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Tenets of PTEN Tumor Suppression

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Since its discovery as the elusive tumor suppressor gene at the frequently mutated 10q23 locus, *PTEN* has been identified as lost or mutated in several sporadic and heritable tumor types. A decade of work has established that PTEN is a nonredundant phosphatase that is essential for regulating the highly oncogenic prosurvival PI3K/AKT signaling pathway. This review discusses emerging modes of PTEN function and regulation, and speculates about how manipulation of PTEN function could be used for cancer therapy.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is one of the most frequently mutated tumor suppressor genes in human cancer. *PTEN* was first discovered by independent groups and recognized as the frequently lost tumor suppressor gene on human chromosome 10q23, a locus that is highly susceptible to mutation in primary human cancers (Li et al., 1997; Steck et al., 1997). The frequency of monoallelic mutations at this locus has been estimated at 50%–80% in sporadic tumors (including endometrial carcinoma, glioblastoma, and prostate cancer) and at 30%–50% in breast, colon, and lung tumors. Complete loss of *PTEN* is observed at highest frequencies in endometrial cancer and glioblastoma and is generally associated with advanced cancers and metastases (Ali et al., 1999). A recent landmark study reveals that *PTEN* loss is a common event in breast cancers caused by *BRCA1* deficiency (Saal et al., 2008).

The importance of PTEN as a tumor suppressor is further supported by the study of *PTEN* germline mutations in a group of autosomal dominant syndromes characterized by developmental disorders, neurological deficits, multiple hamartomas, and an increased risk of breast, thyroid, and endometrial cancers. Collectively, these are referred to as the PTEN hamartoma tumor syndromes (PHTS), which include Cowden syndrome, Lhermitte-Duclos disease, Bannayan-Riley-Ruvalcaba syndrome, and Proteus and Proteus-like syndromes. Various mouse models in which *Pten* is deleted also demonstrate the crucial role of PTEN as a tumor suppressor in multiple tumor types (Di Cristofano et al., 1998; Podsypanina et al., 1999; Suzuki et al., 1998; Trotman et al., 2003; Wang et al., 2003).

Functionally, PTEN is a nonredundant, plasma-membrane lipid phosphatase that antagonizes the phosphatidylinositol-3-kinase (PI3K) signaling pathway (Maehama and Dixon, 1998; Stambolic et al., 1998). Upon stimulation of cells with growth stimuli, class I PI3K family members catalyze the conversion of phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol 3,4,5-trisphosphate (PIP_3), a second messenger that promotes survival, growth, and proliferation. Specifically, PTEN hydrolyzes the 3-phosphate on PIP_3 to generate PIP_2, and thereby negatively regulates PIP_3-mediated downstream signaling. Through its role in phosphatidylinositol homeostasis, PTEN is implicated in cell polarity and migration and thereby provides a potential link between outer membrane phospholipids and pathways that lead to cytoskeleletal reorganization (reviewed in Franca-Koh et al., 2007; see also the SnapShot by A. Carracedo, L. Salmena, and P.P. Pandolfi on the last page of this issue).

Upon *PTEN* loss, PIP_3 accumulates and promotes the recruitment of a subset of proteins that contain a pleckstrin homology domain to cellular membranes, including the serine/threonine kinases AKT1, AKT2, AKT3, and PDK1. Once positioned at cell membranes, AKT isoforms are activated by phosphorylation at two different residues. AKT is thought to be phosphorylated by PDK1 on Thr308 and by the mTOR kinase complex 2 (mTORC2) on Ser473 (as reviewed in Guertin and Sabatini, 2007; Manning and Cantley, 2007). Termination of AKT signaling is thought to be elicited by the protein phosphatase PHLIPP, which directly dephosphorylates phospho-Ser473 on AKT (Brognard et al., 2007; Gao et al., 2005). Activation of AKT kinases promotes cell survival, proliferation, growth, angiogenesis, and cellular metabolism through phosphorylation of myriad cellular substrates including MDM2, GSK3, FOXO, BAD, CASP9, and p27 (Manning and Cantley, 2007). AKT activation also leads to activation of the mTOR kinase complex 1 (mTORC1) through an inhibitory phosphorylation of the TSC tumor suppressor complex and consequent activation of RHEB, a Ras-related small GTPase (Guertin and Sabatini, 2007). As a consequence of PTEN inactivation, activation of mTORC1 in turn leads to enhanced translation of mRNA into protein, a hallmark of many cancers (Tee and Blenis, 2005). In addition to being repressed by a number of tumor suppressor genes directly implicated in human cancer, the mTOR arm of the PTEN/PI3K/AKT pathway is emerging as an effective target for anti-cancer agents, especially in tumors in which the activity of the mTOR pathway is elevated (Faivre et al., 2006; Guertin and Sabatini, 2007).

Many important publications have expertly reviewed the wide spectrum of mutations found in the *PTEN* gene and the multitude of tumor types that arise upon loss of PTEN activity. Also, the contribution of *PTEN* loss to activation of the PI3K/AKT signaling pathway and consequent tumorigenesis is well established. In this review, we present emerging tenets for the regulation of the *PTEN* gene and PTEN protein, discuss uncon-

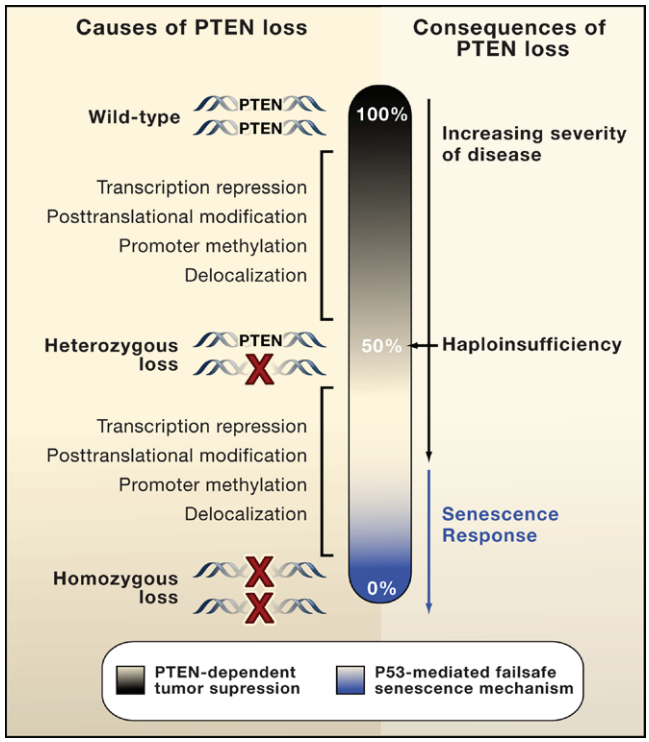

Figure 1. A Continuum of Functional PTEN Loss

We propose that loss of PTEN function by various molecular mechanisms can create gradations of tumor suppression. PTEN function can be compromised via genetic mutations, which result in a stepwise loss of PTEN function to produce states where there is either 50% (heterozygous) or 100% (homozygous) loss. Mechanisms including transcriptional repression, posttranslational modification, epigenetic silencing, and aberrant localization of PTEN can cause subtle and/or dramatic losses of PTEN functionality. Overall, a combination of these events produces a continuum of functional PTEN loss. *Pten* is haploinsufficient for tumor suppression in mice; therefore, 50% of total PTEN is not sufficient for tumor suppression. Whether this is the case in humans remains to be determined. When PTEN levels approach complete loss of expression, cells with intact p53 signaling trigger a strong cellular senescence program, thus acting as a brake on tumor progression.

ventional downstream effectors and pathways of PTEN function, and introduce new potential strategies for PTEN-associated cancer therapies.

Functional PTEN Dose Dictates Cancer Susceptibility

The classical premise of tumor suppression asserts that both copies of a given tumor suppressor gene must be lost for cancer to arise (Knudson, 1971). Although this is definitely the case in certain circumstances and in particular tissue types (such as in a large fraction of retinoblastoma [RB] lesions that experience homozygous loss of the *RB* gene), heterozygous loss of other tumor suppressor genes can have critical biological consequences toward cancer initiation and progression.

Loss of only one allele of *Pten* in mice has been shown to promote the progression of a lethal polyclonal autoimmune disorder with high penetrance (Di Cristofano et al., 1999), thereby suggesting that Pten is functionally haploinsufficient (that is that one functional allele is not enough to sustain a wild-type condition). Similarly, *Pten* heterozygosity appears to be the driving force for epithelial cancers, such as prostate cancer, in mouse models of *Pten* loss (Di Cristofano et al., 2001). Moreover, our group has demonstrated that cellular levels of Pten protein inversely correlate with the occurrence of invasive prostate cancer (Figure 1). This was established by generating a so-called "hypomorphic *Pten* allelic series" in the mouse where *Pten* dose is progressively decreased below heterozygous levels (Trotman et al., 2003). This suggests that *Pten* is a haploinsufficient tumor suppressor gene in specific mouse tissues.

Despite evidence from mouse models, whether *PTEN* is a haploinsufficient tumor suppressor gene in humans remains to be determined. To date, there are only a handful of reports that provide support for this notion. By definition, *PTEN* is haploinsufficient for the development of PTEN hamartoma tumor syndrome, given that heterozygosity leads to characteristic phenotypes, including various developmental disorders and benign polyps. With respect to its role as a tumor suppressor, the increased susceptibility of patients with PTEN hamartoma tumor syndrome to develop tumors may be consistent with haploinsufficiency. Further support for haploinsufficiency is provided by the observation that some tumors derived from patients with Cowden syndrome do not have detectable biallelic mutation of the *PTEN* gene (Dahia, 2000; Marsh et al., 1998). Moreover, primary prostate tumors often show loss or alteration of one *PTEN* allele at presentation (as in 70%–80% of cases of primary prostate cancer [Gray et al., 1998; Whang et al., 1998]) whereas homozygous inactivation is observed at much lower frequencies. Similarly in breast cancer, there is a lack of concordance between the occurrence of monoallelic mutation of PTEN (30%–40%) and the occurrence of biallelic loss (5%) (Ali et al., 1999; Bose et al., 1998; Feilotter et al., 1999). Indeed, complete loss of PTEN is observed in many advanced cancers. However, the observation that monoallelic mutation of *PTEN* without loss or mutation of the second allele is prevalent in breast and prostate cancer lesions is consistent with the notion that monoallelic loss of *PTEN* is sufficient for tumor initiation and progression. Overall, the question of *PTEN* haploinsufficiency remains an important one, and the analysis of more tumor samples is required to clarify whether, and in which types of human tissue, *PTEN* haploinsufficiency is critical.

In line with the notion that *PTEN* haploinsufficiency contributes to tumor progression, there are indications that even a minor impairment in *PTEN* function may lead to the development of cancer. This is illustrated by the identification of Cowden syndrome and tumor-derived *PTEN* mutations that preserve partial or even full PTEN lipid phosphatase function (Waite and Eng, 2002). For example, some C2-domain mutations in PTEN identified in Cowden syndrome as well as other somatic mutations that produce C-terminal truncations retain phosphatase activity in biochemical assays (Han et al., 2000; Waite and Eng, 2002). These truncations ultimately affect PTEN phosphorylation, stability and protein-protein interactions by deletion of phosphorylated residues and the PDZ domain. Moreover, N-terminal mutants are thought to influence PTEN stability yet maintain catalytic activity (Han et al., 2000), and point mutations of the central C2 domain have been demonstrated to impact proper PTEN localization (Trotman et al., 2007). Nevertheless, it must be noted that even though partially functional mutants do indeed exist, the major-

ity of *PTEN* mutations are believed to profoundly inhibit catalytic activity. In conclusion, that a partially functional allele of *PTEN* exists in heterozygosity in disorders that confer increased cancer susceptibility (as observed in PTEN hamartoma tumor syndrome) may point toward a critical role for slight and selective functional impairment of PTEN in tumor susceptibility.

This notion is timely and particularly relevant given that mechanisms for *PTEN* control at the transcriptional and posttranslational levels may be altered in disease. Moreover, mutants of *PTEN* that maintain partial function may have a selective advantage over mutants that confer a complete loss of function, because complete loss of PTEN induces the activation of a p53-dependent cellular senescence response, as discussed below (Chen et al., 2005).

PTEN and Cellular Senescence

While studying the relationship between *Pten* dose and tumor progression in mouse models of prostate specific loss of *Pten*, Chen et al. (2005) observed an unexpected and intriguing phenomenon: complete acute loss of *Pten* did not provide a proliferative advantage as would be expected, but instead promoted a strong senescence response that opposed tumor progression. Senescence, a cellular program that triggers an irreversible growth arrest and limits the replicative life span of cultured primary cells, has been proposed to function as an anti-tumor mechanism set off by tumor suppressor genes in response to triggers including DNA damage and oncogene activation (Campisi and d'Adda di Fagagna, 2007).

Mice with conditional inactivation of *Pten* in the prostate develop invasive cancer; however early-stage tumor development has been associated with slow growth, increased p53 levels, and cellular senescence. As predicted from these findings, combined inactivation of *Pten* and *Trp53* leads to unconstrained tumor growth as demonstrated by the generation of massive invasive prostate tumors. This implies that complete ablation of PTEN can be detrimental to tumor growth in the absence of other mutations and highlights the importance of haploinsufficiency or partial PTEN impairment in tumor progression (Figure 1). Clinically, these findings provide an explanation as to why complete *PTEN* loss is not frequently observed at cancer presentation and, importantly, imply that PTEN-deficient prostate cancer may benefit from drugs that can promote p53 activation and enhancement of p53-dependent cellular senescence.

Regulating the PTEN Message

Genetic loss or mutation of tumor suppressor genes is a frequent event initiating and/or promoting tumorigenesis (Vogelstein and Kinzler, 2004). Tumour suppressor genes are also subject to countless regulatory mechanisms including epigenetic effects, transcriptional modulation, and posttranscriptional and posttranslational modifications that ultimately govern protein levels, activity, localization, binding partners, and function. Disrupted tumor suppressor regulation by one or more of these mechanisms may also have catastrophic consequences for a cell.

In addition to mutations that partially or fully inactivate a given *PTEN* allele, emerging evidence shows that complete or partial loss of PTEN protein expression, through as-yet-unidentified mechanisms, can impact tumor suppression (Figure 2A). For instance, spontaneous cancers that harbor monoallelic mutations of *PTEN* possess at least one functional wild-type *PTEN* allele, yet they further or completely lose PTEN protein immunoreactivity in the absence of detectable mutations of the remaining *PTEN* allele (Leupin et al., 2003; Mutter et al., 2000; Shi et al., 2003; Zhou et al., 2002). These observations imply that epigenetic silencing by aberrant promoter methylation, deregulated transcription, increased degradation, and/or mislocalization of the PTEN protein may disrupt function and promote tumorigenesis. Examples of these consequences are highlighted below.

Transcriptional Regulation

Due to its robust expression levels and long cellular half-life, PTEN is thought to be constitutively expressed and minimally regulated in normal tissues. This notion is in fact misleading given that the exact pattern of regulation of PTEN during development and adult life is unclear. Furthermore, the stability of PTEN may be altered dramatically in pathological settings (Figure 2B).

PTEN was originally cloned as a gene transcriptionally regulated by transforming growth factor β (TGFβ) Li and Sun, 1997). Since this discovery, numerous factors have been demonstrated to upregulate PTEN transcription including the peroxisome proliferation-activated receptor γ (PPARγ) (Patel et al., 2001), and the early growth-regulated transcription factor-1 (EGR-1) (Virolle et al., 2001), which functions downstream of insulin-like growth factor 2 (IGF-2) (Moorehead et al., 2003). Additionally, Stambolic et al. (2001) identified a putative p53-binding element in the promoter sequence of *PTEN* and characterized a p53-mediated cellular survival mechanism that functions through the activation of PTEN transcription. Induction of PTEN may be an important mechanism by which its presence is ensured when it is required to perform its tumor suppressive function. Unlike the tumor suppressor p53, which is acutely and rapidly upregulated in response to potentially oncogenic stresses, PTEN expression is constitutive and essential at all times.

As previously mentioned, suppression of *PTEN* transcription may have an important and underestimated role in cancer (Figure 2B). Indeed, recent studies have demonstrated a link between the oncogenic RAS-MAPK pathway and aberrant transcriptional downregulation of *PTEN* in both fibroblast and epithelial cell types and in human cancer cells. Chow et al. (2007) attribute RAS-mediated PTEN suppression to a TGFβ-dependent mechanism in pancreatic adenocarcinoma and Vasudevan et al. (2007) demonstrate that the oncogenic RAS-RAF-MEK-ERK pathway suppresses PTEN levels through the transcriptional factor, c-Jun (Hettinger et al., 2007). Moreover, other stress kinase pathways including MEKK4 and JNK promote resistance to apoptosis by suppressing PTEN transcription via direct binding of NFκB to the *PTEN* promoter (Xia et al., 2007). Conversely, PTEN was demonstrated to oppose the JNK pathway (Vivanco et al., 2007). This finding suggests that pathways that are negatively regulated by PTEN can in turn regulate PTEN transcription, thereby identifying a new and potentially important feedback loop.

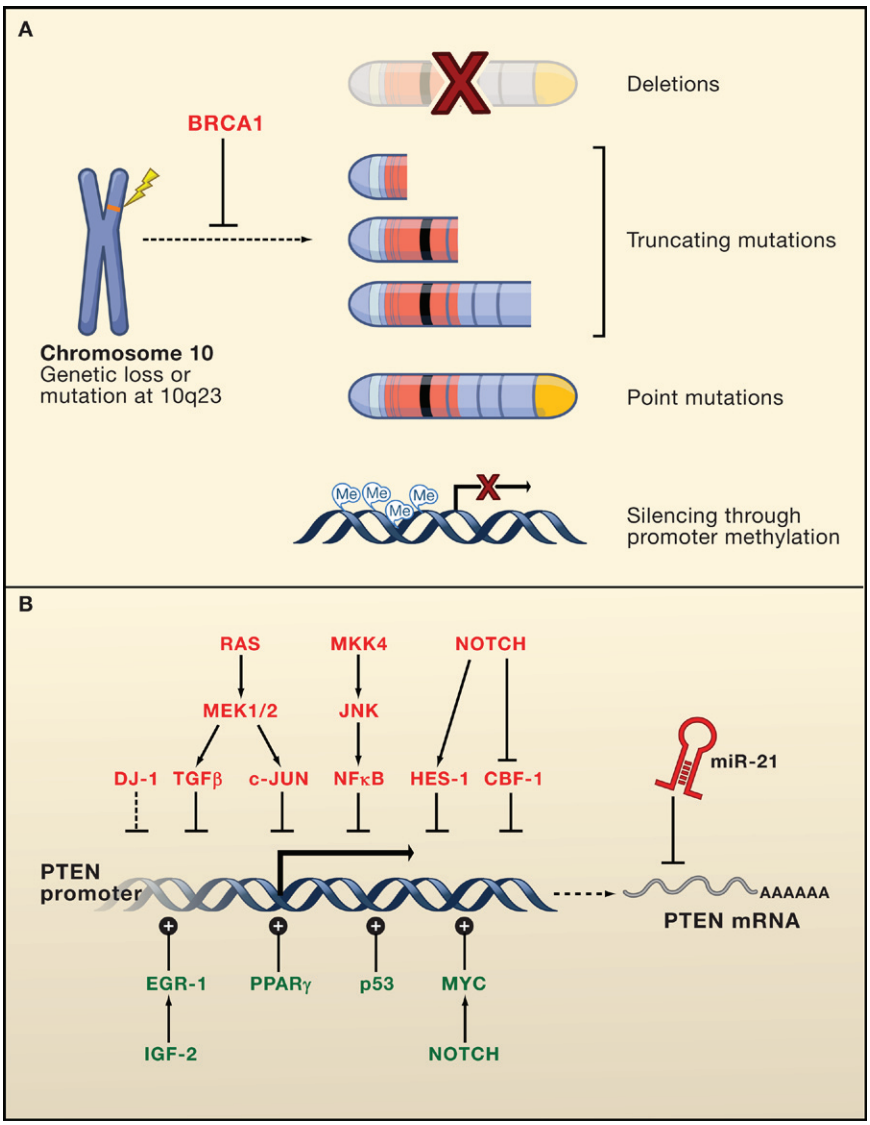

Figure 2. Genetic Alterations and Transcriptional Regulation of *PTEN*

(A) *PTEN* was first identified as a tumor suppressor gene frequently lost on human chromosome 10q23, a locus that is highly susceptible to mutation in primary human cancers and cancer cell lines. Recently, PTEN was found to be mutated in breast cancers characterized by the loss of the DNA repair protein BRCA1 (Saal et al., 2008). Allelic losses can result in complete deletion of the *PTEN* locus; point mutations can produce several PTEN truncation mutants and functional inactivation of PTEN. Several tumor-derived PTEN mutants retain partial or complete catalytic function, suggesting that alternate mechanisms can lead to inactivation of PTEN; for example, the K289E mutation alters the localization of the PTEN protein. PTEN silencing has also been demonstrated to occur through promoter methylation and promoter mutation.

(B) Numerous genes regulate PTEN transcription both positively (EGR-1, PPARγ, Myc, and p53) and negatively (NFκB, c-Jun, HES, and TGFβ signaling). NOTCH1 may be able to activate or repress PTEN transcription depending on the cellular context. Recently, *miR-21* was identified as the first microRNA to regulate the expression of *PTEN*.

Finally, recent studies have identified new pathways for PTEN regulation occurring downstream of NOTCH1. Active NOTCH1 has been reported to increase *PTEN* transcription through mechanisms involving the CBF-1 transcription factor (Chappell et al., 2005; Whelan et al., 2007) and MYC (Palomero et al., 2007). On the other hand, NOTCH1 activation has also been demonstrated to repress *PTEN* transcription through the HES-1 transcription factor (Palomero et al., 2007). The function of NOTCH1 on *PTEN* transcription appears to be complex and multifactorial and is most likely tissue specific. Palomero et al. (2007) have implicated PTEN in the repression of the leukemogenic program promoted by NOTCH1 activation. This work provides the basis for the design of new therapeutic strategies for NOTCH1-related leukemia. Taken together, these findings suggest that the transcriptional control of *PTEN* lies at a key position in a complex network of tumor suppressors and oncogenes that regulates cellular transformation (Figure 2B).

Epigenetic silencing by promoter methylation has also been proposed as a process by which PTEN expression can be suppressed in various types of cancer (Garcia et al., 2004; Goel et al., 2004; Kang et al., 2002; Mirmohammadsadegh et al., 2006) (Figure 1A). These findings must be interpreted with caution given that the promoter of a PTEN pseudogene has been shown to be methylated (Zysman et al., 2002).

PTEN Regulation by MicroRNAs

MicroRNAs (miRNAs) are endogenous short single-stranded RNAs (approximately 22 nucleotides in length) that repress mRNA translation by base-pairing to sequences located in the 3′UTR of target mRNAs. Recently, PTEN was reported to be repressed by the miRNA *miR-21* (Meng et al., 2006, 2007). Importantly, *miR-21* is one of the most frequently upregulated miRNAs in cancer and has been shown to promote proliferation in glioblastoma and to inhibit apoptosis in breast cancer cell lines (Chan et al., 2005; Si et al., 2007; Volinia et al., 2006). These studies suggest that *miR-21* exerts its oncogenic activity in part through the downregulation of *PTEN* expression. Although this particular field is in its infancy, it is exciting to envision that fine-tuners of gene expression such as miRNAs may influence cancer through *PTEN* regulation.

Phosphorylation: The C-Terminal End Rules

Disrupted PTEN protein stability represents an attractive yet elusive mechanism underlying its loss in tumors. Numerous reports suggest that PTEN stability is regulated by its interaction with other proteins and that it is subject to various post-translational modifications (Figure 3A). For instance, phospho-

rylation of PTEN at specific residues in its C-terminal tail have been associated with increased stability (Georgescu et al., 1999; Torres and Pulido, 2001; Vazquez et al., 2000), whereas phosphorylation at other sites may decrease protein stability (Maccario et al., 2007). Furthermore, phosphorylation of PTEN results in decreased catalytic activity toward lipid substrates, which may be explained by changes in its ability to interact with membranes. In total, a cluster of six phosphorylation sites at Thr366, Ser370, Ser380, Thr382, Thr383, and Ser385 are involved in modulating PTEN tumor suppressor functions, subcellular distribution, and stability (Figure 3A). Despite the fact that Ser370 and Ser385 have been identified as the major sites for phosphorylation, their mutation has minimal effects on PTEN function, whereas mutation of the Ser380, Thr382, and Thr383 (collectively referred to as the STT) cluster shows more striking consequences for PTEN. Specifically, mutation of STT destabilizes PTEN and increases its phosphatase activity in cells compared to wild-type PTEN (Vazquez et al., 2000). It has been proposed that a phosphorylated STT cluster renders PTEN in a "closed" state, whereas their mutation to the nonphosphorylatable alanine (Ala) opens the protein conformation. This increases PTEN activity, enhances its interaction with binding partners, and in turn makes PTEN protein more unstable (Leslie and Downes, 2004). Based on this, it has been hypothesized that PTEN is normally maintained in a phosphorylated, inactive state and its activation can be mediated through dephosphorylation of the STT cluster. In general, modification of the C terminus by phosphorylation is believed to promote conformational changes of the PTEN protein (Vazquez et al., 2001), and tumor-derived C-terminal mutants of PTEN are highly susceptible to protein degradation, implying that this region acts as an autostabilizing domain.

PTEN Ubiquitination

Ubiquitin-mediated degradation of PTEN has recently been reported, suggesting an additional process whereby PTEN levels may be downregulated. As described above, PTEN contains two so-called PEST motifs characteristic of short-lived proteins that are subject to ubiquitin-mediated degradation by the proteasome. Paradoxically, PTEN is a long-lived and stable protein in normal cells; however, the PEST domains might play an important role under pathological circumstances. The first study to indicate that PTEN may be regulated through this mechanism demonstrated that the half-life of PTEN is increased by proteasome inhibition (Torres and Pulido, 2001). Thereafter, it was shown that exposure of human bronchial cells to zinc ions promoted ubiquitin-dependent degradation of PTEN (Wu et al., 2003). In contradiction, some experimental conditions demonstrate that inhibitors of the proteasome may actually destabilize PTEN protein (Tang and Eng, 2006).

Interestingly, Trotman et al. (2007) identified Lys13 and Lys289 as two major conserved sites for PTEN ubiquitination (Figure 3A) and demonstrated that ubiquitin conjugation to these sites is indispensable for the nuclear-cytoplasmic shuttling of PTEN. Additionally, by employing a biochemical screening strategy to identify E3 ligases for PTEN mono- and polyubiquitination, Wang et al. (2007) isolated the NEDD4-1 protein. They demonstrated that NEDD4-1 could physically interact with PTEN,

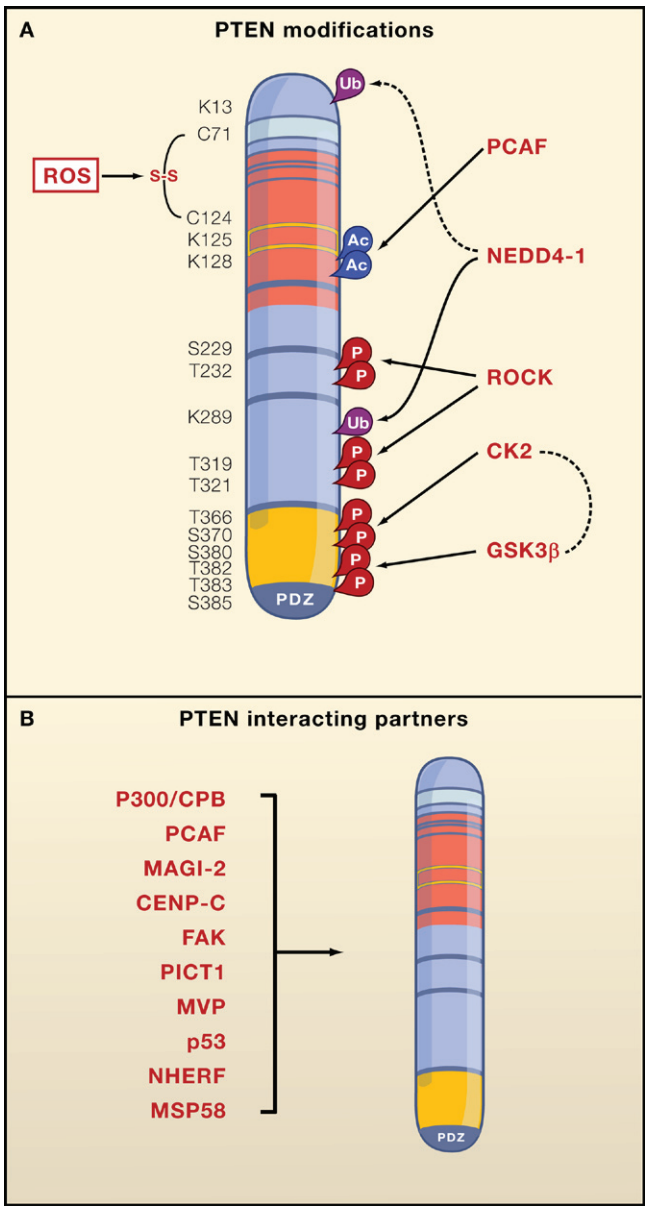

Figure 3. Mechanisms Regulating PTEN

(A) PTEN is subject to several posttranslational modifications including phosphorylation, acetylation, oxidation, and ubiquitination. Phosphorylation of PTEN at T366, S370, S380, T382, T383, and S385 in its C-terminal tail is implicated in modulating PTEN tumor suppressor functions, subcellular distribution, and stability. ROCK has also been reported to promote the localization of PTEN to the plasma membrane by phosphorylating PTEN at residues S229, T322, T319, and T321 in the C2 domain. PTEN interacts with the nuclear histone acetyltransferase-associated PCAF protein, which promotes PTEN acetylation at K125 and at K128. These modifications decrease the catalytic activity of PTEN. The catalytic activity of PTEN is also modulated by oxidation by reactive oxygen species, which induces the formation of a disulfide bond between the active site Cys124 and Cys71. Ubiquitination of PTEN has also been reported on K13 and K289. Mutation of K289 alters PTEN localization.

(B) PTEN interacting proteins include the kinases CK2, GSK3β (which can phosphorylate PTEN at the C terminus), and ROCK (which targets the C2 domain). PICT1 can also bind to the C-terminal tail of PTEN to promote its phosphorylation and stability. PTEN contains a 3 aa C-terminal region that is able to bind to proteins containing PDZ domains. The extreme C-terminal PDZ domain of PTEN mediates binding to NHERF proteins and MAGI-2, an interaction that is inhibited by C-terminal PTEN phosphorylation.

and its overexpression mediates both the mono- and polyubiquitination of PTEN (Wang et al., 2007). Perhaps inhibition of NEDD4-1 activity may provide an avenue for therapy to upregulate PTEN levels in cancer. However, to date, little is known about the regulation of NEDD4-1 nor the physiological circumstances, during which it can target PTEN for degradation. Additional human data and in vivo studies are required to validate that NEDD4-1 is a bona fide oncogene and that acts via the degradation of PTEN. Finally, whether there are other E3 ligases that target PTEN requires further exploration.

PTEN Acetylation and Oxidation

Acetylation and oxidation comprise other mechanisms by which the PTEN protein activity can be regulated (Figure 3A). For example, the interaction of PTEN with nuclear histone acetyltransferase-associated PCAF (as described below) can promote PTEN acetylation at Lys125 and Lys128, which negatively regulate the catalytic activity of PTEN (Okumura et al., 2006). Furthermore, a series of studies demonstrated that the catalytic activity of PTEN could be modulated by reactive oxygen species (ROS) (Kwon et al., 2004; Lee et al., 2002; Leslie et al., 2003; Seo et al., 2005). These studies demonstrate that PTEN activity can be abrogated by oxidative stress-induced formation of a disulfide bond between the active site Cys124 and Cys71 (Lee et al., 2002). Inactivation of PTEN was observed with either hydrogen peroxide (H_2O_2) or endogenous ROS production in macrophages, where ROS are generated in response to cellular stress and receptor activation, and is associated with an oxidant-dependent activation of downstream signaling (Kwon et al., 2004; Leslie et al., 2003). Modulation of PTEN activity by posttranslational modifications represents a provocative and therapeutically exploitable mechanism of PTEN regulation.

PTEN Interacting Proteins

Phosphorylation of the C terminus of PTEN has been attributed to the activities of casein kinase 2 (CK2) and glycogen synthase kinase 3β (GSK3β) (Al-Khouri et al., 2005; Miller et al., 2002; Torres and Pulido, 2001). The C terminus also interacts with PICT1 (protein interacting with carboxyl-terminus tail 1), which binds to the C-terminal tail of PTEN to promote its phosphorylation and stability (Okahara et al., 2004). PTEN contains a 3 aa C-terminal region that is able to bind to PDZ domain-containing proteins (Georgescu et al., 1999; Wu et al., 2000). PDZ domains are involved in the assembly of multiprotein complexes. Hence, this region may control PTEN localization and interaction with other proteins, although the deletion of these amino acids has been reported not to alter the tumor suppressive activity of PTEN (Georgescu et al., 1999). Indeed, the PDZ domain of PTEN mediates interactions with Na^+ H^+ exchanger regulatory factor (NHERF) proteins as well as membrane-associated guanylate kinase inverted 2 (MAGI-2). These interactions serve to recruit PTEN to the membrane, and can be negatively modulated by phosphorylation of PTEN on its C terminus (Takahashi et al., 2006; Vazquez et al., 2000, 2001). Additionally, there is evidence that the C2 domain of PTEN can be phosphorylated by RhoA-associated kinase (ROCK) to activate and target it to the membrane in leukocytes stimulated by chemoattractants (Li et al., 2005). Finally, a repressor of PTEN named DJ-1 was identified in a genetic screen in *Drosophila*. DJ-1 expression is associated with elevated levels of activated AKT and poor clinical outcome in various cancer types, including breast, lung, and ovarian carcinoma; however, its mechanism of action is still unclear (Davidson et al., 2007; Kim et al., 2005). PTEN has many diverse interacting partners suggesting multiple functions for PTEN. Yet, further investigation is needed to understand the physiological relevance of many of these interactions.

PTEN in the Nucleus

It was initially assumed that PTEN was exclusively localized to the cytoplasm. In agreement with this notion, early reports identified a lipid-binding domain and the absence of a canonical nuclear localization signal (NLS) on the PTEN protein, and other studies utilizing overexpression systems and early PTEN antibodies demonstrated that PTEN was exclusively in the cytoplasm (Lee et al., 1999; Li and Sun, 1997; Whang et al., 1998). Soon thereafter, the first lines of evidence for the existence of nuclear PTEN were noted in neuronal and breast tissues or cell lines with little fanfare (Lachyankar et al., 2000; Sano et al., 1999). In general, these early observations of nuclear PTEN were believed to arise due to artifacts of immunohistochemical staining and poor quality antibodies (as reviewed in Lian and Di Cristofano [2005]].

Today it is well documented that a pool of PTEN protein is localized to and functional within the nucleus. Immunocytological and immunohistochemical data with new and reliable PTEN antibodies have established the presence of nuclear PTEN in neurons (Lachyankar et al., 2000), thyroid tissue (Gimm et al., 2000), pancreatic cells (Perren et al., 2000), skin (Whiteman et al., 2002), esophageal squamous cell carcinoma (Tachibana et al., 2002), vascular smooth muscle cells (Deleris et al., 2003), and in the intestinal mucosa (Trotman et al., 2007). Specifically, PTEN is predominantly localized to the nucleus in primary, differentiated, and resting cells, compared to rapidly cycling cancer cell lines where in many cases there is a marked reduction of nuclear PTEN (Gimm et al., 2000; Ginn-Pease and Eng, 2003; Lachyankar et al., 2000; Perren et al., 1999, 2000). Therefore, it is surmised that PTEN localization may be dependent not only on cell cycle stage, but also on differentiation status.

This new knowledge begs the question of what function can be attributed to the nuclear pool of PTEN. Indeed, nuclear-cytoplasmic partitioning of PTEN is a promising biological marker. Indeed, the absence of nuclear PTEN is associated with more aggressive disease in patients with esophageal squamous cell carcinoma (Tachibana et al., 2002), cutaneous melanoma (Whiteman et al., 2002; Zhou et al., 2000), colorectal cancer (Zhou et al., 2002), pancreatic islet cell tumors (Perren et al., 2000) and cases of large B cell lymphoma (Fridberg et al., 2007). Therefore, the absence of nuclear PTEN may serve as a useful prognostic indicator. In keeping with this concept, forced nuclear expression of PTEN opposes anchorage-independent growth in transformation assays (Liu et al., 2005b). Together, the results of these studies indicate that the tumor suppressive function of PTEN is at least in part due to its nuclear function.

PTEN's Nuclear Family

The presence of both PTEN and PIP_3 within the nucleus may imply that PTEN functions as a nuclear lipid phosphatase, similar to its role at cytoplasmic membranes. Unexpectedly, there

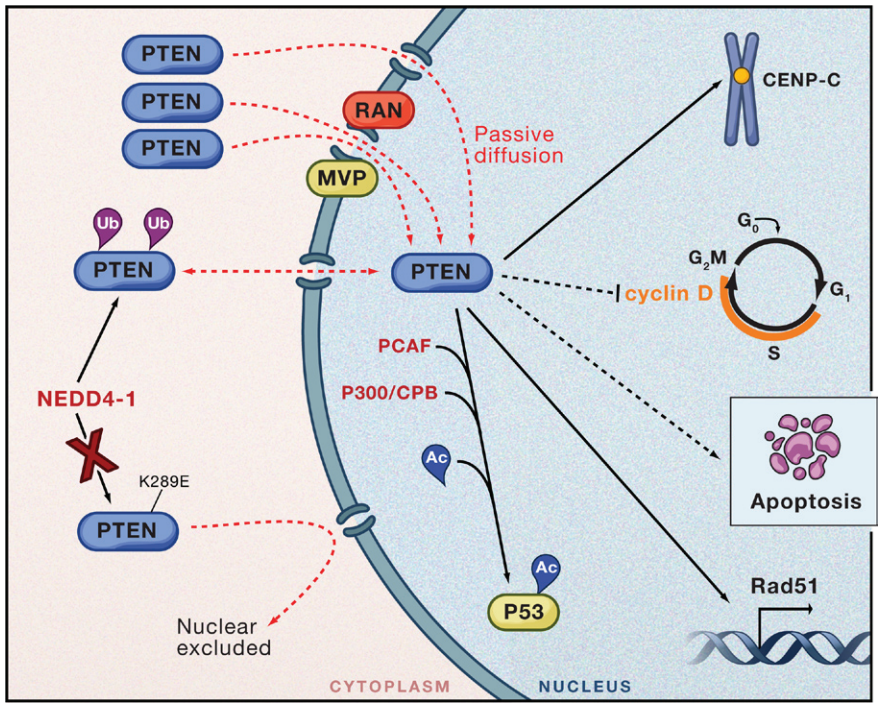

Figure 4. Functions of Nuclear PTEN

PTEN tumor suppressive activity is not restricted to its cytoplasmic function, which is to oppose the PI3K signaling pathway. It has been proposed that PTEN can translocate into the nucleus through various mechanisms including passive diffusion, Ran- or MVP-mediated import, and a monoubiquitination-driven mechanism. In the nucleus, the physiological role of PTEN may not be related to PIP_3 hydrolysis but instead to other functions including regulation of chromosomal integrity, p53 acetylation, cell cycle progression, the induction of apoptosis, and the DNA-damage response through the regulation of RAD51.

is limited evidence supporting this notion (Deleris et al., 2003), and in fact it has recently been reported that only the cytoplasmic (and not the nuclear) pool of PIP_3 is sensitive to PTEN catalysis (Lindsay et al., 2006). Clearly, the hypothesis of a lipid phosphatase function of PTEN within the nucleus requires further experimentation to be substantiated.

How then might nuclear PTEN act as a tumor suppressor? This has become a subject of significant debate, and despite several recent studies, this particular question has yet to be addressed in full. What we have learned is that unlike cytoplasmic PTEN, the effects of nuclear PTEN are likely not mediated directly through AKT (Liu et al., 2005b), yet this is at odds with the finding that enforced nuclear PTEN expression can reduce cellular levels of phosphorylated AKT (Trotman et al., 2007). Nuclear PTEN has been proposed to regulate the cell cycle through the suppression of cyclin D1 activity by transcriptional downregulation or by limiting its nuclear accumulation (Chung et al., 2006; Radu et al., 2003). PTEN in the nucleus may also act as a proapoptotic factor, as suggested by the observation that in cell lines apoptotic stimuli promotes the nuclear accumulation of PTEN (Gil et al., 2006). Nonetheless, it still remains unclear as to whether the suppression of cyclin D1 or apoptosis is indeed due to a specific nuclear function of PTEN, and whether it is lipid phosphatase dependent or independent.

In search of potential nuclear substrates for PTEN, Okumura et al. (2005) employed a yeast-two-hybrid approach with the C terminus of PTEN and identified the nucleolar proto-oncogene MSP58 as a nuclear interacting partner for PTEN (Figure 3B). MSP58 was shown to transform *Pten* null mouse embryonic fibroblasts, although this outcome was abrogated in the presence of wild-type PTEN and, surprisingly, also by a phosphatase inactive PTEN mutant. This report demonstrated that the nuclear tumor suppression function of PTEN is lipid phosphatase independent and, at least in part, mediated through the C-terminal domain. More recently, two studies recently reported the interaction of PTEN with nuclear histone acetyltransferases PCAF and p300/CBP (Li et al., 2006; Okumura et al., 2006). Interestingly, Li et al. (2006) also demonstrated a role for nuclear PTEN in promoting p300/CBP-mediated p53 acetylation in the response to DNA damage. These two studies provide great insight into a new potential mechanism whereby nuclear PTEN controls cellular proliferation by modulating the acetylation of histones and other cellular proteins including p53. As discussed below, p53 maintains a complex relationship with PTEN.

Finally, *PTEN* loss in human primary breast cancer has been associated with extensive centromere breakage and chromosomal translocations, suggesting a role for PTEN in the maintenance of chromosomal integrity (Puc and Parsons, 2005; Shen et al., 2007). In one study, this was attributed to AKT-mediated phosphorylation of CHK1, an event that leads to the sequestration of this DNA-damage checkpoint protein from the nucleus (Puc and Parsons, 2005). In a recent follow up study, Shen et al. (2007) have identified new mechanisms by which PTEN can maintain chromosomal stability. First, PTEN was found to associate with the centromere by docking onto CENP-C, a centromeric binding protein (Figure 4). A phosphatase-inactive mutant of PTEN could also bind CENP-C suggesting that this association is phosphatase independent. A Cowden syndrom and tumor-derived PTEN mutant protein lacking the C terminus inhibited the PTEN-CENP-C interaction and induced chromosomal instability. Second, PTEN was shown to control DNA repair through it ability to regulate the transcription of Rad51, a key protein involved in double-strand break repair (Figure 4). Hence, there is evidence for an important nuclear function of PTEN that is C terminus dependent, phosphatase independent, and multifactorial and complex in nature.

Mechanisms of Nuclear Localization

The appropriate cytoplasmic and nuclear partitioning of PTEN is critical for its diverse biological functions (Figure 4). It was recently reported that nuclear import of PTEN is cell cycle dependent and regulated by the PI3K/Akt/mTOR/S6K signaling cascade (Liu et al., 2007). Importantly, the mechanisms for nuclear import of PTEN are unclear, largely due to the lack of functional and trans-

ferable NLS (Trotman et al., 2007). To date PTEN localization has been attributed to various mechanisms, including a calcium-dependent interaction of the major vault protein with two putative bipartite NLS sequences within PTEN (Chung et al., 2005; Minaguchi et al., 2006), passive diffusion (Liu et al., 2005a), the existence of various nuclear exclusion sequences, and a nuclear localization domain and Ran-dependant mechanism that governs the interplay between these motifs (Gil et al., 2006). Conversely, the nuclear export of PTEN has been reported to occur via a CRM1-dependent mechanism activated by the PI3K/AKT pathway; however, the lack of a conventional nuclear export signal suggests that PTEN may be exported in association with another NLS-containing molecule (Liu et al., 2007).

The examination of a cancer-associated germline mutation of PTEN sheds light on a ubiquitination-dependent mechanism of PTEN nuclear transport. Indeed, a recent study demonstrated that inhibition of ubiquitin conjugation by mutation of critical lysine residues on PTEN has tremendous consequences for PTEN localization and tumorigenesis (Trotman et al., 2007). The notion of ubiquitin dependency for localization requires the existence of enzymes that can actively ubiquitinate PTEN and correspondingly implies that enzymes that can deubiquitinate PTEN can also play an important role in PTEN localization. To date, only the E3-ligase NEDD4-1 has been reported to ubiquitinate PTEN. On the other hand no deubiquitinating enzymes (DUBs) have yet been identified to oppose this function. Importantly, the manipulation of PTEN localization may provide a promising strategy for cancer treatment. Further research into this feature of PTEN regulation will indeed shed light on ways to exploit this mechanism.

AKT-Independent Roles of PTEN

Most of the phenotypes associated with *PTEN* loss can be explained by the activation of the PI3K/AKT pathway, although this is not always the case. For example, *Pten* null prostates eventually develop invasive prostate cancer. However, transgenic models with prostate-specific overexpression of a constitutively active AKT develop only localized and, for the most part, preneoplastic lesions (Majumder et al., 2003; Trotman et al., 2003). Discrepancies between other mouse models of *Pten* loss and *Akt* overexpression has been the subject of a recent review (Blanco-Aparicio et al., 2007). Taken together, it is increasingly evident that PTEN possesses functions that are independent of its ability to specifically suppress the PI3K pathway.

Most recently, Vivanco et al. (2007) utilized a comprehensive transcriptional profiling methodology to identify new PTEN-regulated pathways. The authors demonstrated that the Jun-N-terminal Kinase (JNK) pathway was consistently activated upon PTEN knockdown in an AKT-independent manner. In addition to finding alternative pathways activated subsequent to PTEN loss, these findings provide important clinical clues suggesting that inhibitors of the AKT pathway could be combined with JNK pathway inhibition for improved efficacy in PTEN null cancers.

Lipid Phosphatase-Independent Roles of PTEN

Although PTEN is thought to function predominantly as a lipid phosphatase, lipid phosphatase-independent roles have been reported. For instance, the ability of PTEN to directly dephosphorylate residues on FAK was shown to oppose cell migration by negatively regulating interactions with the cellular matrix (Tamura et al., 1998). However, FAK phosphorylation was unchanged in *Pten* null cells, and a second study was unable to show either a direct interaction of FAK and PTEN or FAK dephosphorylation upon PTEN overexpression (Maier et al., 1999; Sun et al., 1999). Additionally, the physiological relevance of PTEN-mediated FAK dephosphorylation is currently unclear. More recently, as discussed above, PTEN loss has been shown to have detrimental effects on genomic stability (Shen et al., 2007). Numerous studies have demonstrated a complex and uncertain relationship between p53 and PTEN. In one study, PTEN has been reported to bind to p53 directly, and as a consequence it increases p53 protein levels by modulating its stability and increasing its transcription. The half-life of p53 in *Pten* null mouse embryonic fibroblasts was dramatically compromised. Interestingly, this relationship was demonstrated to be independent of PTEN phosphatase function given that catalytically inactive mutants were capable of stabilizing p53 in *Pten* null cells (Freeman et al., 2003). However, other studies have reported that PTEN inactivation results in a rapid and marked upregulation of p53 (Chen et al., 2005). Hence, a thorough analysis of this controversial issue is warranted to understand the role of the PTEN and p53 crosstalk in tumorigenesis.

PTEN and Stem Cell Maintenance

The first lines of evidence demonstrating that PTEN may also play a role in stem cell homeostasis came from the studies of *Pten* loss in murine neuronal tissues (Backman et al., 2001; Groszer et al., 2001; Kwon et al., 2001). Utilizing independent mouse models, three groups demonstrated that specific deletion of *Pten* from the mouse brain led to enlarged brains (macrocephaly) with severely disturbed patterning of brain structures. This phenotype appeared to result from a combination of increased cell number, decreased cell death, and enlarged neuronal cell size. In ex vivo experiments, it was demonstrated that *Pten* loss does not alter neuronal cell fate but, instead, increases the total number of neurons in the fetal brain and the number of neuronal stem cells that were capable of growth in cell culture experiments. This consequence of *Pten* loss was later reported to result from an enhanced self-renewal capacity and G_0-G_1 cell cycle entry and decreased growth factor dependency of *Pten* null neural/stem progenitor cells (Groszer et al., 2006). In conclusion, *Pten* deficiency in neuronal stem cells provides a strong proliferative stimulus and promotes a greatly enhanced self-renewal capacity.

More recently, two groups demonstrated that PTEN has a role in the maintenance of the hematopoietic stem cell (HSC) (Yilmaz et al., 2006; Zhang et al., 2006). Conditional deletion of *Pten* in the murine hematopoietic system resulted in the exhaustion of normal HSCs and promoted the excessive proliferation of leukemogenic stem cells resulting in the development of myeloproliferative disorders and eventually leukemia. Furthermore, Yilmaz et al. (2006) demonstrated that treatment of *Pten*-deficient cells and mice with the mTOR inhibitor rapamycin effectively blocked the growth of the leukemogenic stem cell and at the same time prevented the exhaustion of normal HSCs. These findings may have a tremendous impact for the treatment of stem cells disorders that are leukemogenic in nature, in particular those arising from disorders in the PTEN/PI3K pathway.

Notably, the mechanism for exhaustion or loss of HSCs subsequent to *Pten* deletion is currently unknown. Similar to the observations in mouse prostate (Chen et al., 2005), *Pten* deficiency may trigger a protective p53-dependent senescence apoptotic response that will ultimately deplete the HSC pool. In the same way, the excessive growth of leukemic cells may arise due to selective evasion of this failsafe senescence mechanism because of secondary mutations (in p53, for example) arising from the genomic instability induced by the loss of *Pten*. Importantly, the finding that the mTOR inhibitor rapamycin is very effective at inhibiting both HSC depletion and leukemia has two important implications: (1) the mTOR pathway is highly active in leukemia linked to *Pten* loss, and (2) mTOR is a central player in the mechanism leading to the exhaustion of *Pten*-deficient HSCs.

Finally, the different stem cell phenotypes observed upon *Pten*-deletions may appear to be at odds in different organs. This discrepancy may be explained by a differential capacity of a given organ system to trigger a failsafe mechanism in response to *Pten* loss. For example, it is likely that neuronal stem cells do not activate senescence upon *Pten* loss. In contrast, a senescence response or an apoptotic response in HSCs can lead to reduced proliferation and exhaustion; when selected against by mutation, leukemia undoubtedly arises.

Future Directions

Ten years after its identification, dramatic progress has been made in defining the role of *PTEN* in tumor suppression. *PTEN* is likely to surpass *p53* as the most commonly mutated tumor suppressor gene in human cancer and represents, from a biological standpoint, the first and only known example of a lipid phosphatase with bona fide tumor suppressive activity. In vivo analysis of mouse models have identified critical consequences of *Pten* functional inactivation in cancer pathogenesis. *Pten* mouse mutants have become invaluable tools in cancer research and in preclinical trials by virtue of their pleiotropic tumor-prone phenotype. *Pten* has been identified as a haploinsufficient tumor suppressor protein in mouse models, and the realization that slight variations in the amount of PTEN protein can have tremendous consequences for tumor initiation and cancer susceptibility underscores the importance of defining the regulatory mechanisms that control PTEN function. *Pten* also acts in the nucleus and has been ascribed functions that go beyond its phosphate activity. *Pten* may be essential and nonredundant, although other kinases and phosphatases that are able to modulate the PI3K/AKT pathway may also be critical in tumorigenesis. This progress has in turn raised further exciting questions from both a genetic and mechanistic viewpoint concerning the precise function and the specific regulation of this critical tumor suppressor in human cancer susceptibility. In general, PTEN is robustly expressed in normal cells and mechanisms that regulate PTEN expression, protein levels, and cellular trafficking are largely undiscovered. However, in the course of a wide range of pathological conditions *PTEN* can be downregulated by various mechanisms including genetic mutation, transcription, translation, and posttranslational modification. Furthermore, the PTEN/PI3K/AKT pathway is amenable to pharmacological manipulation, and PTEN itself may become a "druggable" target in the near future, as is currently the case for p53 (Nahi et al., 2008; Vassilev et al., 2004). Functional analysis of PTEN domains through mutation, the discovery of new regulators of PTEN, and a greater understanding of the regulatory networks that influence PTEN expression in vitro and in vivo will be critical in unraveling additional PTEN functions. In view of the key role exerted by PTEN in opposing tumorigenesis and in embryonic development, further elucidation of PTEN function will remain a major focus of research in the years to come.

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REFERENCES

Al-Khouri, A.M., Ma, Y., Togo, S.H., Williams, S., and Mustelin, T. (2005). Cooperative phosphorylation of the tumor suppressor phosphatase and tensin homologue (PTEN) by casein kinases and glycogen synthase kinase 3beta. J. Biol. Chem. *280*, 35195–35202.

Ali, I.U., Schriml, L.M., and Dean, M. (1999). Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. J. Natl. Cancer Inst. *91*, 1922–1932.

Backman, S.A., Stambolic, V., Suzuki, A., Haight, J., Elia, A., Pretorius, J., Tsao, M.S., Shannon, P., Bolon, B., Ivy, G.O., and Mak, T.W. (2001). Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. Nat. Genet. *29*, 396–403.

Blanco-Aparicio, C., Renner, O., Leal, J.F., and Carnero, A. (2007). PTEN, more than the AKT pathway. Carcinogenesis *28*, 1379–1386.

Bose, S., Wang, S.I., Terry, M.B., Hibshoosh, H., and Parsons, R. (1998). Allelic loss of chromosome 10q23 is associated with tumor progression in breast carcinomas. Oncogene *17*, 123–127.

Brognard, J., Sierecki, E., Gao, T., and Newton, A.C. (2007). PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. Mol. Cell *25*, 917–931.

Campisi, J., and d'Adda di Fagagna, F. (2007). Cellular senescence: when bad things happen to good cells. Nat. Rev. Mol. Cell Biol. *8*, 729–740.

Chan, J.A., Krichevsky, A.M., and Kosik, K.S. (2005). MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res. *65*, 6029–6033.

Chappell, W.H., Green, T.D., Spengeman, J.D., McCubrey, J.A., Akula, S.M., and Bertrand, F.E. (2005). Increased protein expression of the PTEN tumor suppressor in the presence of constitutively active Notch-1. Cell Cycle *4*, 1389–1395.

Chen, Z., Trotman, L.C., Shaffer, D., Lin, H.K., Dotan, Z.A., Niki, M., Koutcher, J.A., Scher, H.I., Ludwig, T., Gerald, W., et al. (2005). Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. Nature *436*, 725–730.

Chow, J.Y., Quach, K.T., Cabrera, B.L., Cabral, J.A., Beck, S.E., and Carethers, J.M. (2007). RAS/ERK modulates TGFbeta-regulated PTEN expression in human pancreatic adenocarcinoma cells. Carcinogenesis *28*, 2321–2327.

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 ar-

rest by cyclin D1 transcriptional regulation. Hum. Mol. Genet. *15*, 2553–2559.

Dahia, P.L. (2000). PTEN, a unique tumor suppressor gene. Endocr. Relat. Cancer *7*, 115–129.

Davidson, B., Hadar, R., Schlossberg, A., Sternlicht, T., Slipicevic, A., Skrede, M., Risberg, B., Florenes, V.A., Kopolovic, J., and Reich, R. (2007). Expression and clinical role of DJ-1, a negative regulator of PTEN, in ovarian carcinoma (. Hum. Pathol. *39*, 87–95.

Deleris, P., Bacqueville, D., Gayral, S., Carrez, L., Salles, J.P., Perret, B., and Breton-Douillon, M. (2003). SHIP-2 and PTEN are expressed and active in vascular smooth muscle cell nuclei, but only SHIP-2 is associated with nuclear speckles. J. Biol. Chem. *278*, 38884–38891.

Di Cristofano, A., Pesce, B., Cordon-Cardo, C., and Pandolfi, P.P. (1998). Pten is essential for embryonic development and tumour suppression. Nat. Genet. *19*, 348–355.

Di Cristofano, A., Kotsi, P., Peng, Y.F., Cordon-Cardo, C., Elkon, K.B., and Pandolfi, P.P. (1999). Impaired Fas response and autoimmunity in Pten+/− mice. Science *285*, 2122–2125.

Di Cristofano, A., De Acetis, M., Koff, A., Cordon-Cardo, C., and Pandolfi, P.P. (2001). Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. Nat. Genet. *27*, 222–224.

Faivre, S., Kroemer, G., and Raymond, E. (2006). Current development of mTOR inhibitors as anticancer agents. Nat. Rev. Drug Discov. *5*, 671–688.

Feilotter, H.E., Coulon, V., McVeigh, J.L., Boag, A.H., Dorion-Bonnet, F., Duboue, B., Latham, W.C., Eng, C., Mulligan, L.M., and Longy, M. (1999). Analysis of the 10q23 chromosomal region and the PTEN gene in human sporadic breast carcinoma. Br. J. Cancer *79*, 718–723.

Franca-Koh, J., Kamimura, Y., and Devreotes, P.N. (2007). Leading-edge research: PtdIns(3,4,5)P3 and directed migration. Nat. Cell Biol. *9*, 15–17.

Freeman, D.J., Li, A.G., Wei, G., Li, H.H., Kertesz, N., Lesche, R., Whale, A.D., Martinez-Diaz, H., Rozengurt, N., Cardiff, R.D., et al. (2003). PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. Cancer Cell *3*, 117–130.

Fridberg, M., Servin, A., Anagnostaki, L., Linderoth, J., Berglund, M., Soderberg, O., Enblad, G., Rosen, A., Mustelin, T., Jerkeman, M., et al. (2007). Protein expression and cellular localization in two prognostic subgroups of diffuse large B-cell lymphoma: Higher expression of ZAP70 and PKC-beta II in the non-germinal center group and poor survival in patients deficient in nuclear PTEN. Leuk. Lymphoma *48*, 2221–2232.

Gao, T., Furnari, F., and Newton, A.C. (2005). PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol. Cell *18*, 13–24.

Garcia, J.M., Silva, J., Pena, C., Garcia, V., Rodriguez, R., Cruz, M.A., Cantos, B., Provencio, M., Espana, P., and Bonilla, F. (2004). Promoter methylation of the PTEN gene is a common molecular change in breast cancer. Genes Chromosomes Cancer *41*, 117–124.

Georgescu, M.M., Kirsch, K.H., Akagi, T., Shishido, T., and Hanafusa, H. (1999). The tumor-suppressor activity of PTEN is regulated by its carboxyl-terminal region. Proc. Natl. Acad. Sci. USA *96*, 10182–10187.

Gil, A., Andres-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., Delbridge, 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 homologue deleted on chromosome 10 is associated with G0–G1 in MCF-7 cells. Cancer Res. *63*, 282–286.

Goel, A., Arnold, C.N., Niedzwiecki, D., Carethers, J.M., Dowell, J.M., Wasserman, L., Compton, C., Mayer, R.J., Bertagnolli, M.M., and Boland, C.R. (2004). Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. Cancer Res. *64*, 3014–3021.

Gray, I.C., Stewart, L.M., Phillips, S.M., Hamilton, J.A., Gray, N.E., Watson, G.J., Spurr, N.K., and Snary, D. (1998). Mutation and expression analysis of the putative prostate tumour-suppressor gene PTEN. Br. J. Cancer *78*, 1296–1300.

Groszer, M., Erickson, R., Scripture-Adams, D.D., Dougherty, J.D., Le Belle, J., Zack, J.A., Geschwind, D.H., Liu, X., Kornblum, H.I., and Wu, H. (2006). PTEN negatively regulates neural stem cell self-renewal by modulating G0–G1 cell cycle entry. Proc. Natl. Acad. Sci. USA *103*, 111–116.

Groszer, M., Erickson, R., Scripture-Adams, D.D., Lesche, R., Trumpp, A., Zack, J.A., Kornblum, H.I., Liu, X., and Wu, H. (2001). Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science *294*, 2186–2189.

Guertin, D.A., and Sabatini, D.M. (2007). Defining the role of mTOR in cancer. Cancer Cell *12*, 9–22.

Han, S.Y., Kato, H., Kato, S., Suzuki, T., Shibata, H., Ishii, S., Shiiba, K., Matsuno, S., Kanamaru, R., and Ishioka, C. (2000). Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay. Cancer Res. *60*, 3147–3151.

Hettinger, K., Vikhanskaya, F., Poh, M.K., Lee, M.K., de Belle, I., Zhang, J.T., Reddy, S.A., and Sabapathy, K. (2007). c-Jun promotes cellular survival by suppression of PTEN. Cell Death Differ. *14*, 218–229.

Kang, Y.H., Lee, H.S., and Kim, W.H. (2002). Promoter methylation and silencing of PTEN in gastric carcinoma. Lab. Invest. *82*, 285–291.

Kim, R.H., Peters, M., Jang, Y., Shi, W., Pintilie, M., Fletcher, G.C., DeLuca, C., Liepa, J., Zhou, L., Snow, B., et al. (2005). DJ-1, a novel regulator of the tumor suppressor PTEN. Cancer Cell *7*, 263–273.

Knudson, A.G. (1971). Mutation and cancer: statistical study of retinoblastoma. Proc. Natl. Acad. Sci. USA *68*, 820–823.

Kwon, C.H., Zhu, X., Zhang, J., Knoop, L.L., Tharp, R., Smeyne, R.J., Eberhart, C.G., Burger, P.C., and Baker, S.J. (2001). Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. Nat. Genet. *29*, 404–411.

Kwon, J., Lee, S.R., Yang, K.S., Ahn, Y., Kim, Y.J., Stadtman, E.R., and Rhee, S.G. (2004). Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. Proc. Natl. Acad. Sci. USA *101*, 16419–16424.

Lachyankar, M.B., Sultana, N., Schonhoff, C.M., Mitra, P., Poluha, W., Lambert, S., Quesenberry, P.J., Litofsky, N.S., Recht, L.D., Nabi, R., et al. (2000). A role for nuclear PTEN in neuronal differentiation. J. Neurosci. *20*, 1404–1413.

Lee, J.O., Yang, H., Georgescu, M.M., Di Cristofano, A., Maehama, T., Shi, Y., Dixon, J.E., Pandolfi, P., and Pavletich, N.P. (1999). Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell *99*, 323–334.

Lee, S.R., Yang, K.S., Kwon, J., Lee, C., Jeong, W., and Rhee, S.G. (2002). Reversible inactivation of the tumor suppressor PTEN by H2O2. J. Biol. Chem. *277*, 20336–20342.

Leslie, N.R., Bennett, D., Lindsay, Y.E., Stewart, H., Gray, A., and Downes, C.P. (2003). Redox regulation of PI 3-kinase signalling via inactivation of PTEN. EMBO J. *22*, 5501–5510.

Leslie, N.R., and Downes, C.P. (2004). PTEN function: how normal cells control it and tumour cells lose it. Biochem. J. *382*, 1–11.

Leupin, N., Cenni, B., Novak, U., Hugli, B., Graber, H.U., Tobler, A., and Fey, M.F. (2003). Disparate expression of the PTEN gene: a novel finding in B-cell chronic lymphocytic leukaemia (B-CLL). Br. J. Haematol. *121*, 97–100.

Li, A.G., Piluso, L.G., Cai, X., Wei, G., Sellers, W.R., and Liu, X. (2006). Mechanistic insights into maintenance of high p53 acetylation by PTEN. Mol. Cell *23*, 575–587.

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, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., et al. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science *275*, 1943–1947.

Li, Z., Dong, X., Wang, Z., Liu, W., Deng, N., Ding, Y., Tang, L., Hla, T., Zeng, R., Li, L., and Wu, D. (2005). Regulation of PTEN by Rho small GTPases. Nat. Cell Biol. *7*, 399–404.

Lian, Z., and Di Cristofano, A. (2005). Class reunion: PTEN joins the nuclear crew. Oncogene *24*, 7394–7400.

Lindsay, Y., McCoull, D., Davidson, L., Leslie, N.R., Fairservice, A., Gray, A., Lucocq, J., and Downes, C.P. (2006). Localization of agonist-sensitive PtdIns(3,4,5)P3 reveals a nuclear pool that is insensitive to PTEN expression. J. Cell Sci. *119*, 5160–5168.

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

Liu, J.L., Mao, Z., LaFortune, T.A., Alonso, M.M., Gallick, G.E., Fueyo, J., and Yung, W.K. (2007). Cell cycle-dependent nuclear export of phosphatase and tensin homologue tumor suppressor is regulated by the phosphoinositide-3-kinase signaling cascade. Cancer Res. *67*, 11054–11063.

Liu, J.L., Sheng, X., Hortobagyi, Z.K., Mao, Z., Gallick, G.E., and Yung, W.K. (2005b). Nuclear PTEN-mediated growth suppression is independent of Akt down-regulation. Mol. Cell. Biol. *25*, 6211–6224.

Maccario, H., Perera, N.M., Davidson, L., Downes, C.P., and Leslie, N.R. (2007). PTEN is destabilized by phosphorylation on Thr366. Biochem. J. *405*, 439–444.

Maehama, T., and Dixon, J.E. (1998). The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem. *273*, 13375–13378.

Maier, D., Jones, G., Li, X., Schonthal, A.H., Gratzl, O., Van Meir, E.G., and Merlo, A. (1999). The PTEN lipid phosphatase domain is not required to inhibit invasion of glioma cells. Cancer Res. *59*, 5479–5482.

Majumder, P.K., Yeh, J.J., George, D.J., Febbo, P.G., Kum, J., Xue, Q., Bikoff, R., Ma, H., Kantoff, P.W., Golub, T.R., et al. (2003). Prostate intraepithelial neoplasia induced by prostate restricted Akt activation: the MPAKT model. Proc. Natl. Acad. Sci. USA *100*, 7841–7846.

Manning, B.D., and Cantley, L.C. (2007). AKT/PKB signaling: navigating downstream. Cell *129*, 1261–1274.

Marsh, D.J., Dahia, P.L., Coulon, V., Zheng, Z., Dorion-Bonnet, F., Call, K.M., Little, R., Lin, A.Y., Eeles, R.A., Goldstein, A.M., et al. (1998). Allelic imbalance, including deletion of PTEN/MMACI, at the Cowden disease locus on 10q22–23, in hamartomas from patients with Cowden syndrome and germline PTEN mutation. Genes Chromosomes Cancer *21*, 61–69.

Meng, F., Henson, R., Lang, M., Wehbe, H., Maheshwari, S., Mendell, J.T., Jiang, J., Schmittgen, T.D., and Patel, T. (2006). Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology *130*, 2113–2129.

Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S.T., and Patel, T. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology *133*, 647–658.

Miller, S.J., Lou, D.Y., Seldin, D.C., Lane, W.S., and Neel, B.G. (2002). Direct identification of PTEN phosphorylation sites. FEBS Lett. *528*, 145–153.

Minaguchi, T., Waite, K.A., and Eng, C. (2006). Nuclear localization of PTEN is regulated by Ca2+ through a tyrosil phosphorylation-independent conformational modification in major vault protein. Cancer Res. *66*, 11677–11682.

Mirmohammadsadegh, A., Marini, A., Nambiar, S., Hassan, M., Tannapfel, A., Ruzicka, T., and Hengge, U.R. (2006). Epigenetic silencing of the PTEN gene in melanoma. Cancer Res. *66*, 6546–6552.

Moorehead, R.A., Hojilla, C.V., De Belle, I., Wood, G.A., Fata, J.E., Adamson, E.D., Watson, K.L., Edwards, D.R., and Khokha, R. (2003). Insulin-like growth factor-II regulates PTEN expression in the mammary gland. J. Biol. Chem. *278*, 50422–50427.

Mutter, G.L., Lin, M.C., Fitzgerald, J.T., Kum, J.B., Baak, J.P., Lees, J.A., Weng, L.P., and Eng, C. (2000). Altered PTEN expression as a diagnostic marker for the earliest endometrial precancers. J. Natl. Cancer Inst. *92*, 924–930.

Nahi, H., Selivanova, G., Lehmann, S., Mollgard, L., Bengtzen, S., Concha, H., Svensson, A., Wiman, K.G., Merup, M., and Paul, C. (2008). Mutated and non-mutated TP53 as targets in the treatment of leukaemia. Br. J. Haematol. Published online March 12, 2008. 10.1111/j.1365-2141.2008.07074.

Okahara, F., Ikawa, H., Kanaho, Y., and Maehama, T. (2004). Regulation of PTEN phosphorylation and stability by a tumor suppressor candidate protein. J. Biol. Chem. *279*, 45300–45303.

Okumura, K., Zhao, M., Depinho, R.A., Furnari, F.B., and Cavenee, W.K. (2005). Cellular transformation by the MSP58 oncogene is inhibited by its physical interaction with the PTEN tumor suppressor. Proc. Natl. Acad. Sci. USA *102*, 2703–2706.

Okumura, K., Mendoza, M., Bachoo, R.M., DePinho, R.A., Cavenee, W.K., and Furnari, F.B. (2006). PCAF modulates PTEN activity. J. Biol. Chem. *281*, 26562–26568.

Palomero, T., Sulis, M.L., Cortina, M., Real, P.J., Barnes, K., Ciofani, M., Caparros, E., Buteau, J., Brown, K., Perkins, S.L., et al. (2007). Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. Nat. Med. *13*, 1203–1210.

Patel, L., Pass, I., Coxon, P., Downes, C.P., Smith, S.A., and Macphee, C.H. (2001). Tumor suppressor and anti-inflammatory actions of PPARgamma agonists are mediated via upregulation of PTEN. Curr. Biol. *11*, 764–768.

Perren, A., Weng, L.-P., Boag, A.H., Ziebold, U., Thakore, K., Dahia, P.L.M., Komminoth, P., Lees, J.A., Mulligan, L.M., Mutter, G.L., and Eng, C. (1999). Immunohistochemical Evidence of Loss of PTEN Expression in Primary Ductal Adenocarcinomas of the Breast. Am. J. Pathol. *155*, 1253–1260.

Perren, A., Komminoth, P., Saremaslani, P., Matter, C., Feurer, S., Lees, J.A., Heitz, P.U., and Eng, C. (2000). Mutation and expression analyses reveal differential subcellular compartmentalization of PTEN in endocrine pancreatic tumors compared to normal islet cells. Am. J. Pathol. *157*, 1097–1103.

Podsypanina, K., Ellenson, L.H., Nemes, A., Gu, J., Tamura, M., Yamada, K.M., Cordon-Cardo, C., Catoretti, G., Fisher, P.E., and Parsons, R. (1999). Mutation of Pten/Mmac1 in mice causes neoplasia in multiple organ systems. Proc. Natl. Acad. Sci. USA *96*, 1563–1568.

Puc, J., and Parsons, R. (2005). PTEN loss inhibits CHK1 to cause double stranded-DNA breaks in cells. Cell Cycle *4*, 927–929.

Radu, A., Neubauer, V., Akagi, T., Hanafusa, H., and Georgescu, M.-M. (2003). PTEN induces cell cycle arrest by decreasing the level and nuclear localization of cyclin D1. Mol. Cell. Biol. *23*, 6139–6149.

Saal, L.H., Gruvberger-Saal, S.K., Persson, C., Lovgren, K., Jumppanen, M., Staaf, J., Jonsson, G., Pires, M.M., Maurer, M., Holm, K., et al. (2008). Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. Nat. Genet. *40*, 102–107.

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.A., and Steck, P.A. (1999). Differential expression of MMAC/PTEN in glioblastoma multiforme: relationship to localization and prognosis. Cancer Res. *59*, 1820–1824.

Seo, J.H., Ahn, Y., Lee, S.R., Yeol Yeo, C., and Chung Hur, K. (2005). The major target of the endogenously generated reactive oxygen species in response to insulin stimulation is phosphatase and tensin homolog and not phosphoinositide-3 kinase (PI-3 kinase) in the PI-3 kinase/Akt pathway. Mol. Biol. Cell *16*, 348–357.

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.

Shi, W., Zhang, X., Pintilie, M., Ma, N., Miller, N., Banerjee, D., Tsao, M.S., Mak, T., Fyles, A., and Liu, F.F. (2003). Dysregulated PTEN-PKB and negative receptor status in human breast cancer. Int. J. Cancer *104*, 195–203.

Si, M.L., Zhu, S., Wu, H., Lu, Z., Wu, F., and Mo, Y.Y. (2007). miR-21-mediated tumor growth. Oncogene *26*, 2799–2803.

Stambolic, V., Suzuki, A., de la Pompa, J.L., Brothers, G.M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J.M., Siderovski, D.P., and Mak, T.W. (1998). Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell *95*, 29–39.

Stambolic, V., MacPherson, D., Sas, D., Lin, Y., Snow, B., Jang, Y., Benchimol, S., and Mak, T.W. (2001). Regulation of PTEN transcription by p53. Mol. Cell *8*, 317–325.

Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., et al. (1997). Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. *15*, 356–362.

Sun, H., Lesche, R., Li, D.M., Liliental, J., Zhang, H., Gao, J., Gavrilova, N., Mueller, B., Liu, X., and Wu, H. (1999). PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. Proc. Natl. Acad. Sci. USA *96*, 6199–6204.

Suzuki, A., de la Pompa, J.L., Stambolic, V., Elia, A.J., Sasaki, T., del Barco Barrantes, I., Ho, A., Wakeham, A., Itie, A., Khoo, W., et al. (1998). High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr. Biol. *8*, 1169–1178.

Tachibana, M., Shibakita, M., Ohno, S., Kinugasa, S., Yoshimura, H., Ueda, S., Fujii, T., Rahman, M.A., Dhar, D.K., and Nagasue, N. (2002). Expression and prognostic significance of PTEN product protein in patients with esophageal squamous cell carcinoma. Cancer *94*, 1955–1960.

Takahashi, Y., Morales, F.C., Kreimann, E.L., and Georgescu, M.M. (2006). PTEN tumor suppressor associates with NHERF proteins to attenuate PDGF receptor signaling. EMBO J. *25*, 910–920.

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.

Tang, Y., and Eng, C. (2006). p53 down-regulates phosphatase and tensin homologue deleted on chromosome 10 protein stability partially through caspase-mediated degradation in cells with proteasome dysfunction. Cancer Res. *66*, 6139–6148.

Tee, A.R., and Blenis, J. (2005). mTOR, translational control and human disease. Semin. Cell Dev. Biol. *16*, 29–37.

Torres, J., and Pulido, R. (2001). 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. *276*, 993–998.

Trotman, L.C., Niki, M., Dotan, Z.A., Koutcher, J.A., Di Cristofano, A., Xiao, A., Khoo, A.S., Roy-Burman, P., Greenberg, N.M., Van Dyke, T., et al. (2003). Pten dose dictates cancer progression in the prostate. PLoS Biol. *1*, E59.

Trotman, L.C., Wang, X., Alimonti, A., Chen, Z., Teruya-Feldstein, J., Yang, H., Pavletich, 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.

Vassilev, L.T., Vu, B.T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., et al. (2004). In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science *303*, 844–848.

Vasudevan, K.M., Burikhanov, R., Goswami, A., and Rangnekar, V.M. (2007). Suppression of PTEN expression is essential for antiapoptosis and cellular transformation by oncogenic Ras. Cancer Res. *67*, 10343–10350.

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.

Vazquez, F., Grossman, S.R., Takahashi, Y., Rokas, M.V., Nakamura, N., and Sellers, W.R. (2001). Phosphorylation of the PTEN tail acts as an inhibitory switch by preventing its recruitment into a protein complex. J. Biol. Chem. *276*, 48627–48630.

Virolle, T., Adamson, E.D., Baron, V., Birle, D., Mercola, D., Mustelin, T., and de Belle, I. (2001). The Egr-1 transcription factor directly activates PTEN during irradiation-induced signalling. Nat. Cell Biol. *3*, 1124–1128.

Vivanco, I., Palaskas, N., Tran, C., Finn, S.P., Getz, G., Kennedy, N.J., Jiao, J., Rose, J., Xie, W., Loda, M., et al. (2007). Identification of the JNK signaling pathway as a functional target of the tumor suppressor PTEN. Cancer Cell *11*, 555–569.

Vogelstein, B., and Kinzler, K.W. (2004). Cancer genes and the pathways they control. Nat. Med. *10*, 789–799.

Volinia, S., Calin, G.A., Liu, C.G., Ambs, S., Cimmino, A., Petrocca, F., Visone, R., Iorio, M., Roldo, C., Ferracin, M., et al. (2006). A microRNA expression signature of human solid tumors defines cancer gene targets. Proc. Natl. Acad. Sci. USA *103*, 2257–2261.

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

Wang, S., Gao, J., Lei, Q., Rozengurt, N., Pritchard, C., Jiao, J., Thomas, G.V., Li, G., Roy-Burman, P., Nelson, P.S., et al. (2003). Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. Cancer Cell *4*, 209–221.

Wang, X., Trotman, 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.

Whang, Y.E., Wu, X., Suzuki, H., Reiter, R.E., Tran, C., Vessella, R.L., Said, J.W., Isaacs, W.B., and Sawyers, 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*, 5246–5250.

Whelan, J.T., Forbes, S.L., and Bertrand, F.E. (2007). CBF-1 (RBP-J kappa) binds to the PTEN promoter and regulates PTEN gene expression. Cell Cycle *6*, 80–84.

Whiteman, 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.

Wu, X., Hepner, K., Castelino-Prabhu, S., Do, D., Kaye, M.B., Yuan, X.J., Wood, J., Ross, C., Sawyers, C.L., and Whang, Y.E. (2000). 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 *97*, 4233–4238.

Wu, W., Wang, X., Zhang, W., Reed, W., Samet, J.M., Whang, Y.E., and Ghio, A.J. (2003). Zinc-induced PTEN protein degradation through the proteasome pathway in human airway epithelial cells. J. Biol. Chem. *278*, 28258–28263.

Xia, D., Srinivas, H., Ahn, Y.H., Sethi, G., Sheng, X., Yung, W.K., Xia, Q., Chiao, P.J., Kim, H., Brown, P.H., et al. (2007). Mitogen-activated protein kinase kinase-4 promotes cell survival by decreasing PTEN expression through an NF kappa B-dependent pathway. J. Biol. Chem. *282*, 3507–3519.

Yilmaz, O.H., Valdez, R., Theisen, B.K., Guo, W., Ferguson, D.O., Wu, H., and Morrison, S.J. (2006). Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature *441*, 475–482.

Zhang, J., Grindley, J.C., Yin, T., Jayasinghe, S., He, X.C., Ross, J.T., Haug, J.S., Rupp, D., Porter-Westpfahl, K.S., Wiedemann, L.M., et al. (2006). PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature *441*, 518–522.

Zhou, X.P., Gimm, O., Hampel, H., Niemann, T., Walker, M.J., and Eng, C. (2000). Epigenetic PTEN silencing in malignant melanomas without PTEN mutation. Am. J. Pathol. *157*, 1123–1128.

Zhou, X.P., Loukola, A., Salovaara, R., Nystrom-Lahti, M., Peltomaki, P., de la Chapelle, A., Aaltonen, L.A., and Eng, C. (2002). PTEN mutational spectra, expression levels, and subcellular localization in microsatellite stable and unstable colorectal cancers. Am. J. Pathol. *161*, 439–447.

Zysman, M.A., Chapman, W.B., and Bapat, B. (2002). Considerations when analyzing the methylation status of PTEN tumor suppressor gene. Am. J. Pathol. *160*, 795–800.