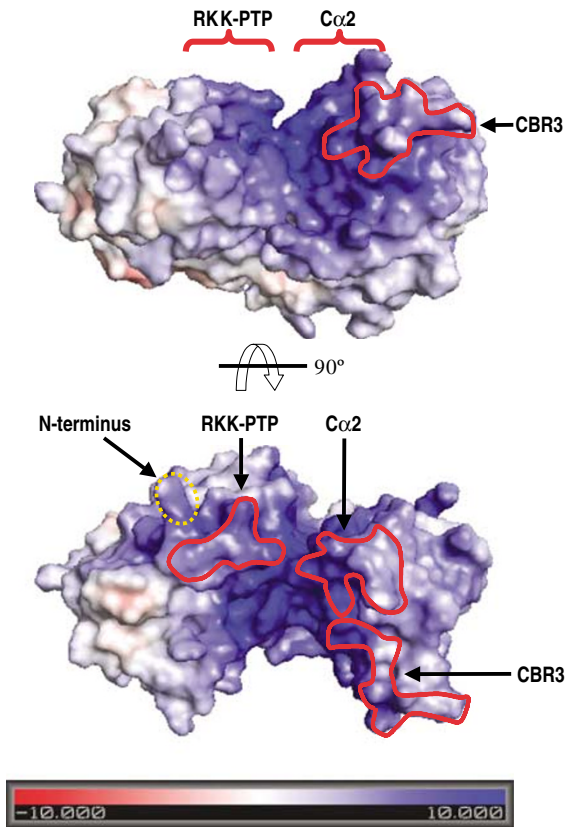
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**Figure 1** Motifs in the PTEN amino-acid sequence involved in PTEN nuclear localization, binding to membranes, and function. **(a)** Sequence alignment of the N-terminal regions of PTEN from different species. The PIP2 binding motif and the NLS-like region are indicated. Mutating the residues into the boxes inhibit PTEN nuclear accumulation. Mutating the residues marked with an asterisk inhibit PIP3 dephosphorylation by PTEN in cells.<sup>17,19</sup> **(b)** Sequence alignment of the N-terminal region of human PTEN with the equivalent regions of human TPTE and TPIP phosphatases. The squares indicate the residues important for PTEN nuclear entry and their conservation in TPTE and TPIP. **(c)** Sequence alignment of the N-terminal PTEN NLS-like sequence with the NLS sequences from v-Jun and the SV40 large-T antigen. The box indicates the residues important for PTEN nuclear entry. **(d)** Amino-acid sequences of the PTEN positively charged motifs that are important for regulation of PTEN nuclear entry and PTEN catalytic activity in cells. Mutating residues in motifs RKK-PTP, C $\alpha$ 2 and CBR3 favoured PTEN nuclear accumulation.<sup>13</sup> Combined mutations of CBR3 and RRK-C2 or CBR3 and RKK-PTP decreased PTEN nuclear localization.<sup>11</sup> Mutating residues in motifs CBR3 and C $\alpha$ 2 disfavours PTEN phosphatase activity towards PIP3 in cells.<sup>21</sup> **(e)** Amino-acid sequence of the PTEN C-terminus. The PDZ-domain binding motif is indicated in italics. The fragment 368–390 is important for PTEN nuclear entry. Residues phosphorylated by CK2 and residues targeted by caspase-3 proteolytic activity are indicated with asterisks. Mutating these residues triggers PTEN nuclear accumulation. The arrows indicate the caspase-3 cleavage sites. Truncation of PTEN at these sites triggers PTEN nuclear accumulation. In all panels, the numbers indicate the amino acid numbering of the proteins

cytoplasm, suggesting the involvement of importin-related proteins in PTEN nuclear entry. The analysis of the amino-acid sequences of PTEN from several species reveals that the PTEN NLS-like domain is conserved between distant organisms, specially in its basic residue-rich region (Figure 1a). Moreover, the mammalian PTEN-related proteins TPTE and TPIP also show high conservation in this region (Figure 1b). The fact that TPTE and TPIP differ from PTEN in the presence of a large N-terminal extension responsible for the localization of these proteins at non-nuclear intracellular compartments,<sup>14,15</sup> suggests that the NLS-like region is essential for the biological activity of these PTEN-related phosphatases. In support of this are the findings that the N-terminal region of PTEN possesses a functional phosphatidylinositol-4,5-bisphosphate (PIP2) binding motif, which overlaps with key residues defined in the PTEN NLS-like domain (see Figure 1a).<sup>16</sup> Binding to PIP2 serves both to recruit PTEN to the plasma membrane and to activate PTEN catalysis, and mutations in the PIP2-binding motif abrogate the dephosphorylation of PIP3 by PTEN in lipid vesicles or in intact cells.<sup>17–19</sup> Remarkably, the positive-charge residues at the PTEN

N-terminal tail that are essential for PTEN nuclear entry resemble a classical NLS sequence, as compared with those from v-Jun and the SV40 large T-antigen (Figure 1c). As shown, the nuclear exclusion motifs at the PTEN core (RKK-PTP, CBR3 and C $\alpha$ 2 motifs) are also enriched in Lys and Arg positive-charge residues (Figure 1d). In contrast, the nuclear exclusion region of the C-terminal tail (residues 368–390) (Gil *et al.*<sup>13</sup> and our unpublished observations) is enriched in negative-charge residues, including Asp residues (some of which are targeted by caspase-3) and Ser and Thr-phosphorylated residues (Figure 1e).<sup>20</sup> Figure 2 shows the location of the nuclear exclusion motifs at the PTEN core surface (RKK-PTP, CBR3 and C $\alpha$ 2) and of the PTEN N-terminus (Arg<sup>14</sup> residue) in its tertiary structure. It has to be mentioned that the PTEN crystal lacks a portion of the PTEN N-terminal tail (residues 1–13), as well as the entire PTEN C-terminal tail (residues 350–403), making impossible the local assignment of these regions.<sup>21</sup> The unstructured properties of the PTEN N- and C-terminal tails favour the hypothesis that these regions may be involved in intra- or inter-molecular protein interactions. As shown, the RKK-PTP



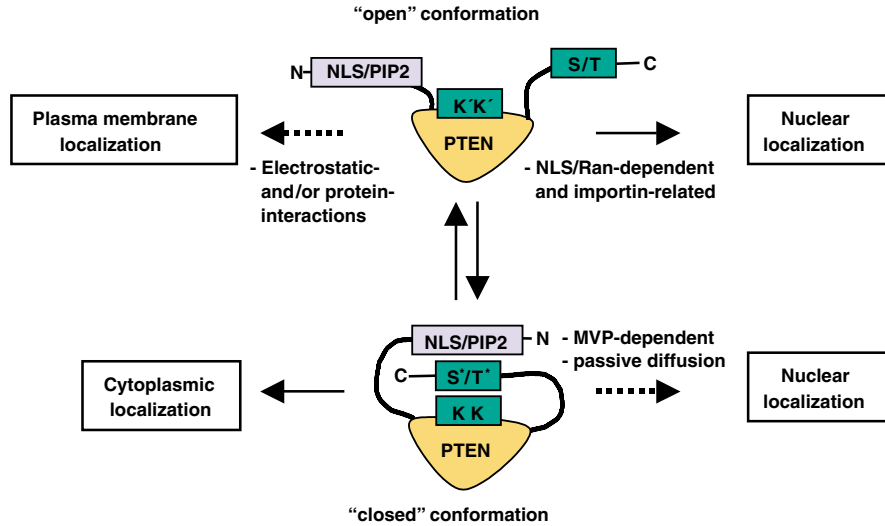
**Figure 2** Surface distribution and electrostatic potential of nuclear exclusion motifs at the PTEN core. The bottom view shows a frontal vision of the three nuclear exclusion motifs (encircled in red). Note the overall positive charge (blue colour) of the surface of the molecule. The top view shows a lateral vision of the RKK-PTP and  $C\alpha 2$  motifs, and a frontal vision of the CBR3 motif. The dashed yellow line in the bottom view indicates the location of the PTEN N-terminus from the crystallized PTEN protein (Arg<sup>14</sup> residue). Residues 1–13 are absent from the PTEN resolved crystalline structure.<sup>21</sup> The figure is drawn in PyMOL

and the  $C\alpha 2$  motifs form two positive-charge patches on the PTEN surface, separated by the interface between the PTP and the C2 domains (Figure 2). These two patches are in the vicinity of the PTEN N-terminus, favouring putative molecular interactions between them and the PTEN NLS-like domain (see below). The CBR3 motif also forms a positive-charge patch next to the  $C\alpha 2$  motif, whereas the RKK–C2 surface exposed positive-charge motif (Figure 1d), which does not behave as a nuclear exclusion motif, is further away from the PTEN N-terminal region.<sup>13,21</sup> Intriguingly, Chung *et al.*<sup>11</sup> found that mutation of the RKK–PTP or the RKK–C2 motifs, in combination with mutation of the CBR3 motif, inhibited PTEN nuclear accumulation, suggesting that these motifs together may also function as NLS-like sequences in the MVP-dependent PTEN nuclear entry model. This raises the possibility that some of the PTEN nuclear entry-regulatory motifs defined in the studies by Chung *et al.*<sup>11</sup> and by Gil *et al.*<sup>13</sup> may have a dual function depending on the mechanisms upon which PTEN enters the nucleus. Also of major interest, mutations at the RKK-PTP,  $C\alpha 2$ , and CBR3 motifs, as well as mutations at the PTEN C-terminal tail, affect PTEN association to membranes by interfering either with PTEN-plasma

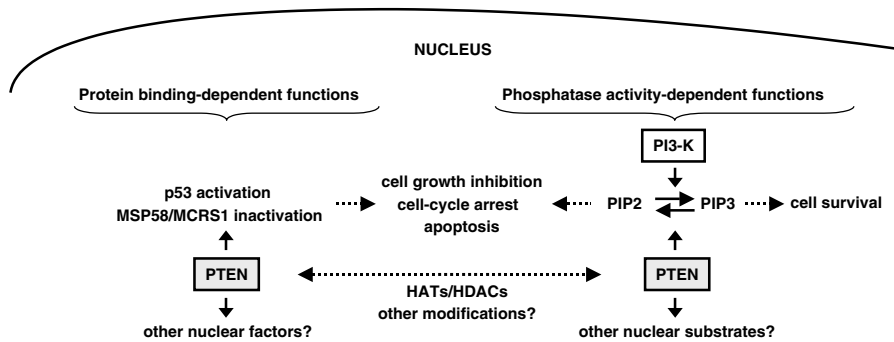
membrane electrostatic interactions or with PTEN binding to PDZ-domain containing- or plasma membrane-proteins.<sup>21–26</sup>

### A Model of PTEN Nuclear Entry

The subcellular compartmentalization of PTEN is a dynamic process involving distinct PTEN regions and many different cellular components.<sup>27</sup> The N-terminal tail of PTEN, in coordination with motifs at the PTEN core and at the PTEN C-terminal tail, seems to orchestrate both the binding of PTEN to the plasma membrane and its nuclear localization. We propose that the N-terminal NLS-like region, the nuclear exclusion motifs at the PTEN core, and the PTEN C-terminal tail, are essential to generate PTEN conformations that control its function and nuclear/cytoplasmic distribution. Our model of PTEN nuclear entry considers the existence of a NLS-like-dependent import of PTEN, which would be mediated by yet not identified importin transporters and the small GTPase Ran. Under certain cell growth conditions, the NLS-like domain could be masked by the nuclear exclusion motifs, in a PTEN ‘closed’ conformation. Adaptive changes of PTEN to variable cell stimulatory conditions, such as changes in its phosphorylation status, binding to regulatory partners, or partial proteolysis, could partially or totally expose the NLS-like domain (PTEN ‘open’ conformation) and trigger PTEN nuclear accumulation (Figure 3). Direct interactions involving the nuclear exclusion motifs and the NLS-like domain could account for this conformational model, although conformational changes affecting the NLS-like region could also occur upon modification of the nuclear exclusion motifs without a direct masking effect. The transition from the ‘closed’ to the ‘open’ conformation could be reversible, if processes such as phosphorylation/dephosphorylation or protein–protein interactions take place. On the other hand, if proteolytic cleavage or mutation of key residues occurs, this transition would be irreversible. Alternative models of PTEN nuclear entry, as those proposed by Chung *et al.*<sup>11</sup> and Liu *et al.*<sup>12</sup> could coexist that finely tune the amount of PTEN in the nucleus under particular cell growth conditions. Classical CRM1-dependent, leptomycin B-sensitive nuclear export, does not seem to be involved in the control of PTEN nuclear/cytoplasmic distribution. However, it remains uncertain whether other export mechanisms may account for PTEN nuclear exit. The ‘open’ conformation would also favour PTEN association to membranes by binding to PDZ-domain partners or to PIP2, or by direct electrostatic interactions between positive-charge membrane binding core motifs and the negative-charge plasma membrane inner surface.<sup>27</sup> The possibility also exists that cytoplasmic, plasma membrane, or nuclear anchoring proteins determine the pool of PTEN molecules that can shuttle between the cytoplasm and the nucleus. The elucidation of the PTEN nuclear transporters, importin-related and/or importin-non-related, will be necessary to understand more clearly the variety of mechanisms by which PTEN enters and accumulates in the nucleus. If different nuclear entry pathways are used for PTEN, the regulation of PTEN nuclear localization is likely to be a complex process. In the case of the N-terminal NLS-like-dependent nuclear entry, a coordinated regulation of both nuclear localization and PIP2 binding is expected. Indeed,



**Figure 3** The distribution of PTEN between the plasma membrane, the cytoplasm, and the nucleus. In this model, PTEN is partitioned in a 'closed' and in an 'open' conformation. In the 'closed' conformation, the NLS-like/PIP2 binding motif at the PTEN N-terminal tail (NLS/PIP2 box) remains masked by the coordinated contribution of the positive-charge core motifs (KK box) and the negative-charge C-terminal tail (S\*/T\* box). Changes in phosphorylation at the C-terminal tail may affect charge distribution and/or conformation. The PTEN 'closed' conformation mostly resides in the cytoplasm, and some nuclear entry can be facilitated by diffusion or other importin-independent mechanisms. Upon alterations of the PTEN C-terminal tail (S/T box) or specific positive-charge core motifs (K'K' box), a conformational change takes place that opens the molecule and exposes the PTEN NLS-like/PIP2 binding region. This PTEN 'open' conformation is transported to the nucleus by a Ran-GTPase-, NLS-like-dependent, importin-related mechanism, or it is targeted to lipids through its lipid binding motifs, electrostatic interactions and/or binding to plasma membrane protein partners. The model is based on observations from Chung *et al.*<sup>11</sup>, Liu *et al.*<sup>12</sup>, Gil *et al.*<sup>13</sup>, Vazquez *et al.*<sup>23</sup>, Das *et al.*<sup>24</sup>



**Figure 4** Pathways that mediate the functions of nuclear PTEN. The scheme illustrates the pathways mediating the functions of PTEN in the cell nucleus, both dependent and independent (protein binding-dependent) of its catalytic activity. The coordinated regulation of both pathways could drive the cell response towards cell growth inhibition and cell cycle arrest or towards apoptosis. Acetylation/deacetylation by histone acetyltransferases (HATs; such as PCAF) and deacetylases (HDACs), or other post-translational modifications could regulate specific functions of nuclear PTEN. The scheme is based on observations from Chung *et al.*<sup>11</sup>, Gil *et al.*<sup>13</sup>, Okumura *et al.*<sup>28</sup>, Freeman *et al.*<sup>30</sup>, Tang *et al.*<sup>32</sup>, Li *et al.*<sup>33</sup>, Chung *et al.*<sup>36</sup>, Liu *et al.*<sup>37</sup>, Deleris *et al.*<sup>38</sup>, Ahn *et al.*<sup>39</sup>

results from a comparative mutational study of the N-terminal region and the nuclear exclusion motifs of PTEN show a differential involvement of distinct PTEN residues in mediating PTEN nuclear accumulation and PIP3 dephosphorylation in cells (our unpublished observations). Modulation of the functionality of the nuclear exclusion motifs at the PTEN core will also affect PTEN binding to the plasma membrane and function. Our observations that PTEN nuclear localization augments during apoptosis, as well as the findings that nuclear PTEN accumulation can be affected during cell differentiation or cell cycle traverse,<sup>8,10,13</sup> indicate that PTEN nuclear entry is indeed subjected to tight regulation in cells. Likely regulatory mechanisms include post-

translational modifications (such as phosphorylation, caspase-3 cleavage, acetylation, or other not yet identified modifications) and protein-protein interactions.<sup>6,28</sup> High-throughput analysis of PTEN interactors, as well as individual validation and functional analysis of PTEN protein-protein interactions, will help to understand the diversity of mechanisms by which the subcellular distribution of PTEN is controlled.<sup>29</sup>

### Functional Pathways of Nuclear PTEN

The accumulation of PTEN in the cell nucleus has been found to inversely correlate with cell proliferation and transformation,

and a variety of biological functions have been attributed to nuclear PTEN that could cooperate to control cell growth and apoptosis (Figure 4).<sup>4–6</sup> Several reports have illustrated that the function of PTEN in the nucleus is independent of its catalytic activity, and rather depends on PTEN physical interaction with nuclear target proteins, such as the tumour suppressor p53 or the oncoprotein MSP58/MCRS1. Remarkably, these interactions are affected by alterations of the PTEN C-terminal tail.<sup>30,31</sup> PTEN also interacts with the histone acetyltransferases PCAF and p300/CBP, and a role for nuclear PTEN in maintaining high p53 acetylation, stability and transcriptional activity has been proposed.<sup>30,32,33</sup> Whether this activity of PTEN is p53-specific, or nuclear PTEN may directly behave as a transcriptional regulator for specific sets of cell growth and/or cell survival genes, deserves further study. PTEN itself is acetylated by PCAF at its catalytic site, which modulates its activity, making possible a nuclear-specific regulatory mechanism of PTEN substrate specificity and/or function (Figure 4).<sup>28</sup> Indeed, the phosphatase activity of PTEN is required for other functions mediated by nuclear PTEN. The involvement of catalytically active PTEN in cell cycle arrest by decreasing cyclin D1 levels in the nucleus has been documented,<sup>34,35</sup> and a specific role for nuclear PTEN in mediating such effect has been found.<sup>36</sup> Interestingly, nuclear PTEN may mediate growth suppression independent of Akt activity, although such process was found to be dependent on PTEN catalytic activity.<sup>37</sup> In addition, several findings point out to a proapoptotic function for nuclear PTEN. Active PTEN purified from vascular smooth muscle cell nuclei dephosphorylated PIP3, and nuclei from NGF-treated PC12 cells incubated with PTEN showed increased apoptotic DNA fragmentation inhibited by PIP3.<sup>38,39</sup> Moreover, apoptotic stimulation augmented nuclear accumulation of PTEN, and overexpression of catalytically active nuclear PTEN enhanced cell apoptotic responses in U87MG and HEK293 cells.<sup>13</sup> Thus, nuclear PTEN may exert distinct phosphatase activity-dependent functions related with its tumour suppressor function, including pro-apoptotic functions, which are likely to be cell type and cell differentiation specific. The lipid- and protein-phosphatase activity of PTEN in the nucleus needs to be explored, as well as the existence of other putative PTEN nuclear substrates than PIP3, to understand the implication of the PI3K/PTEN/Akt nuclear pathway in human cancer.<sup>40</sup> Oncogenic and tumour-suppressor nuclear networks are envisaged that recapitulate the PI3K/PTEN/Akt signalling through the non-nuclear pathways,<sup>41</sup> making the compart-

mentalization of their molecular components, including PTEN, a major checkpoint for the cell fate during development and disease. The possibility to interfere with PTEN function by controlling its subcellular localization emerges as a putative point of intervention in the prevention and/or treatment of human cancer.

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