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NHERFs, NEP, MAGUKs, and More: Interactions That Regulate PTEN

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Abstract

This year marks the 10th anniversary of the discovery of the PTEN/MMAC1/TEP1 tumor suppressor gene (hereafter referred to as PTEN), one of the most commonly mutated genes in cancer. PTEN encodes a lipid phosphatase that dephosphorylates phosphoinositide-3,4,5-triphosphate (PIP₃), thereby counteracting mitogenic signaling pathways driven by phosphoinositide-3-kinases (PI3K). By opposing PI3K signaling, PTEN inhibits the activation of the critical PI3K effector proteins Akt1-3 (also known as protein kinase B or PKB). Given its central role in antagonizing PI3K signaling, one might expect that like PI3K, the activity of the PTEN protein would be highly regulated by numerous protein/protein interactions. However, surprisingly little is known about such interactions. This fact, combined with the generally accepted notion that phosphatases are less exquisitely regulated than kinases, has led to the idea that PTEN may function in a relatively unregulated fashion. Here we review the identities and proposed functions of known PTEN-interacting proteins, and point out avenues of investigation that we hope may be fruitful in identifying important new mechanisms of PTEN regulation in mammalian cells.

Keywords

PTEN; protein/protein interactions; cancer

Sporadic inactivating mutations of PTEN are found in a wide range of common human cancers, and inherited inactivating mutations of PTEN cause the rare cancer predisposition syndromes now collectively known as PTEN Hamartoma Tumor Syndrome [PHTS; Li et al., 1997; Steck et al., 1997; Pilarski and Eng, 2004].

PTEN is a relatively small (55 kDa) protein with at least five different functional domains (Fig. 1). These include the phosphatase domain, the C2 regulatory domain, a phosphoinositide-4,5-bisphosphate (PIP₂) binding domain, two consecutive PEST homology

domains, and a PDZ-binding domain. The primary substrate of PTEN is the mitogenic membrane-associated lipid, PIP₃ [Maehama and Dixon, 1998]. PTEN works to oppose PI3K, a family of kinases that phosphorylate PIP₂ to PIP₃ in response to mitogenic signals and other stimuli. PIP₃ then binds to the Pleckstrin Homology (PH) domain of the Akt proteins and recruits them to the inner leaflet of the plasma membrane where they are phosphorylated by mTOR and other kinases. This activates their serine/threonine kinase activity and initiates a complex signal transduction cascade that modulates cellular survival, growth, migration, and metabolism.

A variety of groups have attempted to identify PTEN interaction partners in the hopes of discovering novel means of PTEN regulation and possibly identifying new PTEN effectors. These studies have been technically challenging, as evidenced by the fact that there have been no reports of interacting proteins that immunoprecipitate with PTEN efficiently enough to be visible with standard protein stains. Instead, the majority of the PTEN-interacting proteins described in the literature have been initially identified by the yeast two-hybrid method or by candidate immunoprecipitation/Western blot approaches. It appears likely that PTEN interactions are weak, transient, and/or destabilized by the detergents that are commonly used for cellular lysis. Furthermore, the predominant membrane localization of PTEN may complicate the purification of PTEN-containing protein complexes. Of note, PTEN is also regulated via post-translational modifications, and in some cases has been shown to interact (probably transiently) with the enzymes that catalyze these modifications. A discussion of the enzymes that catalyze these post-translational modifications is beyond the scope of this review.

Here we summarize the PTEN protein/ protein interactions that have been described in the literature thus far. The majority of them are believed to aid in the intracellular localization of PTEN, with a minority proposed to affect signaling, genomic stability, and other cellular processes. With exceptions as noted, many of these suggested interactions have not yet been independently confirmed.

MEMBRANE LOCALIZATION VIA BINDING TO MAGI PROTEINS

MAGI proteins are members of a class of proteins known as Membrane-Associated Guanylate Kinase Homologues (MAGUKs), large proteins that are thought to serve as membrane-associated scaffold proteins and as guanylate kinases. MAGUKs localize to the inner leaflet of the plasma membrane, often at specific domains such as tight junctions and adherens junctions. MAGI proteins contain regions known as PDZ domains, stretches of approximately 80 amino acids often found as repeating units in a wide variety of proteins, particularly membrane-associated scaffold proteins. As depicted in Figure 1, the carboxylterminus of PTEN contains a putative PDZ-binding domain that permits interaction with PDZ domain-containing proteins.

In the year 2000, three papers were published describing interactions between the PTEN PDZ-binding domain and PDZ domains present in other proteins. A recurring theme among these interactions was the association of PTEN with MAGI proteins. Wu et al. [2000a] performed a yeast two-hybrid screen using the entire human PTEN cDNA (C124S, a

catalytically inactive mutant) as bait. They identified MAGI-2, a PDZ domain-containing protein, as a putative interactor, and mapped the interaction site to the PDZ-binding motif of PTEN and the second PDZ domain of MAGI-2. This interaction between MAGI-2 and PTEN was confirmed in both transfected and untransfected cells. Wu et al. [2000b] also performed a yeast two-hybrid screen with full length, catalytically inactive PTEN as the bait. They identified MAGI-3 as an interacting protein via its PDZ domain, and confirmed the interaction in transfected cells. Finally, Adey et al. [2000] also performed a yeast two-hybrid screen using the carboxyl-terminal 126 amino acids of human PTEN as bait. They identified two PDZ domain-containing interacting proteins— discs-large (hDLG) and hMAST205—as putative PTEN-interacting proteins. These interactions were confirmed using biochemical methods with purified proteins. These three contemporaneously-published papers unambiguously demonstrated that the PDZ-binding motif of PTEN can mediate protein/protein interactions, identified several PDZ domain-containing proteins as PTEN-interacting proteins, and specifically pointed to the MAGI proteins as an important group of PTEN binding partners.

Subsequent studies revealed a third MAGI protein capable of binding to PTEN and provided additional important information regarding the interaction between PTEN and the PDZ domain-containing MAGI proteins. Kotelevets et al. [2005] showed that PTEN could bind to MAGI-1b via a PDZ domain-mediated interaction. They further demonstrated the presence of the PTEN-MAGI-1b complex in both transfected and untransfected mammalian cells, and showed that the MAGI-1b protein serves as a scaffold protein that localizes PTEN to junctional complexes by binding to both PTEN and β -catenin.

Tolkacheva et al. [2001] confirmed the interaction between PTEN and MAGI-2 and demonstrated that phosphorylation of two carboxyl-terminal residues of PTEN (T382 and T383) reduced the affinity of PTEN for MAGI-2. Finally, Subauste et al. [2005] showed that cells lacking vinculin, an important component of the adherens junction, did not express detectable levels of PTEN protein until vinculin expression was restored. These and other related experiments raised the intriguing possibility that localization of PTEN to the adherens junction via binding to MAGI proteins stabilizes the PTEN protein. This hypothesis was supported by Valiente et al. [2005], who also demonstrated that PTEN proteins lacking the PDZ-binding domain have decreased stability. They also identified MAST3/4 and SAST as two additional PDZ domain-containing PTEN interacting proteins, and further suggested that binding to PDZ domain-containing proteins at cell/cell junctions may help stabilize the PTEN protein. The binding of PTEN to MAGI proteins via recognition between a PDZ-binding motif and PDZ domain is the most extensively-studied PTEN interaction demonstrated thus far.

MEMBRANE LOCALIZATION BY BINDING TO BAZOOKA/PAR-3

Recent studies in model organisms have demonstrated that the establishment and maintenance of cellular polarity is dependent on a protein complex known as PAR/aPKC. In mammalian cells, the PAR/aPKC complex is required for the formation of tight junctions. This complex consists of at least four proteins— an atypical protein kinase C isoform (aPKC), Bazooka/PAR-3, PAR-6, and Cdc42. In a yeast two-hybrid screen designed to

identify proteins that bind to Bazooka/PAR-3 (which contains PDZ domains), Von Stein et al. [2005] isolated three independent clones of PTEN. They also showed that the PDZ-binding domain of PTEN binds directly to the second and third PDZ domains of Bazooka/PAR-3, recapitulated this interaction in untransfected *Drosophila* S2 cells, and proved that the two proteins could co-localize using immunocytochemistry. Additionally, they demonstrated that PTEN plays a role in controlling several actin-dependent processes. The association between PTEN and Bazooka/PAR-3 was confirmed by Pinal et al. [2006], who further showed that Bazooka/PAR-3 recruits PTEN to developing adherens junctions and helps regulate the differentiation of the apical membrane into specialized functional domains. It is interesting to note that both the PTEN-Bazooka/PAR-3 and the PTENMAGI interactions function to recruit PTEN to specialized regions of the plasma membrane. Future studies designed to determine whether the interaction with Bazooka/PAR-3 is conserved in human cells (where it is known as PARD3) should be informative. We are also curious to further explore the role this interaction might play in influencing cellular transformation.

MEMBRANE LOCALIZATION BY BINDING TO CAVEOLIN, FAK/PAXILLIN, AND NEP

It has recently been proposed that PTEN localizes to the plasma membrane via invaginations known as caveolae. Formation and maintenance of these invaginations is attributable to the caveolin protein. Caselli et al. [2002] noticed that PTEN, along with many other protein tyrosine phosphatases, contained a putative caveolin-binding motif (Ψ X Ψ XXXX Ψ or Ψ XXXX Ψ XX Ψ , where Ψ is an aromatic residue and X is any amino acid; found at residues 271–278 of PTEN [FHFVWNTF]). Using subcellular fractionation, and co-immunoprecipitation, they demonstrated that endogenous PTEN, together with each of four other phosphatases, can interact with endogenous caveolin in human cells. This study demonstrated another means of PTEN membrane localization and suggested that it may be enriched specifically at caveolae.

Focal Adhesion Kinase (FAK) is a cytoplasmic protein tyrosine kinase that concentrates at focal adhesions whose activity is regulated by cell adhesion. FAK is recruited to the sites of focal adhesions in part because of an interaction with Paxillin, a multidomain adapter molecule that provides binding sites for multiple proteins at focal adhesions. Paxillin was initially identified as a protein that became phosphorylated when chick embryo fibroblasts were transformed with Rous sarcoma virus (RSV). Tamura et al. [1998, 1999] demonstrated that FAK was a PTEN binding protein and PTEN substrate, and that this interaction modulated several FAK-related phenotypes including adhesion, migration, and cell spreading. Haier and Nicolson [2002] further showed that both Paxillin and FAK can bind to PTEN and demonstrated that when cells were grown under adherent conditions, PTEN could regulate integrin-dependent cell adhesion events. Importantly, these interactions were confirmed by Crockett et al. [2005] and Herlevsen et al. [2007]. Like several other PTEN/protein interactions discussed so far, the association of PTEN to FAK/ Paxillin appears to target PTEN to specific regions of the plasma membrane, where it can regulate signaling pathways (Table I).

Neutral Endopeptidase 24.11 (NEP) is an integral membrane protein with an extracellular catalytic domain that cleaves and inactivates neuropeptides. Recent work has also implicated NEP in regulating cell migration and PI3K pathway activation. Sumitomo et al. [2004] demonstrated that NEP can directly interact with PTEN via electrostatic forces between the negative charged phosphorylated region in the carboxyl-terminus of PTEN and a cationic alkaline stretch of NEP's intracellular tail. The authors further showed that NEP recruits PTEN to the plasma membrane and enhances its stability and phosphatase activity. They also demonstrated that NEP can partially inactivate Akt and function as a growth suppressor in human cancer cells. These findings suggest that NEP-mediated membrane localization of PTEN, similar to localization of PTEN to the adherens junction by MAGI proteins, helps stabilize the PTEN protein and enhances its phosphatase activity.

LOCALIZATION TO VAULTS VIA INTERACTION WITH MAJOR VAULT PROTEIN

Vaults are intracellular ribonucleoprotein structures of largely unknown function that are located principally in the cytoplasm. Vaults are composed of three proteins—major vault protein (MVP), vault poly(ADP-ribose) polymerase (VPARP), and the telomerase-associated protein 1. Though their function remains mysterious, it has been proposed that vaults play a role in nucleocytoplasmic transport. Yu et al. [2002] performed a yeast two-hybrid screen using PTEN as bait and found that almost half of the positive clones encoded MVP. They then confirmed this interaction in both transfected and untransfected cells. Furthermore, they showed that the regulatory C2 domain of PTEN was required for the interaction, and that the interaction was Ca^{2+} - dependent. Importantly, Chung et al. [2005] confirmed this interaction and provided evidence that the interaction with MVP is required for nuclear import of the PTEN protein. As the nucleocytoplasmic transport of PTEN is currently an area of intense interest, the role of the PTEN–MVP interaction will undoubtedly continue to shed additional light on the regulation of PTEN.

REGULATION OF P53 ACTIVITY BY INTERACTION WITH PTEN

It is perhaps not surprising that individual tumor suppressor proteins might physically interact and form regulatory networks. Freeman et al. [2003] demonstrated that PTEN binds to endogenous p53 in both mouse and human cells, and also showed this interaction may enhance the transcriptional activity of the p53 protein. Subsequent reports confirmed this interaction [Zhou et al., 2003]. In a related study, Li et al. [2006] demonstrated that PTEN can modulate p53 activity by forming a complex with p300, enabling regulation of p53's acetylation state. Together, these studies suggested that the presence of PTEN serves to activate p53 activity through both direct and indirect protein/ protein interactions. However, it is worth noting that at present there is disagreement in the field regarding the effects of PTEN on p53 activity. Studies by Chen et al. [2005] and Kim et al. [2007] in our group have demonstrated that the presence of PTEN can inhibit the activity of p53 through an as yet unknown biochemical mechanism. The relationship between PTEN and p53 is an active area of investigation, as there are clearly numerous points of intersection between these important tumor suppressor proteins.

MODULATION OF SIGNALING PATHWAYS BY INTERACTION WITH THE PDGF, S1P2, NMDA, AND ANDROGEN RECEPTORS

A recent study by Takahashi et al. [2006] demonstrated that PTEN can bind to the two homologous PDZ domain-containing adapter proteins NHERF1 and NHERF2 (Na^+/H^+ Exchanger Regulatory Factor) and function in a ternary complex with the PDGF receptor (PDGFR) to regulate PI3K signaling in response to PDGF ligand. The binding between PTEN and NHERF1/NHERF2 occurs between the PDZ-binding motif of PTEN and the first PDZ domain of NHERF1/NHERF2. Like the other PDZ-domain interactions, this association was first identified in a yeast two-hybrid screen, and in this case then confirmed using both purified proteins and endogenous proteins in untransfected cells. The authors also observed prolonged PI3K activation following PDGF stimulation in both NHERF^{-/-} MEFs and NHERF-depleted cells. This finding is particularly intriguing in light of a previous report by Mahimainathan and Choudhury [2004] suggesting that PTEN can bind directly to the PDGFR and inhibit PDGF-induced responses. It is also interesting that Le Dai et al. [2004] have identified putative mutations of NHERF in human breast cancer. Taken together, these studies suggest that PTEN can co-localize with the PDGFR via NHERF adapter proteins and/or direct interaction, and may modulate PDGF-induced signaling responses.

PTEN has also been shown to interact with several other receptors. The bioactive lipid sphingosine 1-phosphate signals through the S1P2 G protein-coupled receptor to inhibit cell migration. Sanchez et al. [2005] have demonstrated that PTEN interacts with this receptor in a ligand-dependent fashion and that this interaction is necessary for S1P effects on inhibition of cell migration. Ning et al. [2004] have described a similar role for PTEN in signal transduction mediated through NMDA receptors. They showed that PTEN physically associates with the NR1 and NR2B subunits of the NMDA receptors, and that inhibition of PTEN can inhibit the function of these receptors. Finally, Lin et al. [2004] have found that PTEN can bind to the androgen receptor and suppress its activity in human prostate cancer cells.

MODULATION OF MSP58-INDUCED TRANSFORMATION BY INTERACTION WITH PTEN

MSP58 (also known as MCRS1) is a 58 kDa microspherule protein that is induced after expression of the v-jun oncogene and can confer anchorage-independent growth. Okumura et al. [2005] identified a physical interaction between the carboxyl-terminal region of PTEN and the Forkhead-Associated Domain (FHA) of MSP58. They further showed that PTEN can inhibit MSP58-induced transformation and that the phosphatase activity of PTEN is dispensable for this suppression. This particular interaction demonstrates the ability of PTEN to bind to and regulate the activity of a novel oncogene.

REGULATION OF TGF- β SIGNALING BY INTERACTION WITH SMAD2 AND SMAD3

TGF- β signaling plays important roles in both suppressing and promoting tumorigenesis, depending on the cellular milieu. Smad2 and Smad3 are intracellular signal transduction molecules that are critical for translation of ligand stimulation into transcriptional responses. Hjelmeland et al. [2005] have recently showed that endogenous PTEN can bind to both Smad2 and Smad3 in human keratinocytes, and that this binding occurs in a ligand-dependent fashion. They also found that this interaction inhibits TGF- β -mediated transcriptional responses and a subset of TGF- β induced phenotypes. This finding is particularly interesting in light of the observation that Akt and FOXO family members can also interact with Smad3, and therefore further emphasizes the important cross-regulation that occurs between the TGF- β and PI3K signaling pathways.

MAINTENANCE OF CHROMOSOMAL STABILITY VIA INTERACTION WITH CENP-C

Chromosomal instability is one of the hallmarks of cancer, though the genetic events that lead to instability remain virtually a complete mystery. Shen et al. [2007] recently suggested that mutational inactivation of PTEN might be one factor driving genomic instability. They reported that PTEN-deficient MEFs were characterized by extensive centromere breakage, chromosomal translocations, and chromosomal instability. In an effort to determine the molecular basis for this defect, they performed IP/ Western analysis with CREST antiserum that recognizes multiple centromere proteins and demonstrated that PTEN can bind to the centromere protein CENP-C. This interaction was detected with endogenous proteins in untransfected cells. They went on to show that the physical association of PTEN with centromeres is critical for maintaining genomic stability, and that PTEN deficiency leads to an increase in DNA double strand breaks (DSBs). These data propose a completely new avenue for PTEN research, and suggest that PTEN may play multiple roles in the suppression of malignancy.

REGULATION OF PTEN POST-TRANSLATIONAL MODIFICATION VIA INTERACTION WITH PICT-1 AND THIOREDOXIN-1

It is by now well established that PTEN is regulated by post-translational modifications including phosphorylation, ubiquitination, acetylation, and oxidation. Though a discussion of the enzymes that catalyze these modifications is beyond the scope of this review, the identity, and activity of two proteins that regulate these activities are discussed below.

Protein Interacting with Carboxyl-Terminus 1 (PICT-1) is a novel protein that was recently shown to interact with the carboxyl-terminal domain of PTEN both in a yeast two-hybrid screen and in mammalian cells [Okahara et al., 2004]. Importantly, residues critical to maintaining the stability of the PTEN protein were shown to be necessary for the binding of PTEN to PICT-1. Furthermore, the binding of PICT-1 to PTEN was found to promote the

phosphorylation of PTEN and enhance its stability. As such, PICT-1 is a novel protein that appears to regulate the phosphorylation and therefore the stability of PTEN.

It has been convincingly shown that PTEN can also be regulated by changes in the cellular oxidation state. Meuillet et al. [2004] have proposed that a covalent interaction with the well-studied, small thiol-disulfide oxidoreductase known as Thioredoxin-1 (Trx-1) may be at least partially responsible for this effect. In particular, they demonstrated that reduced Trx-1 can bind to PTEN and inhibit its lipid phosphatase activity. Whether this is the major mechanism of oxidation state-regulated control of PTEN remains a subject of ongoing study.

OTHER INTERACTIONS

Crockett et al. [2005] has reported the interaction of PTEN with Caspase 8, Cyclin E2, IRS4, and PP2A. Herlevsen et al. [2007] identified the protein TRK Fused Gene (TFG) as a PTEN-interacting protein. As the potential functional relationships of these proteins to PTEN remains unknown, further work is required to characterize these proposed interactions.

CONCLUDING REMARKS

As described here, a variety of PTEN-interacting proteins with numerous proposed functions have been identified and characterized. A subset of these interactions have been independently confirmed, including the interactions of PTEN and (1) various PDZ domain-containing proteins such as the MAGUKs and Bazooka/PAR-3; (2) the PDGFR complex; (3) p53; (4) MVP; and (5) FAK/Paxillin. Work is currently ongoing in multiple labs to extend these findings and confirm several of the other more recently identified interactions.

There is clearly much more work to be done. Though a variety of interacting proteins have been identified, this work has been undeniably difficult, perhaps due to the fact that much of PTEN is localized to the cell membrane, or the possibility that PTEN-containing complexes may be unstable in standard cellular lysis buffers. It may be that efforts to identify PTEN complexes using specialized methods to preserve membrane complexes and/or the use of non-detergent lysis techniques could preserve additional PTEN protein/protein interactions. However, others in the field believe that these apparent technical difficulties in identifying PTEN-interacting proteins reflect the possibility that phosphatases such as PTEN are comparatively unregulated and therefore have relatively few bona fide protein partners. We believe that a gene as important to maintaining cellular homeostasis and cancer as PTEN is likely to be carefully regulated at numerous levels, including by multiple as yet unknown protein/protein interactions. The first decade of PTEN research was fruitful and productive; we expect that the second decade of investigation will provide substantial additional insights into the mechanisms and pathways that regulate this critical tumor suppressor protein.

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Fig. 1.

PTEN functional domains. Human PTEN includes at least five different functional domains—a PIP₂ binding domain (1–15), a phosphatase domain (22–185), a C2 regulatory domain (190–351), PEST homology domains (350–375, 379–396), and a PDZ binding domain (401–403).

TABLE I

Proteins Shown to Interact With PTEN

Name	Function	Region of PTEN	Demonstrated with endogenous proteins?	Reference(s)
MAGI-2	Membrane-associated scaffold	PDZ-BD	Yes	Wu et al. [2000a], Tolkacheva et al. [2001], Subauste et al. [2005]
MAGI-3	Membrane-associated scaffold	PDZ-BD	No	Wu et al. [2000b]
hDLG	Tumor suppressor	PDZ-BD	No	Adey et al. [2000]
hMAST205	Kinase	PDZ-BD	No	Adey et al. [2000]
MAGI-1b	Membrane-associated scaffold	PDZ-BD	Yes	Kotelevets et al. [2005]
SAST	Kinase	PDZ-BD	No	Valiente et al. [2005]
MAST3	Kinase	PDZ-BD	No	Valiente et al. [2005]
Bazooka/PAR-3	Cell polarity/zonula adherens	PDZ-BD	Yes	Von Stein et al. [2005], Pinal et al. [2006]
Caveolin-1	Caveolae	C2	Yes	Caselli et al. [2002]
FAK/Paxillin	Focal adhesions/signaling	N.D. ^a	Yes	Tamura et al. [Tamura et al., 1998, Tamura et al., 1999]. Haier and Nicolson [2002], Crockett et al. [2005], Herlevsen et al. [2007]
NEP	Neuropeptidase	C2	Yes	Sumitomo et al. [2004]
MVP	Vaults	C2	Yes	Yu et al. [2002], Chung et al. [2005], Herlevsen et al. [2007]
p53	Tumor suppressor	C2	Yes	Freeman et al. [2003], Zhou et al. [2003]
NHERF	PDGFR binding protein	PDZ-BD	Yes	Takahashi et al. [2006]
PDGFR	Mitogenic signaling	C2	Yes	Mahimainathan and Choudhury [2004]
S1P2R	Signaling/cell migration	N.D.	No	Sanchez et al. [2005]
NMDAR	Neuronal signaling	N.D.	Yes	Ning et al. [2004]
AR	Androgen signaling	Phosphatase/C2	Yes	Lin et al. [2004]
MSP58	Oncogene	C2	Yes	Okumura et al. [2005]
Smad2/3	TGF- β signaling	Phosphatase	Yes	Hjelmeland et al. [2005]
CENP-C	Centromere/genomic stability	C2	Yes	Shen et al. [2007]
PICT-1	Unknown	C2	Yes	Okahara et al. [2004]
Thioredoxin-1	Oxidation state	C2	Yes	Meuillet et al. [2004]
Caspase 8, CyclinE2, IRS4, PP2A	Various	N.D.	Yes	Crockett et al. [2005]
TFG	Unknown	N.D.	No	Herlevsen et al. [2007]

^aNot determined.