

Perspective

Regulation of PTEN Function as a PIP3 Gatekeeper Through Membrane Interaction

Francisca Vazquez*

Peter Devreotes

Department of Cell Biology; Johns Hopkins University School of Medicine; Baltimore, Maryland USA

*Correspondence to: Francisca Vazquez; Department of Cell Biology; Johns Hopkins University School of Medicine; 725 N. Wolfe Street, WBSB116; Baltimore, Maryland 21205 USA; Tel.: 443.287.5028; Fax: 410.955.4129; Email: fvazquez@jhmi.edu

Original manuscript submitted: 06/06/06

Manuscript accepted: 06/07/06

Previously published online as a *Cell Cycle* E-publication:

<http://www.landesbioscience.com/journals/cc/abstract.php?id=3005>

KEY WORDS

PTEN, tumor suppressor, PI3K signaling, signal transduction, cancer therapy, plasma membrane, phosphorylation

ACKNOWLEDGEMENTS

We thank Pere Puigserver, Jonathan Francka-Koh and Stacey Willard for critical reading of the manuscript. Work in the author's laboratories was supported by The National Institutes of Health and the Department of Defense Prostate Cancer program.

ABSTRACT

PTEN, one of the most frequently mutated genes in human cancer, acts as a tumor suppressor by dephosphorylating the plasma membrane lipid second messenger phosphoinositide-3,4,5-trisphosphate (PIP3) generated by the action of PI3Kinases. *PTEN* activity to prevent elevated levels of PIP3 and tumorigenesis depends on its interaction with the lipid bilayer. *PTEN* binds dynamically to the plasma membrane through a complex mix of protein-lipid and protein-protein interactions and the translocation is regulated by several mechanisms including C-terminal tail phosphorylations. Here we have summarized our current view of the interaction of *PTEN* with the plasma membrane and what the implications are for cancer biology.

INTRODUCTION

PTEN (phosphatase and tensin homolog deleted in chromosome 10) is one of the most commonly mutated genes in human cancer. Somatic alterations of *PTEN* locus are found in a wide range of tumors but are particularly common in high-grade gliomas, melanomas, prostate and endometrial cancers.¹ Germline mutations in the *PTEN* gene are associated with the hamartoma diseases; Cowden and Bannayan-Riley-Ruvalcaba syndromes. Cowden syndrome is also associated with an increased predisposition to breast and thyroid malignancies.² Increased tumorigenesis in the prostate, endometrium, breast, thyroid, liver and gastrointestinal track has also been observed in heterozygous *pten*^{+/-} mouse models.³⁻⁵

The tumor suppressor function of *PTEN* is strongly linked to its activity as a lipid phosphatase for the second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3).^{6,7} PIP3 is generated by the action of the Phosphatidylinositol-3-kinase (PI3K) family of enzymes.⁸ The finding that inactivating mutations in the *PTEN* gene as well as activating mutations in *PI3KCA*—the gene encoding the p110 α catalytic subunit of PI3K—are very common events in tumors, indicates that elevated levels of PIP3 confer a strong advantage to cancer cells.^{9,10} The downstream effects of increased PIP3 levels are diverse and cell type specific. Increased proliferation, survival and motility are some of the main cellular effects associated with the increased PIP3 levels that could contribute to its tumorigenic effects.¹

Cells that lack *PTEN* have constitutively higher levels of PIP3 and activated downstream targets. Thus, *PTEN* acts as PIP3 gatekeeper by maintaining basal levels of PIP3 below a threshold for signaling activation. It is evident that *PTEN* must access the plasma membrane in order to keep PIP3 low, yet it appears largely cytosolic. Recent evidence shows that a small but critical fraction of *PTEN* interacts dynamically with the plasma membrane.¹¹ In this review, we summarize the current research and our view of how and when *PTEN* binds to the lipid bilayer to dephosphorylate PIP3 and what are the implications for cancer biology.

NATURE OF PTEN MEMBRANE BINDING SITES

There is evidence indicating that *PTEN* binds directly to plasma membrane lipids. The crystal structure revealed two globular domains. The first half comprises the phosphatase domain and the second contains a C2 domain, a Ca²⁺-independent membrane-targeting module found in many proteins involved in signal transduction or membrane trafficking. A short N-terminal leader and a C-terminal phosphorylation sites containing tail were left out of the structure (Fig. 1A).¹² In vitro binding assays with phospholipid vesicles indicated that two of the three C2-domain loops contain basic residues that are important for binding to anionic lipids.¹² Mutations of these residues in the C2 domain largely

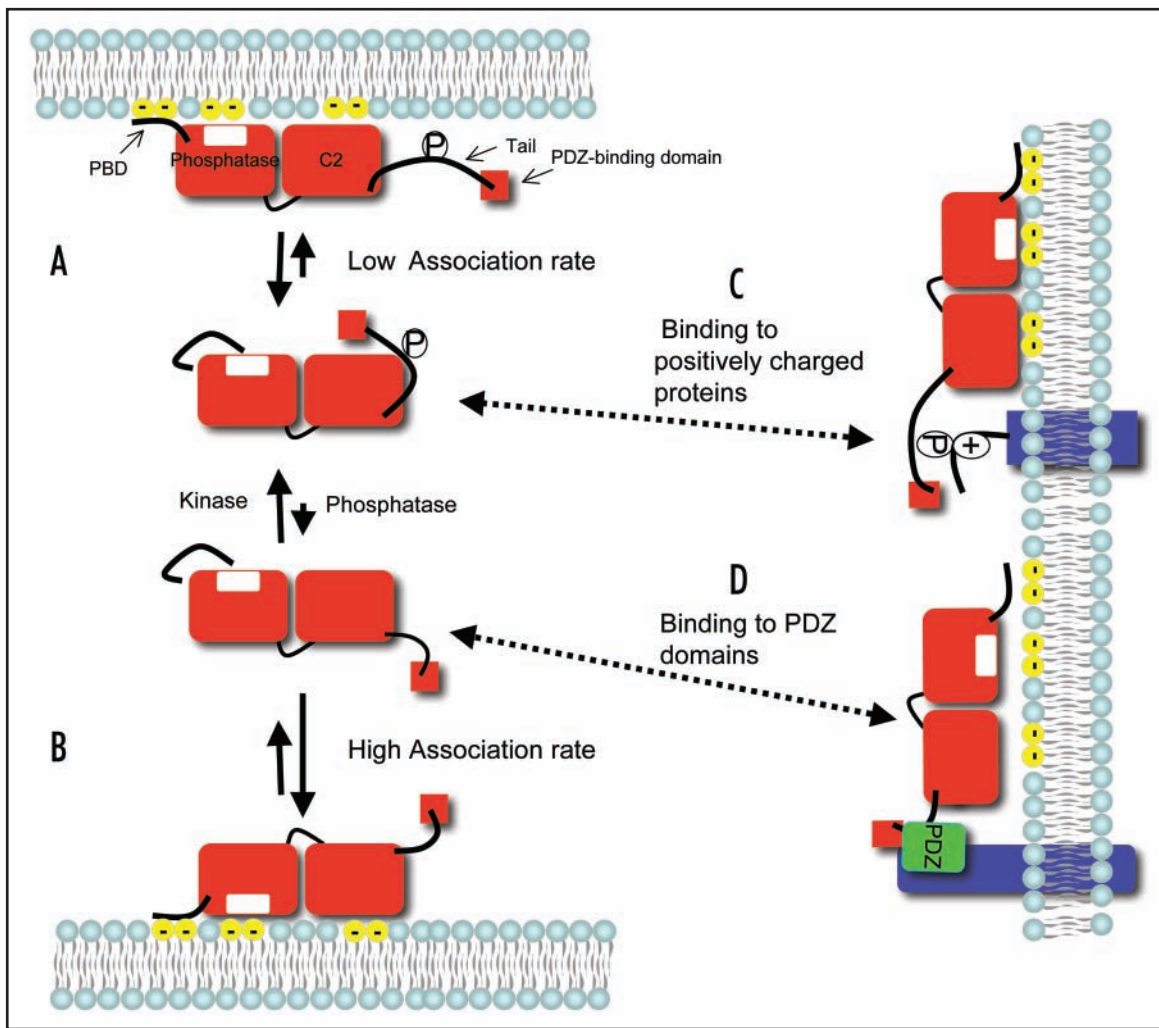


Figure 1. Model of potential modes of PTEN membrane binding. a) Phosphorylation of the c-terminal tail masks the membrane binding domains resulting in a low membrane association rate. b) Dephosphorylation of the tail increases the membrane association step resulting in a higher fraction of PTEN at the plasma membrane. Both phosphorylated and unphosphorylated PTEN dissociate from the membrane at a similar rate. c) Binding of PTEN to membrane proteins with positively charged cytoplasmic tails, like NEP, results in a displacement of the tail intramolecular interactions and exposure of the membrane binding domains. d) Dephosphorylation of the tail exposes the PDZ binding domain.

reduce interaction with lipid vesicles *in vitro* and growth suppressing activity of PTEN in cells.¹³ Although phosphatase activity per se is not required for membrane binding,¹¹ basic residues within the phosphatase domain were also shown to contribute to membrane binding.¹⁴ Thus, clusters of positively charged residues on the surface of the C2 and phosphatase domains are critical for PTEN binding to anionic lipids.

In addition to the phosphatase and C2 domains, deletion or mutations of highly conserved stretch of basic and hydrophobic residues at the N-terminus, dubbed the PIP2 binding domain (PBD), completely eliminated PTEN binding to the plasma membrane *in vivo* and to lipid vesicles *in vitro*.^{11,15} Interestingly, phosphatidylinositol (4,5)-bisphosphate (PIP2) enhances PTEN recruitment to lipid vesicles and PIP3 phosphatase activity and mutations in the PBD domain abolish these effects.^{16,17} Moreover, in *Dictyostelium discoideum*, deletion of the PBD enhances activity against a water-soluble substrate, inositol (2,3,4,5)-tetrakisphosphate (IP4).¹⁸ Taken together, these results suggest that the PBD may occlude the active site when PTEN is in the cytosol but that the molecule attains an “open” conformation when bound to PIP2 at the plasma membrane

(Fig. 1). Consistent with this idea, the PBD was not included in the crystal structure, because of its unstructured or loosely folded structure.¹² Importantly, mutations or deletions of the PBD abrogate PTEN activity *in vivo*^{11,19} and tumor-derived mutations found in this motif, such as K13E found in glioblastoma and endometrioid carcinoma or S10N found in lymphoma,²⁰⁻²² are predicted to impair PTEN tumor suppressor function by interfering with membrane binding.

There are several lines of evidence suggesting that PTEN is also recruited to the plasma membrane by interaction with proteins. The last three amino acids (threonine 401, lysine 402 and valine 403) constitute a PDZ binding motif (Fig. 1), a protein-protein interaction module found in a few hundred human proteins. Indeed, it has been shown that PTEN can interact with some of these PDZ domain containing proteins including MAGI1b, 2 and 3, hDlg, NHERF1, NHREF2, MAST205, MAST3 and SAST.²³⁻²⁷ PDZ domain-containing proteins are often part of multiprotein complexes that assemble signaling proteins at specific sites at the plasma membrane.²⁸ Five mutations identified in glioblastoma and one identified in leiomyosarcoma are predicted to disrupt the PDZ

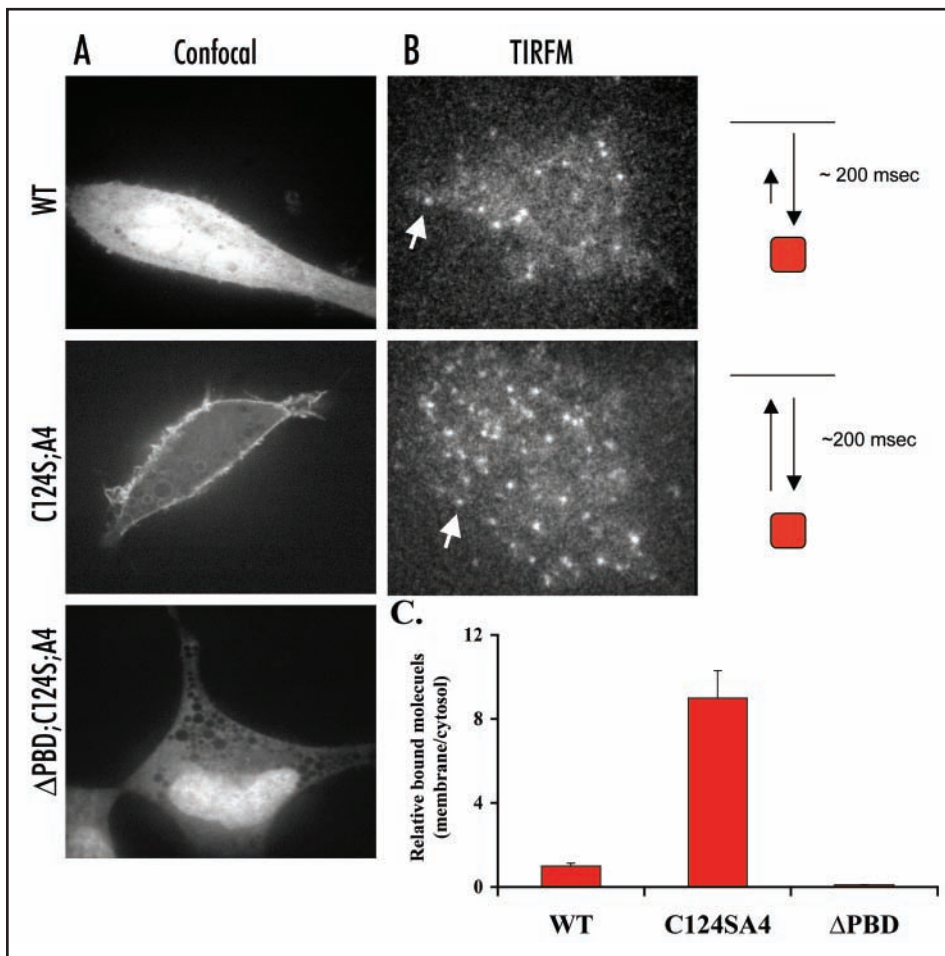


Figure 2. PTEN membrane association is controlled by c-terminal tail phosphorylations. HEK293 cells transfected with PTEN-YFP and mutant forms (A) Confocal microscopy and (B) TIRFM images are shown. With TIRFM only a small region close to the slide surface is excited and can be used to detect proteins at the plasma membrane on the basal surface of the cell. The arrow indicates single-molecules of PTEN-YFP at or close to the plasma membrane. (C) Quantification of the number of relative bound molecules to cytosolic levels. Both PTEN-YFP and PTEN;C124S;A4-YFP molecules bind to the membrane for less than 200 msec. Thus, the differences in the steady-state levels of molecules bound would result from an increase in the association time.

binding domain,^{29,30,31-33} suggesting that binding to PDZ containing complexes may play a role in PTEN tumor suppressor function. Recent reports suggest that under certain physiological conditions PTEN is recruited from the cytosol to PDZ containing complexes. For example, one study found that in polarized epithelial cells PTEN was recruited to adherens junctions through binding to the PDZ domain-containing scaffold protein MAGI-1b, where it played a role in stabilizing functional complexes.³⁴ A fraction of PTEN is in a large complex when purified from liver or brain extracts rather than tissue cultured cells^{27,35} suggesting that in intact tissues where cells are in an appropriate microenvironment, and are for example fully polarized, PTEN may be recruited to the plasma membrane through PDZ interactions. Furthermore, *D. Melanogaster* PTEN is recruited to the apical cortex of epithelial cells and neuroblasts and to cell-cell junctions of photoreceptors through a direct interaction with Bazooka, the *D. Melanogaster* homologue of Par-3.^{36,37}

Another protein that has been shown to recruit PTEN to the plasma membrane using a different mode of interaction is the transmembrane Neutral Endopeptidase 24.11 (NEP). A polybasic stretch within the cytoplasmic domain of NEP binds to the cluster of

phosphorylation sites in the tail of PTEN through electrostatic interactions. When overexpressed, NEP can recruit PTEN to the plasma membrane at a very high stoichiometry. Although there is an inverse correlation between expression levels of PTEN, NEP and activated AKT, PTEN is able to dephosphorylate PIP3 in cells that lack NEP expression.³⁸ Therefore, although NEP is not essential for PTEN activity, it might play a contributing role.

While lipid interactions play an essential role in interaction of PTEN with the plasma membrane, protein-protein interactions are likely to play a regulatory role, for example in determining steady-state levels of PTEN at the plasma membrane or recruiting PTEN to specific membrane sites to decrease the local concentration of PIP3. Which, if any, of these PTEN-interacting proteins contribute to PTEN tumor suppression function remains to be determined through genetic analyses in animal models where more complex functional contributions can be determined.

REGULATION OF PTEN MEMBRANE INTERACTIONS

Despite the aforementioned lipid and protein interaction domains, PTEN is mainly cytoplasmic and nuclear. Our studies as well as that of others suggest that PTEN levels at the plasma membrane are kept low by “masking” the membrane binding sites through intramolecular interactions and/or by direct electrostatic repulsion^{11,14,35} (Fig. 1).

The best-characterized mode of masking membrane-binding domains of PTEN is the C-terminal tail phosphorylation. The

PTEN tail, consisting of residues 350 to 403, is constitutively phosphorylated.^{39,40} Although other sites can also be phosphorylated, serines 380 and 385, and threonine 382 and 383 are the functionally relevant phosphorylation sites.^{35,39,40} Alanine substitution of the phosphorylation sites exposes new sites to protease digestion and also increases the ability of PTEN to interact with PDZ-domain containing proteins suggesting a conformational change.^{35,41} Furthermore, these perturbations greatly enhance interaction of PTEN with the plasma membrane.¹⁴ Based on these results, we propose that the phosphorylated tail folds-back and creates intramolecular interactions with another portion of the protein and occludes the membrane binding sites (Fig. 1). Negative charges of the phosphorylation sites could also contribute by directly repelling interaction with positively charged lipids, as has been proposed.¹⁴

We have also found that a specific mutation of the phosphatase pocket C124S enhances membrane localization. However, other mutations that inactivate phosphatase activity, like G129R or D92A do not have this effect, suggesting that inactivation of the phosphatase activity per se is not the cause. Consistent with these results, PIP3 levels do not affect PTEN binding to the membrane. Interestingly,

the C124S mutation synergizes with the aforementioned alanine substitutions of the tail phosphorylation sites (collectively named A4) to cause nearly all of PTEN to associate with the membrane^{11,14} (Fig. 2).

If most of PTEN is found in the cytosol, how and when does PTEN access the plasma membrane to dephosphorylate PIP3? The fact that in all cells tested, deletion of PTEN causes an increase in basal PIP3 levels suggests that the enzyme is constitutively active. Using total reflection internal microscopy (TIRFM) we detected single molecules of a functional PTEN-YFP at the plasma membrane at levels that were undetectable by confocal or wide-field microscopy¹¹ (Fig. 2). The number of molecules at the plasma membrane was proportional to the cytosolic amount and did not saturate at the levels of PTEN-YFP used, which ranged from below to above the levels of endogenous PTEN. Interestingly, the dwell time of PTEN at the plasma membrane in HEK293 cells is less than 400 msec demonstrating that the binding is very dynamic. The steady-state number of molecules binding is dramatically decreased by deletion of the PBD (Fig. 2). Since deletion of the PBD is required for its activity, we concluded that low and dynamic interaction of PTEN molecules with the plasma membrane is necessary and sufficient for PTEN to function, at least in cells in culture.¹¹ However, the low levels of PTEN at the plasma membrane indicate that the steady-state fraction of functionally active molecules at a particular time is very small. This strategy may allow PTEN to keep basal levels of PIP3 low but not blunt increases in PIP3 levels when PI3K is stimulated.

The amount of PTEN competent to bind to the membrane may be regulated. This could be achieved in different cell types by differences not only in expressed protein levels but also in the ratio of phosphorylated to unphosphorylated molecules. For example, in intestinal epithelial cells phosphorylated PTEN was found to be enriched in the stem cell compartment where it colocalized with activated Akt.⁴² Additional mechanisms of increasing the active fraction of PTEN could exist. In this regard, an interesting possibility is that by directly binding to the tail phosphorylation sites NEP could displace the intramolecular interactions and expose PTEN membrane binding sites (Fig. 1). The idea that PTEN can dephosphorylate itself has been suggested by the observation that a catalytically inactive PTEN is more phosphorylated at threonine 383 than active PTEN.⁴³ Although this is an interesting model, it is unclear how and when this function of PTEN would be regulated. Whatever the mechanism, the levels of PTEN acting on the membrane would dictate cell sensitivity to PI3K stimulation which could be important for cancer predisposition (see below).

Along with this temporal regulation, there is evidence suggesting that PTEN membrane association is spatially regulated. This type of regulation would be very important in polarizing the distribution of PIP3 in the cell. The polarized distribution of PIP3 is important in several physiological processes critical for tumor development, for example cell migration, cytokinesis, or epithelial polarity. Polarized membrane localization of PTEN has been observed in *D. discoideum* cells where PTEN is enriched at the cleavage furrow during cytokinesis and on the rear of migrating cells.⁴⁴ This localization mechanism is conserved when human PTEN is expressed in the same cells and functionally substitutes for endogenous PTEN.¹¹ In certain *D. Melanogaster* cell lineages PTEN has also been shown to localize in membrane specific regions.^{36,37} However, these two types of polarity appear to be generated by different mechanisms. In *D. Melanogaster* cells, the binding to PDZ domain containing proteins recruits

PTEN to the specific membrane regions, as discussed in the previous section. In *D. Discoideum* cells, however, PDZ domains are not present and the mechanism of membrane binding has yet to be determined. Whether PTEN is also recruited to a specific region of the membrane in human tissues and whether loss of this polarity contributes to tumor progression awaits further investigation.

IMPLICATIONS FOR CANCER BIOLOGY

As more information about the “cancer genome” becomes available, it will be important not only to determine which genetic alterations are casual but also which pathways they alter.⁴⁵ Understanding the network that regulates PTEN activity is necessary to be able to connect particular genetic lesions with PTEN function. There is a wide range of tumors where hemizygous inactivation of *PTEN* is more common than mutation of both alleles.^{1,46} These observations combined with results in mouse models, where small changes in PTEN expression levels have important consequences on the rate of prostate carcinogenesis, suggest that PTEN may be a haploinsufficient tumor suppressor in certain tumor types.⁴⁷ It is conceivable that changes of PTEN activity not only expression could also contribute to tumor development. In particular, alterations in genes that encode proteins that are important for the regulation of PTEN membrane interaction could lead to an increase in PI3K pathway activity that may contribute to the neoplastic process. Some candidates have already been proposed. For example, NEP whose expression is decreased in androgen-independent metastatic prostate cancer, could regulate the PI3K pathway through PTEN.³⁸

Many drug candidates that target the PI3K pathway at multiple steps are currently under development.⁴⁸ The fact that PTEN levels at the plasma membrane can be modulated to regulate its activity offers a new potential therapeutic opportunity. This mode of therapy could be particularly important in tumors with insufficient or decreased PTEN expression and in tumors with increased PIP3 levels caused by means other than PTEN gene inactivation, for example PI3KCA activating mutations. Increase in PTEN activity could result in a reset of the basal PIP3 levels in these tumors. Because the PI3K pathway controls many important physiological processes, for example insulin signaling, drugs that target this enzyme have the concern of many potential side effects.⁴⁹ Induction of PTEN activity could have the particular advantage that PI3K stimulation would not be inhibited and thus physiological induction of PIP3 levels would not be completely blunted. Thus, pharmacological interventions that increase PTEN activity could be used to prevent tumor progression or treat tumors that retain at least one copy of the PTEN gene.

References

1. Chow LM, Baker SJ. PTEN function in normal and neoplastic growth. *Cancer Lett* 2006.
2. Pilarski R, Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the *PTEN* hamartoma tumour syndrome. *J Med Genet* 2004; 41:323-6.
3. Di Cristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP. *Pten* is essential for embryonic development and tumour suppression. *Nat Genet* 1998; 19:348-55.
4. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, Ho A, Wakeham A, Irie A, Khoo W, Fukumoto M, Mak TW. High cancer susceptibility and embryonic lethality associated with mutation of the *PTEN* tumor suppressor gene in mice. *Curr Biol* 1998; 8:1169-78.
5. Podsypanina K, Ellenson LH, Nemes A, Gu J, Tamura M, Yamada KM, Cordon-Cardo C, Catoretti G, Fisher PE, Parsons R. Mutation of *Pten/Mmac1* in mice causes neoplasia in multiple organ systems. *Proc Natl Acad Sci USA* 1999; 96:1563-8.
6. Machama T, Dixon JE. *PTEN*: A tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol* 1999; 9:125-8.
7. Vazquez F, Sellers WR. The *PTEN* tumor suppressor protein: An antagonist of phosphoinositide 3-kinase signaling. *Biochim Biophys Acta* 2000; 1470:M21-35.
8. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002; 296:1655-7.

9. Parsons R. Human cancer, PTEN and the PI-3 kinase pathway. *Semin Cell Dev Biol* 2004; 15:171-6.
10. Samuels Y, Ericson K. Oncogenic *PI3K* and its role in cancer. *Curr Opin Oncol* 2006; 18:77-82.
11. Vazquez F, Matsuoka S, Sellers WR, Yanagida T, Ueda M, Devreotes PN. Tumor suppressor *PTEN* acts through dynamic interaction with the plasma membrane. *Proc Natl Acad Sci USA* 2006; 103:3633-8.
12. Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, Dixon JE, Pandolfi P, Pavletich NP. Crystal structure of the *PTEN* tumor suppressor: Implications for its phosphoinositide phosphatase activity and membrane association. *Cell* 1999; 99:323-34.
13. Georgescu MM, Kirsch KH, Kaloudis P, Yang H, Pavletich NP, Hanafusa H. Stabilization and productive positioning roles of the C2 domain of *PTEN* tumor suppressor. *Cancer Res* 2000; 60:7033-8.
14. Das S, Dixon JE, Cho W. Membrane-binding and activation mechanism of PTEN. *Proc Natl Acad Sci USA* 2003; 100:7491-6.
15. Downes CP, Walker S, McConnachie G, Lindsay Y, Batty IH, Leslie NR. Acute regulation of the tumour suppressor phosphatase, *PTEN*, by anionic lipids and reactive oxygen species. *Biochem Soc Trans* 2004; 32:338-42.
16. McConnachie G, Pass I, Walker SM, Downes CP. Interfacial kinetic analysis of the tumour suppressor phosphatase, *PTEN*: Evidence for activation by anionic phospholipids. *Biochem J* 2003; 371:947-55.
17. Campbell RB, Liu F, Ross AH. Allosteric activation of PTEN phosphatase by phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 2003; 278:33617-20.
18. Iijima M, Huang YE, Luo HR, Vazquez F, Devreotes PN. Novel mechanism of PTEN regulation by its phosphatidylinositol 4,5-bisphosphate binding motif is critical for chemotaxis. *J Biol Chem* 2004; 279:16606-13.
19. Walker SM, Leslie NR, Perera NM, Batty IH, Downes CP. The tumour-suppressor function of *PTEN* requires an N-terminal lipid-binding motif. *Biochem J* 2004; 379:301-7.
20. Gronbaek K, Zeuthen J, Guldberg P, Ralfkiaer E, Hou-Jensen K. Alterations of the *MMAC1/PTEN* gene in lymphoid malignancies. *Blood* 1998; 91:4388-90.
21. Yokoyama Y, Wan X, Shinohara A, Takahashi S, Takahashi Y, Niwa K, Tamaya T. Expression of PTEN and PTEN pseudogene in endometrial carcinoma. *Int J Mol Med* 2000; 6:47-50.
22. Minaguchi T, Yoshikawa H, Oda K, Ishino T, Yasugi T, Onda T, Nakagawa S, Matsumoto K, Kawana K, Taketani Y. *PTEN* mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. *Clin Cancer Res* 2001; 7:2636-42.
23. Adey NB, Huang L, Ormonde PA, Baumgard ML, Pero R, Byreddy DV, Tavtigian SV, Bartel PL. Threonine phosphorylation of the *MMAC1/PTEN* PDZ binding domain both inhibits and stimulates PDZ binding. *Cancer Res* 2000; 60:35-7.
24. Wu X, Hepner K, Castellino-Prabhu S, Do D, Kaye MB, Yuan XJ, Wood J, Ross C, Sawyers CL, Whang YE. Evidence for regulation of the *PTEN* tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. *Proc Natl Acad Sci USA* 2000; 97:4233-8.
25. Wu Y, Dowbenko D, Spencer S, Laura R, Lee J, Gu Q, Lasky LA. Interaction of the tumor suppressor *PTEN/MMAC* with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. *J Biol Chem* 2000; 275:21477-85.
26. Valiente M, Andres-Pons A, Gomar B, Torres J, Gil A, Tapparel C, Antonarakis SE, Pulido R. Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. *J Biol Chem* 2005; 280:28936-43.
27. Takahashi Y, Morales FC, Kreimann EL, Georgescu MM. *PTEN* tumor suppressor associates with *NHERF* proteins to attenuate *PDGF* receptor signaling. *Embo J* 2006; 25:910-20.
28. Kim E, Sheng M. PDZ domain proteins of synapses. *Nat Rev Neurosci* 2004; 5:771-81.
29. Peraud A, Watanabe K, Schweddeheimer K, Yonekawa Y, Kleihues P, Ohgaki H. Genetic profile of the giant cell glioblastoma. *Lab Invest* 1999; 79:123-9.
30. Saito T, Oda Y, Kawaguchi K, Takahira T, Yamamoto H, Tamiya S, Tanaka K, Matsuda S, Sakamoto A, Iwamoto Y, Tsuneyoshi M. *PTEN/MMAC1* gene mutation is a rare event in soft tissue sarcomas without specific balanced translocations. *Int J Cancer* 2003; 104:175-8.
31. Tohma Y, Gratas C, Biernat W, Peraud A, Fukuda M, Yonekawa Y, Kleihues P, Ohgaki H. *PTEN (MMAC1)* mutations are frequent in primary glioblastomas (de novo) but not in secondary glioblastomas. *J Neuropathol Exp Neurol* 1998; 57:684-9.
32. Zhou XP, Li YJ, Hoang-Xuan K, Laurent-Puig P, Mokhtari K, Longy M, Sanson M, Delattre JY, Thomas G, Hamelin R. Mutational analysis of the *PTEN* gene in gliomas: Molecular and pathological correlations. *Int J Cancer* 1999; 84:150-4.
33. Schmidt EE, Ichimura K, Goike HM, Moshref A, Liu L, Collins VP. Mutational profile of the *PTEN* gene in primary human astrocytic tumors and cultivated xenografts. *J Neuropathol Exp Neurol* 1999; 58:1170-83.
34. Kotelevts L, van Hengel J, Bruyneel E, Marcel M, van Roy F, Chastre E. Implication of the MAGI-1b/PTEN signalosome in stabilization of adherens junctions and suppression of invasiveness. *Faseb J* 2005; 19:115-7.
35. Vazquez F, Grossman SR, Takahashi Y, Rokas MV, Nakamura N, Sellers WR. Phosphorylation of the PTEN tail acts as an inhibitory switch by preventing its recruitment into a protein complex. *J Biol Chem* 2001; 276:48627-30.
36. Pinal N, Goberdhan DC, Collinson L, Fujita Y, Cox IM, Wilson C, Pichaud F. Regulated and polarized PtdIns(3,4,5)P3 accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. *Curr Biol* 2006; 16:140-9.
37. von Stein W, Ramrath A, Grimm A, Muller-Borg M, Wodarz A. Direct association of Bazooka/PAR-3 with the lipid phosphatase PTEN reveals a link between the PAR/aPKC complex and phosphoinositide signaling. *Development* 2005; 132:1675-86.
38. Sumitomo M, Iwase A, Zheng R, Navarro D, Kaminetzky D, Shen R, Georgescu MM, Nanus DM. Synergy in tumor suppression by direct interaction of neutral endopeptidase with *PTEN*. *Cancer Cell* 2004; 5:67-78.
39. Vazquez F, Ramaswamy S, Nakamura N, Sellers WR. Phosphorylation of the PTEN tail regulates protein stability and function. *Mol Cell Biol* 2000; 20:5010-8.
40. Torres J, Pulido R. The tumor suppressor *PTEN* is phosphorylated by the protein kinase CK2 at its C terminus. Implications for PTEN stability to proteasome-mediated degradation. *J Biol Chem* 2001; 276:993-8.
41. Tolkacheva T, Boddapati M, Sanfiz A, Tsuchida K, Kimmelman AC, Chan AM. Regulation of PTEN binding to MAGI-2 by two putative phosphorylation sites at threonine 382 and 383. *Cancer Res* 2001; 61:4985-9.
42. Tian Q, He XC, Hood L, Li L. Bridging the BMP and Wnt pathways by PI3 kinase/Akt and 14-3-3zeta. *Cell Cycle* 2005; 4:215-6.
43. Raftopoulos M, Etienne-Manneville S, Self A, Nicholls S, Hall A. Regulation of cell migration by the C2 domain of the tumor suppressor *PTEN*. *Science* 2004; 303:1179-81.
44. Janetopoulos C, Borleis J, Vazquez F, Iijima M, Devreotes P. Temporal and spatial regulation of phosphoinositide signaling mediates cytokinesis. *Dev Cell* 2005; 8:467-77.
45. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004; 10:789-99.
46. Oki E, Kakeji Y, Baba H, Tokunaga E, Nakamura T, Ueda N, Futatsugi M, Yamamoto M, Ikebe M, Machara Y. Impact of loss of heterozygosity of encoding phosphate and tensin homolog on the prognosis of gastric cancer. *J Gastroenterol Hepatol* 2006; 21:814-8.
47. Trotman LC, Niki M, Dotan ZA, Koutcher JA, Di Cristofano A, Xiao A, Khoo AS, Roy-Burman P, Greenberg NM, Van Dyke T, Cordon-Cardo C, Pandolfi PP. Pten dose dictates cancer progression in the prostate. *PLoS Biol* 2003; 1:E59.
48. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB. Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nat Rev Drug Discov* 2005; 4:988-1004.
49. Cully M, You H, Levine AJ, Mak TW. Beyond *PTEN* mutations: The PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 2006; 6:184-92.