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Mini-Review

PTEN-associated complexes: An overview

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ABSTRACT

PTEN is a tumor suppressor best characterized for its role as a lipid phosphatase in antagonizing the PI3-kinase pathway. Several recent studies have identified proteins that form high molecular weight complexes with PTEN in different subcellular compartments. PTEN is critical for early embryonic development, cell proliferation, cell survival and stem cell function. The discovery of PTEN complex components may help our understanding of its biological functions. In this review, PTEN complex components, functions and their regulation will be discussed.

KEYWORDS: PTEN, interaction, complex, phosphorylation, PI3-kinase

INTRODUCTION

PTEN (phosphatase and tensin homologue deleted on chromosome 10) is one of the most commonly mutated tumor suppressors in human cancers [1, 2], including glioblastomas [3, 4], endometrial carcinomas [5] and prostate cancers [6]. PTEN contains an N-terminal phosphatase domain, which dephosphorylates the lipid substrate phosphatidylinositol-3,4,5-trisphosphate (PtdIns (3,4,5)P3) *in vivo*, and by this mechanism PTEN antagonizes the effects of phosphatidylinositol 3-kinase (PI3-kinase) [7, 8]. Although based on *in vitro* analysis PTEN also has dual-specific protein phosphatase activity [9], its intracellular targets are yet to be defined. In addition to PTEN's catalytic domain, the C-terminus, which is composed of the C2 domain, the C-terminal tail and the PDZ (PDS-95/Disc-large/Zo-1) binding motif, also plays important roles in regulating PTEN function.

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Study of PTEN interacting partners and complex formation

Protein-protein interactions can modulate known and novel PTEN functions through regulating complex formation, protein stability and subcellular localization. Several methodologies have been used to identify such interaction partners and assess their complex assembly with PTEN, including yeast two hybrid screens, affinity pull-down assays and size exclusion chromatography.

The yeast two hybrid (YTH) screen was originally designed by Stanley Fields and uses a reporter assay read-out for the detection of protein-protein interactions in yeast [10]. In this system the bait and prey of interest are fused to the binding domain and activating domain of a transcription factor, respectively; and the binding of the two proteins results in the activation of the downstream reporter gene. This method has been utilized for the identification of novel PTEN binding partners with full length or the C-terminus of PTEN as a bait screened against various cDNA libraries. This has led to the identification of MAGI2, MAGI3, MVP, MSP58, NHERF1, PICT-1 and LKB1 as potential PTEN interacting proteins [11-17].

In addition to YTH, affinity pull-down assays have been used for identification of the PTEN

interactome. This method uses affinity tagged PTEN, such as Flag, HA, His or GST, to pull-down PTEN interacting proteins. In a study by Crockett and colleagues, using His-tagged PTEN as a bait, 79 interacting proteins from a lymphoma cell line were identified using mass spectrometry analysis [18]. Moreover, tandem affinity purification of Flag-HA tagged PTEN led to the identification of PCAF as an interacting partner and acetylating enzyme of PTEN [19]. Recently, pull-down of GST tagged PTEN from cytoplasmic extracts of a human glioblastoma cell line led to the purification and identification of P-REX2a, a guanine nucleotide exchange factor, which interacts with and regulates PTEN lipid phosphatase activity [20].

Studies initiated by Vazquez and colleagues have suggested that PTEN may function within large complexes [21]. Size exclusion chromatography analysis, which allows separation of proteins by size, on lysates isolated from various tissues and cells lines have demonstrated the presence of PTEN in a very high molecular weight complex of >650KDa, in addition to a lower molecular weight monomer (50-100KDa) [15, 21-24]. Using this methodology several novel and known binding partners of PTEN have been shown to exist in these large complexes. In this brief review, we will specifically focus on the components of these complexes and their potential functions.

PTEN complexes in different subcellular compartments

Membrane

PTEN localization to the plasma membrane is critical for binding to and dephosphorylation of its lipid substrate, PtdIns(3,4,5)P3, resulting in negative regulation of the PI3-kinase pathway. PTEN's recruitment to the plasma membrane is mediated by 1) the PtdIns(4,5)P2 binding site at the N terminus 2) the electrostatic interactions with lipids through its C2 domain [25, 26] and 3) PDZ domain- dependent and -independent protein-protein interactions [11, 12]. Size exclusion chromatography analysis has shown co-fractionation of several of these proteins such as hDLG (21), MAGI-1, NHERF1 [15] and NEP [24] with PTEN in high molecular weight fractions. Potential roles for the PTEN-associated complex at the membrane have been suggested. Takahashi and colleagues demonstrated that PTEN directly interacts with NHERF proteins at the membrane, allowing for complex formation with PDGFRβ [15]. The PDZ binding domain of PTEN was shown to be critical for interaction with these proteins, since upon deletion of residues 401-403, the interaction of PTEN with the NHERF proteins was significantly reduced. NHERF2 and PDGFRβ were both shown to co-fractionate with PTEN in complex fractions. This complex assembly appears to negatively regulate signaling initiated by PDGF through the PI3-kinase.

Sumitomo and colleagues identified an additional complex between PTEN and NEP (neutral endopeptidase) [24]. They demonstrated that NEP co-fractionates with PTEN in low and high molecular weight fractions, and NEP induction, through addition of tetracycline, shifted PTEN into higher sized complexes. NEP is a membrane protein and its expression has been shown to negatively correlate with cancer, suggesting that NEP can confer one of its tumor suppressive functions through its interaction with PTEN and inhibition of the PI3-kinase pathway. Importantly, the role of NEP in inhibiting PI3-kinase pathway is independent of its catalytic function but dependent on its interaction with PTEN.

Nucleus

PTEN was initially characterized as a cytosolic protein; however, several studies have also identified a nuclear pool of PTEN with distinct functions in regulating cell cycle progression and chromosome stability through interacting with p53 [27] and CENP-C [28], respectively. Interestingly, altered nuclear-cytoplasmic shuttling of PTEN has been associated with cancer progression [29, 30]. Size exclusion chromatography analysis on nuclear and cytosolic extracts has demonstrated the presence of the PTEN-associated complex in both of these compartments. The efficiency of complex assembly in different cell compartments appears to be cell line-specific since an enrichment of nuclear PTEN complex was observed in PC3

cells [22] while the complex assembly appeared equal in both nuclear and cytosolic compartments in 293T cells [23].

Analysis of extracts isolated from PC3 PTEN inducible cells demonstrated a significant increase in complex assembly from nuclear fraction. In addition, two-dimensional DIGE (2D-DIGE) analysis of high molecular weight fractions from PTEN null or inducible PC3 cells identified hnRNP C as a potential component of the PTEN-associated complex. HnRNP C was shown to be recruited to higher molecular weight fractions in the presence of PTEN. In addition, a specific interaction was detected between these two proteins in the nucleus [22]. Although the functional significance of this interaction is still under investigation, this complex assembly may be important in regulating known hnRNP C functions in RNA regulation, especially in splicing.

Cytosol

As discussed, PTEN's role in negatively regulating the PI3-kinase pathway through dephosphorylating PtdIns(3,4,5)P3 has been well established. In addition, recent findings suggest that PTEN can also interact and form a complex with the regulatory (p85) and catalytic (p110β) subunits of PI3-kinase [23]. The results demonstrate co-fractionation of p85 and p110β with PTEN in high molecular weight fraction in 293T cytosolic extracts. This suggests a novel mechanism by which PTEN complex assembly with these components, independently of its phosphatase activity, can regulate the PI3-kinase pathway through sequestering p85/p110β and preventing their interaction with receptor tyrosine kinases and their downstream signaling. These findings may have a therapeutic significance, since the interaction of PTEN with p85 is shown to be enhanced upon treatment with trastuzumab, a HER2 inhibitor. As a result, the complex assembly between PTEN, p85 and p110β might play a significant role in inhibiting the PI3-kinase pathway, especially in the presence of trastuzumab treatment.

Although the focus and study of this complex has been in the cytosol, additional data demonstrate co-fractionation of p85 and p110β with PTEN in high molecular weight fractions from nuclear extracts as well [23]. Phosphatidylinositol lipids, such as PtdIns(3,4,5)P3 and the subunits of PI3-kinase have been detected in the nuclei of cells [31]. In the presence of certain cell stimuli, PI3-kinase can translocate to the nucleus and is activated, through direct interaction with the PI3-kinase enhancer (PIKE) [32]. In addition, a role for PTEN has been proposed in negatively regulating AKT phosphorylation in the nucleus and promoting cell apoptosis [33]. These findings bring up a possibility for the formation of a complex composed of PTEN, p85 and p110β in the nucleus through which PI3-kinase signaling can be negatively regulated.

Regulation of complex assembly by phosphorylation

PTEN phosphorylation has emerged as a common, connecting theme in regulating PTEN's complex assembly (Figure 1). Phosphorylation at the C-terminal tail on residues S380, T382, T383 and S385 have been shown to be critical in PTEN function through regulating its stability [34-37], subcellular localization [38, 39] and protein-protein interactions [16, 21]. Therefore, several groups have investigated the role of PTEN phosphorylation on complex assembly and have consistently detected reduced levels of phosphorylated PTEN in higher molecular weight fractions but a relative enrichment in monomer fractions [21-23]. Moreover, generation of an antibody specific to unphosphorylated PTEN at these residues has further confirmed the existence of this population in high molecular weight fractions [23]. This conclusion is also strengthened by higher complex forming potential of the A4-PTEN (S380A, T382A, T383A and S385A, mimicking dephosphorylated PTEN) when compared to E4-PTEN (S380E, T382E, T383E and S385E, mimicking phosphorylated PTEN) [22]. Taken together, these results identify phosphorylation as a key negative regulator of PTEN complex assembly.

How does phosphorylation regulate PTEN complex assembly in different cell compartments? Several studies suggest that dephosphorylation of PTEN on S380, T382, T383 and S385 residues

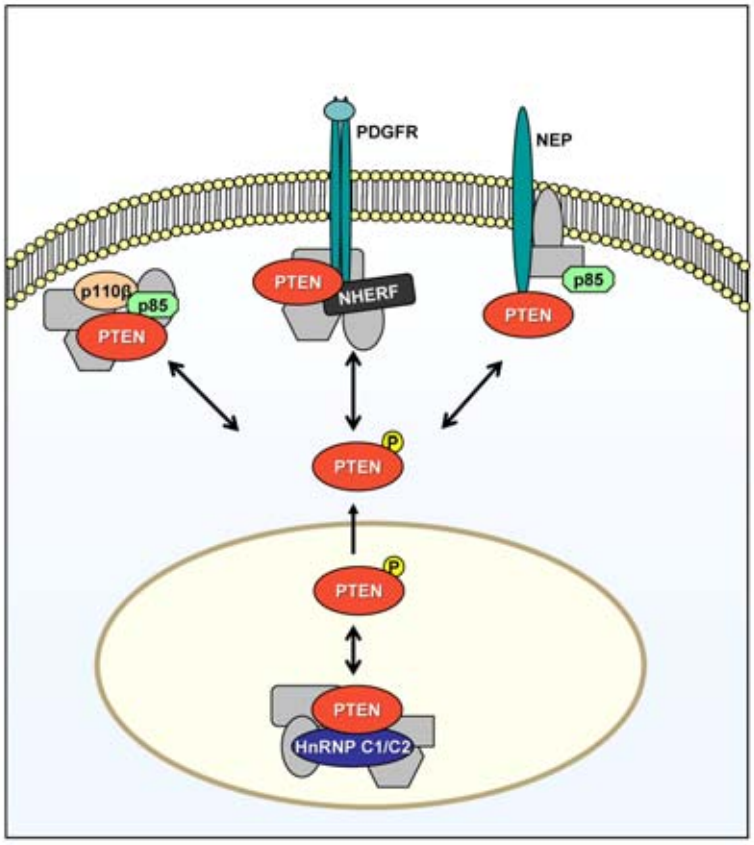

Figure 1. PTEN associated complexes. The assembly of PTEN into large complexes in different subcellular compartments is depicted here. Phosphorylation (P) of PTEN at the C-terminal tail negatively regulates complex assembly. Note: the proteins drawn in this diagram are not shown to scale.

induces a conformation change to the C-terminal tail, which allows for protein- protein interactions and potential complex assembly [21, 25, 39]. This explains the increased interaction observed between unphosphorylated PTEN and several of its complex components such as p85 [23], PDGFR, NHERF proteins [15] and NEP [24].

Phosphorylation of PTEN can also regulate its nuclear complex assembly through influencing PTEN localization [38]. A recent report suggests that phosphorylation of PTEN on the S380 residue decreases its nuclear retention and causes an increase in cytosolic pool of PTEN. These results are consistent with the observation that unphosphorylated PTEN is enriched in the high molecular weight complexes by forming a nuclear complex with hnRNP C [22]. Interestingly, physiological stimuli, such as oxidative stress, can regulate the accumulation of nuclear PTEN by inhibiting PTEN nuclear export [38], facilitating PTEN complex assembly in the nucleus.

CONCLUDING REMARKS

In summary, many PTEN interacting partners have been identified recently and several of these proteins such as, NHERF/PDGFR, NEP, p85/p110β and hnRNP C have been shown to form high molecular weight complexes with PTEN. These complexes highlight the existence of novel PTEN functions in different subcellular compartments. As mentioned, the PTEN-associated complex is >650KDa in size, therefore, PTEN is expected to associate with additional components, such as proteins, lipids and potentially RNA. Further purification of the components of these complexes will be necessary to shed light on their functions and their impacts on PTEN regulated signaling pathways.

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