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REVIEW

PTEN: a default gate-keeping tumor suppressor with a versatile tail

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The tumor suppressor PTEN controls a variety of biological processes including cell proliferation, growth, migration, and death. As a master cellular regulator, PTEN itself is also subjected to deliberated regulation to ensure its proper function. Defects in PTEN regulation have a profound impact on carcinogenesis. In this review, we briefly discuss recent advances concerning PTEN regulation and how such knowledge facilitates our understanding and further exploration of PTEN biology. The carboxyl-tail of PTEN, which appears to be associated with multiple types of posttranslational regulation, will be under detailed scrutiny. Further, a comparative analysis of PTEN and p53 suggests while p53 needs to be activated to suppress tumorigenesis (a dormant gatekeeper), PTEN is probably a constitutive surveillant against cancer development, thus a default gatekeeper.

***Keywords*:** PTEN, tumor suppressor, posttranslational regulation, ubiquitination, p53

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PTEN as a tumor suppressor and master cellular regulator

PTEN (phosphatase and tensin homolog deleted on chromosome ten), also known as MMAC1 (mutated in multiple advanced cancers-1) or TEP1 (tensin-like Phosphatase-1), was originally identified in 1997 by three independent groups led by Parsons, Steck, and Sun [1-3]. Sun and colleagues cloned *PTEN* as a putative protein tyrosine phosphatase induced by TGFβ [3], whereas the Parsons lab [1] and Steck lab [2] cloned *PTEN* as a gene that is frequently mutated or deleted in various human cancers, and therefore a potential tumor suppressor. The tumor suppressive function of PTEN was soon confirmed by mouse gene targeting studies [4-7]. Indeed, genetic mutation/deletion of *PTEN* is so common in many types of human cancers, making it one of the most frequently mutated tumor suppressors, second only to *p53* [8, 9]. In addition to somatic mutation, germline mutation of *PTEN* has been shown to be responsible for cancer-prone diseases such as Cowden syndrome and Bannayan-Zonana syndrome [10, 11]. All this evidence establishes PTEN as an important and potent tumor suppressor.

The molecular basis of the antitumor function of PTEN (or one of the major mechanisms, as discussed later) was also quickly defined owing to the stunning finding from Dixon and colleagues that PTEN is a lipid phosphatase for the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP3) [12]. By converting PIP3 into phosphatidylinositol 4,5-bisphosphate (PIP2), PTEN negatively regulates the PI3 kinase-Akt signaling pathway. This pathway dictates multiple downstream signaling events, including inhibition of apoptosis via phosphorylation of the pro-apoptotic protein BAD, stimulation of nutrient response and protein synthesis via the mTOR pathway, and regulation of metabolism and cell cycle via phosphorylation of glycogen synthase kinase 3 (GSK3) and the Forkhead transcription factor (see [9, 13] for detailed reviews). The versatile function of the PI3 kinase-Akt pathway in controlling cell proliferation, growth, and survival is manifested by the fact that this pathway is perturbed in almost every single type of human cancer; it also explains why PTEN, as the only defined negative regulator of PI3K-Akt signaling, is such a dominant tumor suppressor.

The biological functions and the tumor suppressive role of PTEN are not always due to its dominant inhibitory activity in the PI3 kinase-Akt pathway. The protein sequence of PTEN indicates PTEN is a putative protein

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phosphatase, a feature of PTEN recognized right after its gene was cloned and before its lipid phosphatase activity was identified. The identity of the physiological protein substrate(s) of PTEN has been elusive, and the few that have been proposed [14-17] are still under debate. However, this does not mean that the protein phosphatase function of PTEN is irrelevant physiologically, because the G129E PTEN mutant (a mutant that loses the PIP3 phosphatase activity but can dephosphorylate artificial peptide substrates [18, 19]) but not the catalytically inactive mutant (C124S) can still regulate cellular processes such as migration and focal adhesion, suggesting requirement of the PTEN protein phosphatase activity in these events. The role of PTEN in negatively regulating cell migration is probably the reason why PTEN can suppress tumor invasion and metastasis. Indeed, in a human bladder cancer cell line with a functionally impaired PTEN, it was found that transfection of wild-type *PTEN* inhibited both soft agar colony formation and cancer cell invasion, whereas transfection of the G129E mutant inhibited cancer cell invasion but failed to suppress colony formation, indicating a role for lipid phosphatase activity and protein phosphatase activity in inhibiting cell growth and cell migration, respectively [20]. The putative protein phosphatase activity of PTEN might also regulate the cell cycle, because the G129E mutant retains the capability to arrest cell cycle of MCF-7 cells as wild-type PTEN, while the G129R and H123Y mutants (they lack both phosphatase activities as the C124S mutant) do not [21]. More recently, PTEN was also suggested to be involved in maintaining chromosomal integrity, a tumor suppressive mechanism that might be independent of its lipid phosphatase activity as well [22].

Regulation of PTEN function

Why is PTEN function regulated?

Given that PTEN is such a crucial and multifunctional regulator in cells, it is conceivable that PTEN *per se* should also be delicately regulated. Understanding how PTEN is regulated is important not only in normal physiology but also from the perspective of cancer biology: it is possible that a decrease or total loss of PTEN function in human cancers might be caused indirectly by deregulation of PTEN in addition to a direct mutation or deletion of the *PTEN* gene. At the transcriptional level, such a cancer-associated PTEN deregulation can occur via transcription silencing by DNA methylation [23-25]; it can also be a consequence of loss of p53, as it has been reported that p53 can upregulate PTEN transcription [26]. In this review, we will focus only on posttranslational regulation of PTEN.

Experimentally, how the lipid phosphatase activity of PTEN was discovered immediately suggested that PTEN activity should be regulated posttranslationally. In the original publication from Dixon and colleagues, it was shown that overexpression of the catalytically inactive PTEN mutant (C124S) resulted in a further increase of PIP3 levels when cells were treated with insulin [12]. This and other results not only demonstrated that PTEN could decrease PIP3 levels but also indicated a dominant negative effect of the inactive PTEN mutant. How can the PTEN mutant be dominant negative? Competition for the substrate PIP3 with the endogenous wild-type PTEN is not possible considering the excessive amount of PIP3, a lipid small molecule, over PTEN, in cells. Thus it is only likely that the overexpressed PTEN mutant competes for certain regulatory/stimulatory factor(s) with the endogenous PTEN. In other words, endogenous PTEN activity is posttranslationally regulated by additional components.

Further, the PIP3 phosphatase activity of PTEN indicates that its cellular localization needs to be regulated posttranslationally. In many cell types (for example, cancerous or other immortalized cell lines, as used in earlier studies) PTEN appears to be predominantly cytosolic, but its PIP3 phosphatase activity requires it to be a plasma membrane protein, at least transiently in coordination with its biochemical activity. In certain specific tissues such spatial regulation of PTEN and the biological relevance have been unambiguously defined. For example, in chemotactic neutrophils, polarized PTEN is regulated by small GTPases, RhoA and Cdc42 in a phosphorylation dependent manner [27]. During epithelial morphogenesis, the apical plasma membrane localization of PTEN has been shown to be a crucial event [28]. Whether the apical plasma membrane PTEN localization is via its PIP2-binding ability or by other mechanisms is still an open issue. To make the story more complicated, recent evidence confirmed an early observation that PTEN can also be a nuclear protein. For example, PTEN nuclear localization appears to be reversely correlated with tumor progression [29], and positively correlated with cell differentiation in some tissues such as neurons [30]. These results suggest that PTEN nuclear localization is closely regulated and thus PTEN must perform an important biological function(s) in the nucleus.

Posttranslational modification of PTEN

One important mechanism for regulation of protein function is through posttranslational modification.

Indeed, PTEN has been reported to be modified in multiple ways including phosphorylation [31-34], oxidation [35-37] and the recently reported nitrosylation [38]. Progress along these lines has been limited although it is clear that all these modifications exert negative regulatory effects on PTEN function. The difficulty is partly due to the fact that the direct upstream regulators or the physiological cues that induce/remove these modifications have been elusive. As regulation of PTEN by these modes of modification has been discussed in depth in previous reviews [39-41], they will not be covered specifically in this review. Another mode of posttranslational modification of PTEN, ubiquitination, will be discussed in detail later in this review.

PTEN regulation from a structural point of view

To further appreciate the biochemical regulation of PTEN, a closer examination of its structure is necessary. As shown schematically in Figure 1, PTEN protein contains multiple domain structures. The phosphatase domain is the catalytic center of PTEN; predictably, some cancer-derived missense mutations are clustered in this region [8]. In the center of the protein, PTEN has a C2 domain that is required for its interaction with the membrane [19]. Importantly, it has been shown that the N-terminal residues 6 to 15 are also involved in membrane interaction, particularly with the phospholipid PIP2 [42]. Conceivably, both these regions are indispensable for the cellular function of PTEN to downregulate PI3K signaling.

PTEN protein also has a few regions susceptible to limited proteolysis suggesting that they might be highly flexible or unstructured. All these regions are not required for the enzymatic activity of PTEN. Intriguingly, these regions are highly conserved, signifying important regulatory function. One of these regions, spanning from residues 286 to 309, splits an otherwise classical C2 domain into two segments [19]. Whether this conserved loop region within the C2 domain can regulate the membrane translocation function of the C2 domain is an interesting question. A more defined regulatory role of this same region will be discussed later in this review.

Another long flexible fragment, the C-terminal tail (residues 352 to 403) of PTEN, is obviously involved in multiple modes of PTEN regulation. This region has several recognizable motifs, including a cluster of putative phosphorylation sites, a PDZ binding motif, and two PEST motifs (Figure 1). All these motifs are implicated in the physiological regulation of PTEN function. For example, several C terminal serine and threonine residues of PTEN have been shown to be phosphorylated in cells. It is widely accepted that such phosphorylation is required to maintain PTEN as a cytosolic protein; once these residues are dephosphorylated, PTEN can translocate to the plasma membrane and act as an active PIP3 phosphatase. Significantly, it appears that after such dephosphorylation, the protein stability of PTEN also decreases [31, 43,

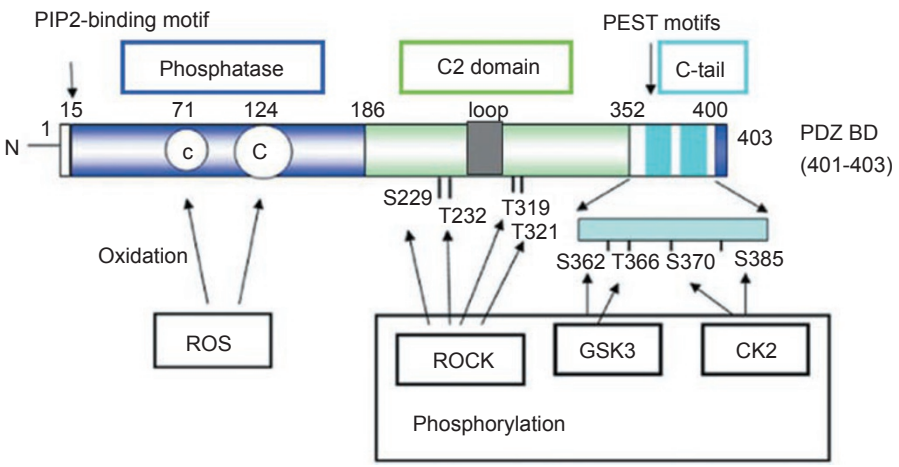

Figure 1 The domain structure of PTEN. The numbers denote the amino acid positions of individual domains or motifs of human PTEN. The three major domains of PTEN, the phosphatase domain ("Phosphatase", residues 15-186), the C2 domain (186-351), and the C-terminal fragment ("C-tail", 352-403) are labeled. The PIP2 binding motif spans from residues 6 to 15, and the PEST motifs from 350 to 375. "Loop" stands for the conserved but flexible region (286-309) within the C2 domain. "PDZ BD" stands for PDZ domain-binding motif. The figure also shows the cysteine residues in the phosphatase domain that are subjected to oxidation by reactive oxygen specifies (ROS), as well as multiple serine and threonine residues that can be phosphorylated by their correspondent kinases.

44], providing a potential feedback regulation of PTEN function. Therefore, it is important to understand how phosphorylation and dephosphorylation of these residues are regulated, which is still an open question. The PDZ binding motif of PTEN has been shown to associate with many PDZ domain-containing proteins including MAST205 (mircotubule-associated serine-threonine kinase 205 kDa) and MAGI-2 [45, 46]. There are also other proteins such as PICT1 that associate with the C terminus of PTEN in a PDZ domain-independent manner [32]. It is reasonable to assume that association of PTEN with these proteins is required for either regulation of these proteins by PTEN or vice versa. However, the effect of PTEN on the function of these proteins has not been investigated intensively, and whether these binding proteins directly regulate the enzymatic activity of PTEN has not been carefully examined either. Presumably these questions can be addressed biochemically. Interestingly, what has been reported is that interaction of PTEN with some of these partners affects the stability of PTEN (e.g., MAGI-2 and PICT1 can stabilize PTEN [32, 33, 43, 47]). Therefore, both the phosphorylation sites and certain binding proteins of the PTEN C-terminal region appear to be involved in regulation of PTEN stability. This makes the presence of two PEST motifs, putative signatures for proteins subjected to ubiquitin-mediated proteasomal degradation, in the same C-terminal region of PTEN more compelling!

Regulation of PTEN by ubiquitination

In addition to the reasons aforementioned, the study of PTEN protein stability was also inspired by the fact that PTEN degradation can be accelerated under certain conditions [48] and the provocative observations that many cancer-derived PTEN mutants manifested dramatic loss of protein stability in transfected cells [49]. Furthermore, subtle changes in wild type PTEN expression levels have been shown to pose dramatic long-term pathological effects in mouse models: PTEN expression level correlates inversely in an exquisite dose-dependent manner with prostate cancer progression, its incidence, latency, and the changes in the molecular signature of the PI3 Kinase-AKT pathway [50]. Loss of PTEN stability associated with its C-terminal tail phosphorylation was proposed to be through the ubiquitin-proteasomal pathway [31, 43].

In the ubiquitin-mediated proteasomal degradation pathway, the most import component is usually the enzyme known as ubiquitin ligase or E3, which determines both substrate specificity and reaction rate (read [51] for review for the ubiquitin-mediated proteasomal degradation pathway). Recently, our laboratory identified NEDD4-1 (neural precursor expressed developmentally downregulated 4-1), a HECT-domain protein, as the first E3 ubiquitin ligase for PTEN using a biochemical purification approach [52]. NEDD4-1 could potentiate oncogenic Ras-induced cell transformation in a PTEN-dependent manner, suggesting that aberrant upregulation of NEDD4-1 can posttranslationally suppress PTEN in cancers. Further, a reverse correlation of the protein levels of NEDD4-1 with PTEN was also observed in a mouse prostate cancer model and multiple human cancer samples, suggesting NEDD4-1 is a potential oncogene. More recently, it was reported that PTEN is able to decrease NEDD4-1 transcription [53], providing a negative feedback mechanism.

Intriguingly, in addition to promoting polyubiquitination and therefore degradation of PTEN, NEDD4-1 can also catalyze monoubiquitination of PTEN, which was shown by Pandolfi's laboratory to be critical for PTEN nuclear import (Figure 2) [54]. Importantly, certain Cowden syndrome-derived PTEN mutants, K13E and K289E, are defective in nuclear translocation. Because these mutants possess intact lipid phosphatase activity, it is highly likely that their loss of nuclear import might result in a decrease of PTEN tumor suppressive function [54]. Also, both K13 and K289 are ubiquitination sites of PTEN, and K289 is a major site for NEDD4-1 activity. Interestingly, K289 locates in the flexible/unstructured but conserved loop region within the C2 domain of PTEN, indicating that this loop region is involved in ubiquitination-mediated PTEN nuclear import. Although lacking experimental evidence, it is tempting to propose that ubiquitination of PTEN at K289, while being able to promote PTEN nuclear import, might also disrupt the function of the C2 domain for membrane localization and thus the more conventional function of PTEN to antagonize membrane PI3 kinase signaling. The molecular basis of PTEN nuclear translocation and the regulatory mechanisms have been reviewed recently in details elsewhere [55, 56].

On the other hand, induction of both PTEN proteasomal degradation and PTEN nuclear translocation by the same ubiquitin ligase is intriguing and somewhat puzzling because they might lead to opposing outcomes. In many cell types, the majority of NEDD4-1 is cytoplasmic, thus nuclear translocation of PTEN triggered by NEDD4-1-catalyzed monoubiquitination prevents PTEN from further polyubiquitination and degradation in the cytoplasm. Therefore the two functions of NEDD4-1 have quite opposite effects on PTEN, which strongly suggests that they should be differentially regulated in cells. The intrinsic biochemical property of NEDD4-1 makes such differential regulation achievable.

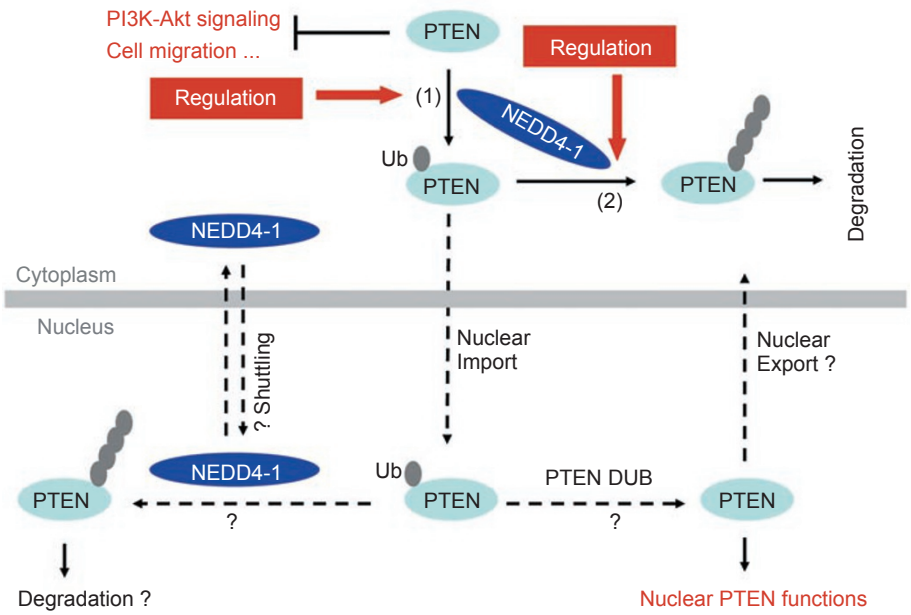

Figure 2 Regulation of PTEN by NEDD4-1-mediated ubiquitination. NEDD4-1-mediated PTEN monoubiquitination and polyubiquitination have different functional consequences. They are two separable reactions (labeled as (1) and (2)), and therefore might be subject to distinctive regulations. Ub stands for ubiquitin; DUB stands for deubiquitinating enzyme.

Enzymatically, NEDD4-1 acts with a distributive rather than processive kinetics towards PTEN [57], thus monoubiquitination and polyubiquitination of PTEN by NEDD4-1 are two separated steps; in other words, these two steps can be differentially controlled in cells (Figure 2). How these two activities of NEDD4-1 toward PTEN are triggered differentially under physiological conditions is an important question for future research. Furthermore, it has been reported that NEDD4-1 can also shuttle between the cytoplasm and nucleus [58, 59], adding one more layer of regulation to PTEN stability and subcellular localization.

The C-terminal tail of PTEN and ubiquitination

The C-terminal tail of PTEN is involved in NEDD4-1-catalyzed PTEN ubiquitination in an unexpected manner. Although the C-terminal tail possesses PEST motifs, surprisingly, we found that this fragment is not required for ubiquitination of PTEN by NEDD4-1. More strikingly, deletion of this fragment renders PTEN a stronger binding partner and better ubiquitination substrate of NEDD4-1 than full-length PTEN. Therefore, instead of mediating PTEN interaction with NEDD4-1, the C-terminal fragment of PTEN possesses a self stabilizing capability by antagonizing NEDD4-1-mediated PTEN polyubiquitination and degradation in cells [57]. Consistently, it was reported recently that the C-terminal fragment of PTEN intramolecularly interacts with the C2 domain of PTEN [60]. We reason this intramolecular interaction probably masks NEDD4-1 binding with PTEN. Work from our laboratory and others corroborate the notion that C-terminus of PTEN is essential for regulation of both activity and protein stability of PTEN in multiple ways (Figure 3). By forming intramolecular interactions with the C2 domain, the C-terminal tail may negatively regulate PTEN membrane binding potential thus exerting an inhibitory effect on the lipid phosphatase activity. The same interaction also protects PTEN from rapid degradation mediated by NEDD4-1, making PTEN a rather stable protein in resting state. It is possible that other events related to the C-terminal fragment, such as its phosphorylation and interaction with PDZ domain-containing proteins affect PTEN stability via positively or negatively modulating the interaction of the C-terminal tail and the C2 domain, and thus the accessibility of NEDD4-1 to PTEN, a formally testable scenario (Figure 2).

Given the critical role of the C-terminal tail in controlling PTEN degradation, deletion of this fragment by genetic mutation will result in rapid degradation of the truncated PTEN protein and subsequent loss of the tumor suppressive function (Figure 3), even though the C-terminal truncated PTEN is enzymatically intact as the full-length PTEN [19]. We believe this mechanism accounts for the fact that PTEN C-terminal truncation

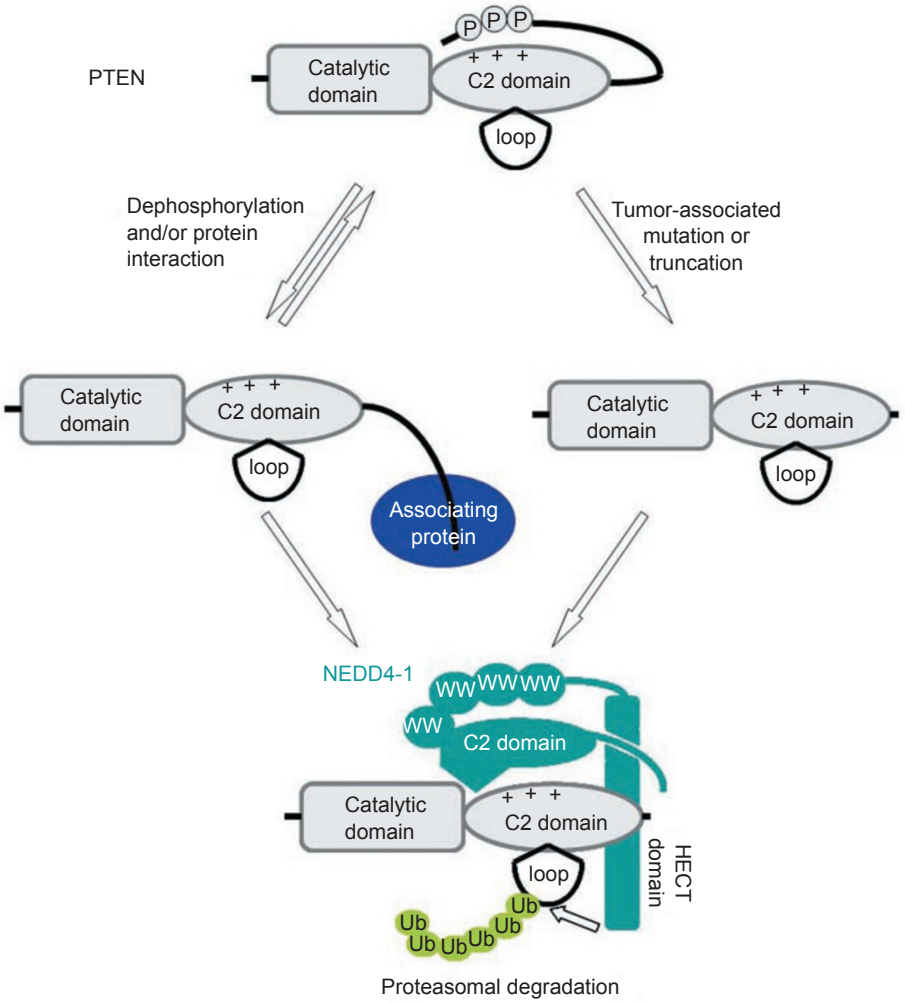

Figure 3 Regulation of PTEN stability by its C-terminal fragment. The C-terminal fragment of PTEN binds intramolecularly with the C2 domain. This binding can be strengthened by the interaction between the phosphorylation cluster of the C-terminal tail and a positively-charged surface of the C2 domain. The C-terminal associated proteins of PTEN might also modulate, either positively or negatively (as shown in the figure), this intramolecular interaction. NEDD4-1 can interact and ubiquitinate PTEN efficiently when this intramolecular interaction is released. "Loop" stands for residues 286 to 309 of human PTEN, which reside within the C2 domain and contain K289, a major ubiquitination site of NEDD4-1. "WW" and "Ub" stand for WW motif and ubiquitin, respectively.

mutation is frequently associated with human cancers, and propose that NEDD4-1 is a promising therapeutic target for treating such cancers.

A comparative analysis of PTEN versus p53

A comparison of PTEN with p53 also helps in understanding the function and regulation of PTEN. Unlike the tumor suppressor p53, which was initially thought to be an oncoprotein, the tumor suppressor identity of PTEN was unambiguously established in a rather short period of time after its cloning (reviewed in [61]). Like p53, PTEN is involved in many common cellular processes such cell growth, apoptosis [4, 62], differentiation [63], and maintenance of genomic stability [22, 64]. *PTEN* gene is also mutated with high frequency [8, 65-67] particularly in certain types of cancers such as primary glioblastoma [68], endometrial carcinoma [69-71], and late stage sporadic prostate cancers [72]. Further, both p53 and PTEN are regulated by a variety of posttranslational mechanisms, and the similarity is particularly striking concerning regulation by ubiquitination. Mdm2, a ubiquitin ligase for p53, induces both p53 degradation (polyubiquination) and nuclear export (monoubiquitination) [73]. Similarly, NEDD4-1, a ubiquitin ligase for PTEN, catalyzes both

PTEN degradation (polyubiquination) and nuclear import (monoubiquitination).

Yet, as a tumor suppressor, PTEN also has its unique features. In contrast to *p53* whose germline mutation has been shown to be associated with a single familiar cancer syndrome (Li-Fraumeni syndrome), many syndromes such as Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus syndrome (PS) and Proteus-like syndrome (PSL) have been linked to *PTEN* germline mutation [67]. Further, *PTEN* is essential for both development and cancer suppression [4, 5, 74], whereas *p53* knockout mice are developmentally normal but die of cancers months after birth [75]. This much wider clinical spectrum of *PTEN* germline mutations and the additional roles of PTEN in development manifest fundamental differences between p53 and PTEN.

At the molecular level, one obvious difference between these two crucial tumor suppressors has to be their default levels/activities. Under normal conditions, PTEN is a stable protein and the function of NEDD4-1 is probably suppressed. Cellular PTEN protein is constitutively or partially active, because PTEN expression levels are inversely correlated with the phospho-AKT levels in many tissues [4, 50]. In contrast, via Mdm2-mediated ubiquitination and proteasomal degradation, p53 protein is usually expressed at very low level, and only increases its expression and thereby executes its activity when cells respond to various types of stress [76, 77]. Therefore, it appears that the function of PTEN is constantly present or required in cells, whereas p53 activity is needed only under specific situations.

This notion is further supported by the striking difference of cancer mutation spectra between *PTEN* and *p53*, which may reflect the different selection pressure on these tumor suppressor genes in human cancers. Most *p53* cancer mutations (80%) are missense mutations that are clustered in the DNA binding domain, resulting in loss of the transcriptional activity of p53. Moreover, the severity of the mutation on transcriptional activity is tightly correlated with cancer onset in patients bearing *p53* germline mutation [78]. This implies that the selection pressure for *p53* in cancer is to eliminate its transcriptional activity. Hence, the transcriptional activity of p53 is essential for its tumor suppressor function. On the other hand, *PTEN* somatic mutations scatter along the entire gene. According to an early documentation [8], these mutations consist of 49% of frameshift mutation, 30% of missense mutation, and 15% of nonsense mutation. Surprisingly, only a small percent of the somatic mutations are the missense mutations at the core phosphatase domain. Importantly, 72% of total *PTEN* cancer mutations result in PTEN truncation [8]. Interestingly, one hot spot for frameshift and nonsense mutations is near the end of the C2 domain and beginning of the flexible C terminus, resulting in loss of the functional C-terminal fragment of PTEN protein. Although many of these mutants no longer contain the intact C2 domain and thus lose the enzymatic activity [49], some of these mutants still possess a complete C2 domain. As we discussed earlier, such mutations do not abrogate the enzymatic activity of PTEN, but they accelerates NEDD4-1-mediated PTEN degradation. Therefore, the selection pressure for such PTEN mutations in cancer seems to be elimination of the PTEN protein physically. This is consistent with the observation that usually one can readily detect wild-type PTEN in normal tissues by immunohistochemistry but not mutant PTEN in cancer tissues [79]. In contrast, wild type p53 is hardly detectable in normal tissues without stress while the mutant p53 (inactive) is readily seen in many cancer tissues. For all these reasons, we can categorize PTEN tumor suppressor as a default gatekeeper (because it is always awake in cells to suppress tumorigenesis) and p53 as a dormant gatekeeper (because it only functions in response to emergency).

Preliminary studies indicate that PTEN and p53 might also communicate with each other in many ways. For example, p53 upregulates PTEN transcription via a p53-binding element upstream of exon 1 of the Pten gene [26]; conversely, PTEN was reported to be able to stabilize p53 in a phosphatase activity-independent manner through either direct physical interaction [80, 81] or maintenance of p53 acetylation [82]. Intriguingly, there appears to be a fail-safe mechanism that cells utilize to awake the dormant tumor suppressive function of p53 when the function of the default gatekeeper PTEN is lost. This fail-safe mechanism is likely operated via the ability of PTEN to regulate CHK1 localization through AKT-mediated phosphorylation of CHK1 and subsequent ubiquitination and cytoplasmic sequestration. Loss of PTEN protein was found to cause altered CHK1 cytoplasmic localization, leading to a defective checkpoint response and subsequent p53 activation [64]. Indeed, *in vivo* evidence from mouse genetics has provided strong support for the existence of a functioning network consisting of both the default and dormant gatekeepers. It has been revealed that conditional inactivation of p53 did not produce prostate tumors in mice while complete inactivation of Pten in the prostate caused non-lethal invasive prostate cancer after long latency. Strikingly, inactivation of both *Pten* and *p53* generated lethal prostate cancer phenotype with extremely early onset [83]. In this system, the reason why loss of the default gatekeeper *Pten* did not produce

fully-fledged prostate malignancy is probably because that acute *Pten* loss triggered a cellular senescence program via activation of the dormant gatekeeper p53. Thus, the p53-dependent senescence might be a crucial barrier for the development and progression of lethally invasive prostate cancer after cells lost the default tumor suppressor PTEN, establishing p53 as the key player in this fail-safe anticancer mechanism.

Perspectives

Almost all of the early studies on PTEN focused on its tumor suppressive roles by antagonizing PI3K-Akt signaling. Recent research revealed more unexpected roles of PTEN in both cancer biology and normal physiology. Several areas that will probably be actively pursued in the near future include defining and dissecting developmental and tissue-specific functions of PTEN. In particular, the tissue-specific role of PTEN in cell stemness demands further investigation, considering PTEN has been shown to be essential for maintenance of hematopoietic stem cells but to be a negative regulator of neuronal stem cell maintenance [84-87]. These studies require sophisticated *PTEN* animal models because (1) conventional homozygous *PTEN* gene deletion results in early embryonic lethality, and (2) PTEN has multiple functions independent of its PIP3 phosphatase activity. In addition, as a master cell regulator, the various functions of PTEN have to be precisely controlled, thus understanding how PTEN itself is regulated is the key to appreciate the time- and spatial-specific functions of PTEN. Questions concerning this aspect include direct regulation of PTEN function at its biochemical activity level (which, surprisingly, is a totally unexplored area); physiological regulation of PTEN ubiquitin ligase NEDD4-1 (considering PTEN is generally a stable protein); whether PTEN has multiple E3 ligases as p53 does; and regulation of PTEN subcellular localization (linked to it, the exact role of nuclear PTEN). Further, because PTEN is subjected to multiple fashions of posttranslational modification, to identify the enzymes required for the modification, to determine how these events are regulated and the functional impact on PTEN in a tissue/context-specific manner should also provide insights into understanding the physiological and pathological functions of PTEN.

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