The nuclear affairs of PTEN

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Summary
PTEN encodes a major tumor-suppressor protein that is a dual-specificity phosphatase. Inactivation of PTEN has been shown to be involved in heritable and sporadic cancers. Mutation or deletion of PTEN, historically the most commonly identified mechanisms of inactivation of tumor suppressors, is found only in the minority of sporadic non-cultured primary cancers, which indicates that there might be other, novel mechanisms of inactivation. Despite the absence of a classic nuclear localization signal, PTEN enters the nucleus by several mechanisms, including simple diffusion, active shuttling, cytoplasmic-localization-signal-dependent export and monoubiquitylation-dependent import. Cytoplasmic PTEN has a well-known role as a negative regulator of the PI3K/AKT pathway; however, it is becoming clear that cytosolic PTEN is not the same as nuclear PTEN. Nuclear PTEN plays a role in chromosome stability, DNA repair, cell cycle arrest and cellular stability. The balance between these functions is an important factor in determining whether a cell remains benign or becomes neoplastic.

Key words: PTEN, Nuclear, Subcellular localization, Cowden, PHTS, Bifurcation

Introduction
Phosphatase and tensin homolog (mutated in multiple advanced cancers 1) (PTEN), located on 10q23.3, encodes a 403-residue dual-specificity phosphatase that has both lipid and protein phosphatase activity. PTEN classically converts phosphatidylinositol-3,4,5-trisphosphate (PIP3) in the cytoplasm to phosphatidylinositol-4,5-bisphosphate (PIP2), thereby directly antagonizing the activity of PI3 kinase (PI3K) (Leevers et al., 1999) (Fig. 1). Its inactivation results in constitutive activation of the PI3K/AKT pathway and in subsequent increases in protein synthesis, cell cycle progression, migration and survival (Li and Sun, 1998) (Fig. 1). Recombinant PTEN has been shown to dephosphorylate serine, threonine and tyrosine residues of peptides in vitro (Myers et al., 1997) and focal adhesion kinase in vivo (Tamura et al., 1998). In addition, various studies have demonstrated that the protein phosphatase activity of PTEN inhibits activation of mitogen-activated protein kinase (MAPK) via several pathways.

Although numerous somatic mutations have been localized to the PTEN gene, these only occur in a minority of tumors, which indicates that alternative mechanisms, both genetic and non-genetic, of PTEN inactivation must exist. One such mechanism is the regulation of subcellular localization. In this Commentary, we focus on nuclear localization of PTEN, and the importance of this in normal physiology and disease. Other non-traditional mechanisms of regulation are discussed or reviewed elsewhere (Pezzolesi et al., 2006; Teresi et al., 2007; Zbuk and Eng, 2007).

Nuclear PTEN
Early studies of PTEN localization, using immunohistochemical staining techniques, found it in the cytoplasm; however, these focused primarily on overexpressed protein or tumorigenic cell lines and tissues (Furnari et al., 1997; Li and Sun, 1997; Whang et al., 1998). Subsequently, multiple reports placed PTEN in the nucleus of normal primary neurons and endothelial cells (Sano et al., 1999), myoepithelial cells of normal breast ducts (Perren et al., 1999) and normal follicular thyroid cells (Gimm et al., 2000). In pancreatic tissue, Perren and co-workers also observed nuclear PTEN in normal islet cells but predominantly cytoplasmic PTEN in sporadic endocrine pancreatic tumors (Perren et al., 2000). The loss of nuclear PTEN specifically has now been found in a variety of sporadic tumors (Brenner et al., 2002; Depowski et al., 2001; Dreher et al., 2004; Perren et al., 2000). Perhaps the first compelling evidence for a role of nuclear PTEN came from studies of melanoma. These indicated an association between a lack of nuclear PTEN and mitotic index: nuclear PTEN is apparent in quiescent cells, but in actively dividing cells PTEN localizes mostly to the cytosol (Whiteman et al., 2002). Other studies have observed similar trends in different tissue types (Gimm et al., 2000; Perren et al., 2000). This has led to the belief that, in normal, quiescent tissue, PTEN is localized primarily in the nucleus, whereas, in neoplastic tissue, cytoplasmic PTEN predominates. Thus, PTEN in the nucleus appeared to be required for tumor-suppressing ability.

At the time, however, many felt that the presence of PTEN in the nucleus was merely an artifact, because all the data had relied solely on immunohistochemical techniques. Subsequent subcellular fractionation and fluorescence microscopy in the human breast carcinoma cell line MCF-7 (Fig. 2), however, showed that localization of PTEN is cell cycle dependent, with higher nuclear PTEN levels being found in G0-G1 phase and lower levels being found in S phase (Gimm-Pease and Eng, 2003). This was consistent with the PTEN localization patterns observed in normal and tumor tissues: normal, quiescent tissues consisting mostly of cells in G0-G1 phase exhibit a predominantly nuclear localization of PTEN, whereas actively dividing cancerous tissues have a higher percentage of cells in S phase and therefore exhibit increased levels of cytoplasmic PTEN. A change in intracellular localization of PTEN thus seems to be involved in neoplastic transformation and might modulate its activity.
PTEN interacts with proteins in the cytoplasm that sequester it to prevent nuclear translocation. PTEN might thus enter the nucleus by passive diffusion and its cytoplasmic sequestration could influence nucleo-cytoplasmic distribution.

Active, RAN-mediated nuclear import of PTEN has been documented in U87MG human glioblastoma cells (Gil et al., 2006). Gil and co-workers found that PTEN is excluded from the nucleus of cells expressing the GTPase-deficient, dominant-negative RANQ69L mutant. Additionally, this group demonstrated that mutations affecting phosphorylation of PTEN, which had previously been shown to affect stability of PTEN (Vazquez et al., 2000), alter its nucleo-cytoplasmic localization. In their experiment, mutation of a single phosphorylation site (S370A) caused PTEN to localize to the cytoplasm, similar to the wild-type protein, whereas the T382A mutant had a roughly equal nucleo-cytoplasmic distribution. Mutations of other single phosphorylation sites (S380A, T383A or S385A) produced strong nuclear distributions, as did mutants that have multiple defective phosphorylation sites (S370A/S385A, S380A/T382A/T383A, S370A/S385A/S380A/T382A/T383A). These findings differ from work in MCF-7 human breast cancer cells, in which expression of similar mutants did not result in altered nucleo-cytoplasmic localization of PTEN (Chung et al., 2005). Although these reports are contradictory, the model systems used are different. Gil and co-workers used glioblastoma cells lacking PTEN protein expression. The phosphorylation mutants were therefore expressed without a background of endogenous PTEN with which to interact. By contrast, Chung and co-workers used MCF-7 breast cancer cells that already express PTEN. These contradictory data might simply be a result of different model systems (brain cancer versus breast cancer). Alternatively, endogenous PTEN could interact with the PTEN mutants and alter their localization.

A third potential mechanism of PTEN import involves MVP. PTEN binds to MVP, a protein hypothesized to be a general carrier molecule for nuclear-cytoplasmic transport (Mossink et al., 2003), in yeast two-hybrid screens (Yu et al., 2002). Further analysis confirmed that this interaction occurs in both 293T and HeLa cells and localized the binding sites to the C2 domain of PTEN and the EF-hand pair (a calcium-binding motif) of MVP. Studies in MCF-7 cells identified four non-traditional NLS-like sequences: NLS1 to NLS4 (Chung et al., 2005). Mutations in single individual sequences do not affect PTEN localization; however, when combinations of two sequences are targeted, specifically NLS2 and NLS3 or NLS2 and NLS4, PTEN is excluded from the nucleus. These double mutants also lack the ability to bind to MVP. Further characterization of the interactions between PTEN and MVP indicated that Ca^{2+} positively regulates binding (Minaguchi et al., 2006). Bipartite NLS motifs exist in other tumor-suppressor proteins, such as p53 (Efthymiadias et al., 1997) and the retinoblastoma protein pRb (Liang and Clarke, 1999), and links between the nuclear transport of proteins and the development of cancer have been demonstrated (Kau et al., 2004).

Trotman and co-workers have described another potential mechanism of PTEN nuclear import (Trotman et al., 2007). They observed that a particular PTEN mutation (K289E) in a Cowden syndrome (CS) patient results in the nuclear exclusion of PTEN. The authors determined that K289 is a major target for ubiquitylation. Because mono-ubiquitylation has been implicated in the transport of other proteins (Li et al., 2003; Massoumi et al., 2006; Pfaffker et al., 2004), they performed further analyses of this mutation, leading to the hypothesis that mono-ubiquitylation of

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**Fig. 1.** ‘Classic’ PTEN pathway. Binding of ligand to membrane receptors activates PI3K. Activated PI3K phosphorylates P(3,4,5)P (PIP3) to produce P(3,4,5)P (PIP3). PIP3 recruits PDK1 to the plasma membrane. PDK1 phosphorylates and activates AKT, which regulates various cellular processes. The lipid phosphatase activity of cytoplasmic PTEN dephosphorylates PIP3, thereby decreasing PIP3 levels and increasing levels of PIP2, resulting in a concomitant decrease in AKT activity.

**Fig. 2.** Fluorescence microscopy of GFP-PTEN. Fluorescence microscopy of the human breast carcinoma cell line MCF-7 expressing exogenous wild-type PTEN tagged with green fluorescent protein. Fluorescent PTEN is noticeable in both the cytosolic and nuclear compartments in the cell. This micrograph demonstrates predominant nuclear localization.
PTEN at K289 results in its nuclear import, whereas polyubiquitylation causes proteasome-mediated degradation. The same group identified NEDD4-1 as the E3 ubiquitin ligase responsible, showing that it negatively regulates PTEN (Wang et al., 2007). They speculate that the NEDD4-1–PTEN relationship is similar to that between p53 and MDM2 – with a subtle twist. The p53 protein has a short half-life and its levels are regulated by inhibition of degradation. By contrast, PTEN is a relatively stable protein, so expression levels might be modulated by acceleration of degradation. Monoubiquitylation of PTEN by NEDD4-1 would lead to nuclear localization of PTEN, cell cycle arrest and genomic stability; however, polyubiquitylation of PTEN would lead to degradation and a loss of its tumor-suppressor activity.

Denning and co-workers recently identified a cytoplasmic localization signal (CLS) in the N-terminal domain of PTEN (Denning et al., 2007). Mutation of any residue, except residue 22, within the short N-terminal motif spanning residues 19-25 results in increased nuclear localization of PTEN. The CLS might function as either a CLS or a non-classical NES; however, the mechanism remains elusive.

The variety of mechanisms for the nuclear import and regulation of PTEN that have been identified suggests that the mechanism(s) by which PTEN enters the nucleus might be both cell-type-specific and situation-specific (Fig. 3). There appears to be a delicate interplay between the stability/degradation of the PTEN protein and its subcellular localization. Future investigations into these regulatory mechanisms should provide exciting avenues of investigation and might identify potential therapeutic targets given the localization of PTEN in normal and cancerous cells.

Functions of nuclear PTEN

The lipid phosphatase activity of PTEN regulates AKT phosphorylation and p27 expression levels, whereas its protein phosphatase activity regulates MAPK phosphorylation and cyclin D1 expression (Weng et al., 2002; Weng et al., 2001). This bifurcation of effects is further complicated by the alterations in subcellular localization of PTEN that have been observed (Fig. 4).

In MCF-7 cells expressing wild-type or a nuclear-localization mutant of PTEN, levels of phosphorylated AKT (AKT-\(\text{\text{p}}\)) decrease and p27\(^{\text{\text{kip1}}}\) levels increase (Chung and Eng, 2005). This indicates that these downstream targets of PTEN activity are regulated by cytoplasmic PTEN. By contrast, expression of PTEN capable of nuclear localization results in a decrease in MAPK phosphorylation and cyclin D1 levels, but expression of a PTEN nuclear-localization-defective mutant has no effect. These results suggest that PTEN that is specifically localized in the nuclear compartment regulates these two signaling events. Nuclear PTEN also induces a G0-G1 arrest in these cells: cells expressing wild-type PTEN protein exhibit a slower growth rate and higher G1:S ratio than cells expressing the nuclear-localization-defective mutant. This increase in the number of cells in G0-G1 phase is probably a direct result of downregulation of cyclin D1 by nuclear PTEN. It might lead to decreased tumor growth, explaining another aspect of PTEN tumor-suppressor function (Chung et al., 2006; Denning et al., 2007; Trotman et al., 2007).

Recent work has described another exciting role for nuclear PTEN: maintaining chromosomal stability (Shen et al., 2007). Fluorescence in situ hybridization (FISH) analysis of Pten-deficient mouse embryo fibroblasts (MEFs) demonstrated spontaneous chromosomal instability in virtually all metaphase spreads examined. The chromosomal alterations included breaks, fragments, translocations and fusions, with a high level of centromeric instability. Further analysis led to the discovery of a physical association between PTEN and centromere-specific binding protein C (CENP-C) required for centromere stability. It also determined that PTEN is necessary and sufficient for induction of RAD51, which leads to double-stranded break (DSB) repair. This work was supported by patient data: PTEN protein from a CS patient with an R189X mutation (Eng, 2003) does not bind to CENP-C. Expression of the R189X mutant in wild-type MEFs results in numerous chromosomal alterations, indicating that this can induce massive chromosomal abnormalities in normal cells. These results have obvious implications for the development of potential anti-cancer therapies because many chemotherapeutic agents act through DNA-damage repair mechanisms.

PTEN might also affect apoptosis. In U87MG human glioblastoma cells, it appears to accumulate in the nucleus following the induction of apoptosis by TNF\(\alpha\) or doxorubicin (Gil et al., 2006). Following the treatment of cells with TNF\(\alpha\), the percentage of cells displaying condensed nuclei, a hallmark of apoptosis, is significantly higher in cells that have predominantly nuclear PTEN than those that have predominantly cytoplasmic PTEN. These effects do not depend on PTEN catalytic activity because expression of the phosphatase-dead mutants C124S and G129E does not alter the results. By contrast, studies in MCF-7 cells have determined that cytoplasmic PTEN is pro-apoptotic (Chung and Eng, 2005): expression of wild-type or a nuclear-localization-deficient PTEN increases the activity of caspase 3/7 compared with untransfected cells. Again, the contradictory studies used different cell types and different background levels of endogenous PTEN. Nevertheless, these results suggest that the equilibrium between nuclear and cytoplasmic PTEN might be important in the regulation of apoptosis.

Undoubtedly, future studies will shed more light on the roles of both cytoplasmic and nuclear PTEN in the regulation of carcinogenesis. It is interesting to note that these preliminary studies seem to indicate that the lipid phosphatase activity of PTEN predominates in the cytoplasmic compartment, whereas the protein phosphatase activity is generally nuclear. Whether this is a general
mechanism remains to be examined. Clearly, future investigations will identify additional functions of PTEN that depend on its subcellular localization.

**Perspectives**

Investigations of the role of nuclear PTEN are still in their infancy; yet we have begun to reveal how subcellular localization of PTEN might modulate its activity. Accumulating evidence suggests that there are multiple mechanisms of PTEN nuclear import that depend on the cell type and cellular environment. It is clear that the role of nuclear PTEN is not the same as that of cytoplasmic PTEN. Nuclear PTEN plays a strong role in regulating cellular homeostasis and stability. It contributes to chromosome stability, DNA repair and cell cycle arrest. Loss of nuclear PTEN results in chromosomal alterations and unregulated cell cycling (Fig. 4). Tipping the localization balance from nuclear to cytoplasmic PTEN clearly affects its tumor-suppressor function, which can, at worst, lead to the development of neoplasia.

Whereas somatic PTEN mutations in tumors are relatively rare, germline PTEN mutations are found in ~85% of CS patients (Eng, 2003; Marsh et al., 1998). Although these mutations have been documented in the exonic, intronic and promoter regions of the protein (Waite and Eng, 2002), to date, no mutation has been shown to result in mis-localization of the PTEN protein among 2200 research subjects (C.E., K.A.W. and Tammy M. Sadler, unpublished). Such a mutation might be embryonic lethal given the important role of nuclear PTEN in cell proliferation; however, this remains to be seen. Interestingly, changes in nuclear PTEN protein are observed in tumors that have somatic mutations, which supports the idea that germline mutations that result in altered PTEN localization are lethal.

It is interesting to note that, in a subset of patients, no coding, promoter or deletion mutations in PTEN are apparent. Alternative mechanisms of PTEN dysregulation, such as alterations in subcellular localization, that result in clinical manifestations must therefore exist. Continued examination of samples from CS patients will undoubtedly uncover novel mutations that reveal additional mechanisms of PTEN localization. How these mechanisms interact dynamically will be essential to determine. Additional functions will certainly be uncovered and these should help us further elucidate the importance of this protein in human health and disease.

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**References**

Boulikas, T. (1993). Nuclear localization signals (NLS). *Crit. Rev. Eukaryot. Gene Expr.* 3, 193-227.

Brenner, W., Farber, G., Herget, T., Lehr, H. A., Hengstler, J. G. and Thuroff, J. W. (2002). Loss of tumor suppressor protein PTEN during renal carcinogenesis. *Int. J. Cancer* 99, 53-57.

Chung, J. H. and Eng, C. (2005). Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. *Cancer Res.* 65, 8096-8100.

Chung, J. H., Ginn-Pease, M. E. and Eng, C. (2005). Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) has nuclear localization signal-like sequences for nuclear import mediated by major vault protein. *Cancer Res.* 65, 4108-4116.

Chung, J. H., Ostrowski, M. C., Romigh, T., Minaguchi, T., Waite, K. A. and Eng, C. (2006). The ERK1/2 pathway modulates nuclear PTEN-mediated cell cycle arrest by cyclin D1 transcriptional regulation. *Hum. Mol. Genet.* 15, 2553-2559.

Denning, G., Jean-Joseph, B., Prince, C., Durden, D. L. and Vogt, P. K. (2007). A short N-terminal sequence of PTEN controls cytoplasmic localization and is required for suppression of cell growth. *Oncogene* 26, 3930-3940.

Depowski, P. L., Rosenthal, S. I. and Ross, J. S. (2001). Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. *Cancer Res.* 61, 5348-5353.

Dreher, T., Zentgraf, H., Abel, U., Kappeler, A., Michel, M. S., Bleyl, U. and Grobholz, R. (2004). Reduction of PTEN and p27kip1 expression correlates with tumor grade in prostate cancer. *Anal. Prostate cancer.* 15, 22134-22139.
Eng, C. (2003). PTEN: one gene, many syndromes. *Hum. Mutat.* 22, 183-198.

Furutani, F. B., Lin, H., Huang, H. S. and Cavenee, W. K. (1997). Growth suppression of glialoma cells by PTEN requires a functional phosphatase catalytic domain. *Proc. Natl. Acad. Sci. USA* 94, 12479-12484.

Gil, A., Andretis-Pons, A., Fernandez, E., Valiente, M., Torres, J., Cervera, J. and Pulido, R. (2006). Nuclear localization of PTEN by a Ran-dependent mechanism enhances apoptosis: Involvement of an N-terminal nuclear localization domain and multiple nuclear exclusion motifs. *Mol. Biol. Cell* 17, 4002-4013.

Gimm, O., Perren, A., Weng, L. P., Marsh, D. J., Yeh, J. J., Ziebold, U., Gil, E., Hinze, R., Debriide, L., Lees, J. A. et al. (2000). Differential nuclear and cytoplasmic expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. *Am. J. Pathol.* 156, 1693-1700.

Ginn-Pease, M. E. and Eng, C. (2003). Increased nuclear phosphatase and tensin expression of PTEN in normal thyroid tissue, and benign and malignant epithelial thyroid tumors. *Am. J. Pathol.* 156, 1693-1700.

Kau, T. R., Way, J. C. and Silver, P. A. (2004). Nuclear transport and cancer: from mechanism to intervention. *Nat. Rev. Cancer* 4, 106-117.

Levers, S. J., Vanhaesebroeck, B. and Waterfield, M. D. (1999). Signalling through phosphoinositide 3-kinases: the lipids take centre stage. *Curr. Opin. Cell Biol.* 11, 219-225.

Li, D. M. and Sun, H. (1997). TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. *Cancer Res.* 57, 2124-2129.

Li, D. M. and Sun, H. (1998). PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells. *Proc. Natl. Acad. Sci. USA* 95, 15406-15411.

Li, M., Brooks, C. L., Wu-Baer, F., Chen, D., Baer, R. and Gu, W. (2003). Mono- versus polyubiquitination: differential control of p53 fate by Mdm2. *Science* 302, 1972-1975.

Liang, S. H. and Clarke, M. F. (1999). A bipartite nuclear localization signal is required for p53 nuclear import regulated by a carboxyl-terminal domain. *J. Biol. Chem.* 274, 32699-32703.

Lin, F., Wagner, S., Campbell, R. B., Nickerson, J. A., Schiffer, C. A. and Ross, A. H. (2005). PTEN enters the nucleus by diffusion. *J. Cell. Biochem.* 96, 221-234.

Marsh, D. J., Coulson, V., Lunetta, K. L., Rocca-Serra, P., Dabhi, P. L., Zheng, Z., Liaw, D., Caron, S., Duboue, B., Lin, A. Y. et al. (1998). Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germ line PTEN mutation. *Hum. Mol. Genet.* 7, 501-515.

Massoumi, R., Chmielarska, K., Neennecke, K., Pfeifer, A. and Fassler, R. (2006). Cyld inhibits tumor cell proliferation by blocking Bcl-3 dependent NF-kappaB signaling. *Cell* 128, 665-677.

Minaguchi, T., Waite, K. A. and Eng, C. (2006). Nuclear localization of PTEN is regulated by Cap2 by through a tyrosyl phosphorylation-independent conformational modification in major vault protein. *Cancer Res.* 66, 1167-11682.

Mossink, M. H., van Zon, A., Scheper, R. J., Sonneveld, P. and Wiemer, E. A. (2003). Vaults: a ribonucleoprotein particle involved in drug resistance? *Oncogene* 22, 7458-7467.

Myers, M. P., Stolarov, J. P., Eng, C., Li, J., Wang, S. I., Wigler, M. H., Parsons, R. and Tonks, N. K. (1997). PTEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc. Natl. Acad. Sci. USA* 94, 9052-9057.

Pezzolesi, M. G., Li, Y., Zhou, X. P., Pilarski, R., Shen, L. and Eng, C. (2006). Mutation-positive and mutation-negative patients with Cowden and Bannayan-Riley-Ruvalcaba syndromes associated with distinct 10q haplotypes. *Am. J. Hum. Genet.* 79, 923-934.

Pilakka, S. M., Pilafki, K. S., Wetsomam, A. M. and Macara, I. G. (2004). Ubiquitin charging of human class II ubiquitin-conjugating enzymes triggers their nuclear import. *J. Cell Biol.* 167, 649-659.

Sano, T., Lin, H., Chen, X., Langford, L. A., Koul, D., Bondy, M. L., Hess, K. R., Myers, J. N., Hong, Y. K., Yung, W. K. et al. (1999). Differential expression of MMAC1/PTEN in glioblastoma multiforme: relationship to localization and prognosis. *Cancer Res.* 59, 1820-1824.

Shen, W. H., Balajee, A. S., Wang, J., Wu, H., Eng, C., Pandolfi, P. P. and Yin, Y. (2007). Essential role for nuclear PTEN in maintaining chromosomal integrity. *Cell* 128, 157-170.

Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R. and Yamada, K. M. (1998). Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* 280, 1614-1617.

Teresi, R. E., Zhuk, K. M., Pezzolesi, M. G., Waite, K. A. and Eng, C. (2007). Cowden syndrome-affected patients with PTEN promoter mutations demonstrate abnormal protein translation. *Am. J. Hum. Genet.* 81, 756-767.

Tromtan, L. C., Wang, X., Alimonti, A., Chen, Z., Terausa-Feldstein, J., Yang, H., Peklich, N. P., Carver, B. S., Cordon-Cardo, C., Erdjument-Bromage, H. et al. (2007). Ubiquitination regulates PTEN nuclear import and tumor suppression. *Cell* 128, 141-156.

Vazquez, F., Ramaswamy, S., Nakamura, N. and Sellers, W. R. (2000). Phosphorylation of the PTEN tail regulates protein stability and function. *Mol. Cell. Biol.* 20, 5010-5018.

Waite, K. A. and Eng, C. (2002). Protein PTEN: form and function. *Am. J. Hum. Genet.* 70, 829-844.

Wang, X., Tromtan, L. C., Koppie, T., Alimonti, A., Chen, Z., Gao, Z., Wang, J., Erdjument-Bromage, H., Tempst, P., Cordon-Cardo, C. et al. (2007). NEDD4-1 is a proto-oncogenic ubiquitin ligase for PTEN. *Cell* 128, 129-139.

Weng, L. P., Brown, J. L. and Eng, C. (2001). PTEN coordinates G1 arrest by down-regulating cyclin D1 via its protein phosphatase activity and up-regulating p27 via its lipid phosphatase activity in a breast cancer model. *Hum. Mol. Genet.* 10, 599-604.

Weng, L. P., Brown, J. L., Baker, K. M., Ostrowski, M. C. and Eng, C. (2002). PTEN blocks insulin-mediated ETS-2 phosphorylation through MAP kinase, independently of the phosphoinositide 3-kinase pathway. *Hum. Mol. Genet.* 11, 1687-1696.

Whang, Y. E., Wu, X., Suzuki, H., Reiter, R. E., Tran, C., Vessella, R. L., Said, J. W., Isaacs, W. B. and Sawyer, C. L. (1998). Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. *Proc. Natl. Acad. Sci. USA* 95, 1972-1975.

Whitehead, D. C., Zhou, X. P., Cummings, M. C., Pavey, S., Hayward, N. K. and Eng, C. (2002). Nuclear PTEN expression and clinicopathologic features in a population-based series of primary cutaneous melanoma. *Int. J. Cancer* 99, 63-67.

Yu, Z., Fotouhi-Ardakani, N., Wu, L., Maoui, M., Wang, S., Banville, D. and Shen, S. H. (2002). PTEN associates with the vault particles in HeLa cells. *J. Biol. Chem.* 277, 40247-40252.

Zbinden, K. M. and Eng, C. (2007). Hamartomatic polyposis syndrome. *Nat. Clin. Pract. Gastroenterol. Hepatol.* 4, 502-507.