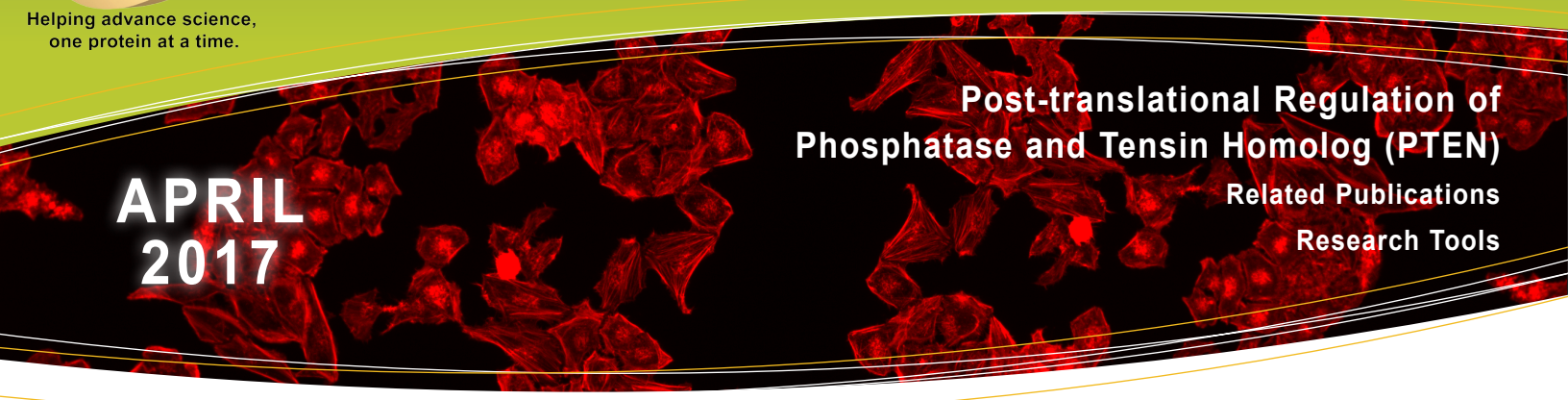
The Protein Experts
Helping advance science, one protein at a time.

CYTOSKELETON NEWS

NEWS FROM CYTOSKELETON INC.

APRIL 2017



Post-translational Regulation of Phosphatase and Tensin Homolog (PTEN)

Related Publications

Research Tools

Meetings

American Association for Cancer Research
April 1-5th, 2017
Washington, D.C.
Booth #3446

Emerging Concepts of the Neuronal Cytoskeleton
April 2-6th, 2017
Puerto Varas, Chile
Supported by Cytoskeleton

Boston Area Mitosis Meeting
May 6th, 2017
Boston, MA
Supported by Cytoskeleton

Cell and Molecular Mechanisms of Chromosome Segregation
May 11th, 2017
Supported by Cytoskeleton

Cytoskeleton Products

Actin Proteins
Activation Assays
Antibodies
ECM Proteins
ELISA Kits
G-LISA® Kits
Pull-down Assays
Motor Proteins
Small G-Proteins
Tubulin & FtsZ Proteins

Contact Us

P: 1 (303) 322.2254
F: 1 (303) 322.2257
E: cserve@cytoskeleton.com
W: cytoskeleton.com

Post-translational Regulation of Phosphatase and Tensin Homolog (PTEN)

The phosphatidylinositol (3,4,5)-trisphosphate phosphatase and tensin homolog (PTEN) is a tumor supressor protein discovered 20 years ago by two independent laboratories[1]. PTEN is also known to regulate diverse cellular functions such as adhesion, migration, proliferation, growth, and survival. PTEN is composed of five domains: an N-terminal phosphatidylinositol (4,5)-bisphosphate (PIP2)-binding domain, a catalytic tensin-type phosphatase domain, a C2 tensin-type domain that binds phospholipids, a C-terminal tail domain, and a PDZ-binding domain[2] (Fig. 1). The role of PTEN as a tumor suppressor is attributed to its lipid phosphatase activity which inhibits the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway integral for cell survival and growth by converting phosphatidylinositol (3,4,5)- trisphosphate (PIP3) into PIP2[1-3].

PTEN expression and enzymatic activity are tightly regulated transcriptionally, post-transcriptionally, and post-translationally[3]. Indeed, a wealth of data has shown that post-translational modifications (PTMs) of PTEN such as phosphorylation, ubiquitination, SUMOylation, acetylation, and oxidation can dynamically alter its stability, activity, localization, and interaction with other proteins. Defective post-translational regulation of PTEN leads to loss of PTEN activity and this appears to occur more often in tumor vs normal cells. Clinically, PTEN mutations and functional deficiencies are prevalent in many types of human cancers. This newsletter discusses the functional regulation of PTEN by PTMs.

Phosphorylation

Various phosphorylation sites have been identified in at least 3 of PTEN's domains and are mediated by multiple kinases (Fig. 1). In the C-terminal tail domain, phosphorylation of PTEN plays an important role in regulating PTEN activity. Ser362 and Thr366 are phosphorylated by glycogen synthase kinase 3β[4,5]. Casein kinase 2 phoshorylates Ser370 and Ser385[4,5], while also modifying Ser380, Thr382, and Thr383 to a lesser extent[5] (however, see Al-Khouri et al.[4]). C-terminal tail-phosphorylated PTEN adopts a closed conformational structure, which inhibits its interaction with membrane phospholipids and other membrane-anchored proteins[6]. Lacking the ability to bind to the plasma membrane renders PTEN inactive against its membrane-associated substrate PIP3. Interestingly, closed, phosphorylated PTEN appears to be less susceptible to ubiquitin ligases, and thus the proteasomal degradation of PTEN[5].

Phosphorylation also occurs in the C2 domain of PTEN (Fig. 1). RAK, a member of the Src tyrosine kinase family, enhances PTEN activity by phosphorylating Tyr336, which prevents

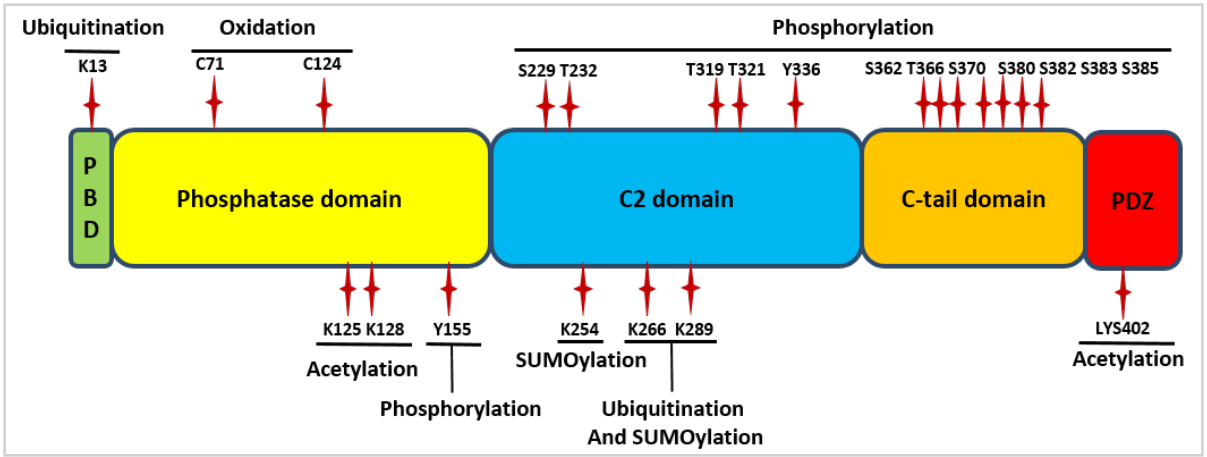


Figure 1. Domains of PTEN and PTMs within domains at specific sites. PBD, PIP2 binding domain. Adapted from reference #19.

Continued from Page 1

the interaction of PTEN with the E3 ligase NEDD4-1, thus protecting PTEN from poly-ubiquitination and proteasomal degradation[7]. Also in the C2 domain, RhoA-associated kinase (ROCK), a downstream effector of the Rho GTPase signaling pathway, phosphorylates PTEN on Ser229, Thr232, Thr319, and Thr321. These modifications regulate PTEN's intracellular localization and PTEN-mediated chemotaxis[9].

Within the phosphatase domain of PTEN, phosphorylation of Tyr155 enhances E3 ligase WWP2 binding to PTEN and mediates its ubiquitination-dependent degradation[8]. The kinase responsible for this phosphorylation has yet to be described.

Ubiquitination and SUMOylation

While poly-ubiquitination of PTEN causes proteasomal degradation in the cytosol, mono-ubiquitination of PTEN at Lys13 and Lys289 (Fig. 1) promotes its localization to the nucleus where it can exert its tumor suppressor function by antagonizing AKT activity[10]. Lys266 can also be mono-ubiquitinated[11]. PTEN's lysine residues are also SUMOylated by both SUMO1 and SUMO2/3[11-13]. PTEN's Lys254 and Lys266 are SUMOylated by SUMO1[12]. Lys289 is also SUMOylated[11]. SUMO1 modification enhances PTEN interaction with the membrane and thus the binding to its substrate PIP3[12]. SUMO2/3-modified PTEN localizes to the nucleus where it controls DNA repair[13].

Acetylation

The phosphatase-active domain of PTEN is modified by acetylation (Fig. 1). Acetylation at Lys125 and Lys128 affects PTEN binding to its substrate PIP3, inhibiting its catalytic phosphatase activity[14]. Another acetylation site is Lys402 within the C-terminal PDZ-binding domain, although its effects on PTEN function and localization are unclear[15].

Oxidation

Oxidation is another PTM shown to have a regulatory effect on PTEN function. H_2O_2 -mediated oxidation of Cys124 in PTEN's active site leads to the formation of a disulfide bond between Cys124 and Cys71, resulting in a decrease in PTEN phosphatase activity[16] (Fig. 1). Animal studies have shown that mice lacking the peroxidase peroxiredoxin1 (PRDX1) are more susceptible to tumor development due to abnormally high levels of reactive oxygen species, which leads to oxidation-induced inactivation of PTEN and hyperactivation of the AKT pathway[17]. Normally, PRDX1 binds PTEN and prevents its oxidation[17].

Summary

Studies of PTMs have provided researchers with new insights into protein regulation and are vital for identifying new therapeutic targets for human diseases. At Cytoskeleton Inc., we have developed a line of comprehensive and user-friendly Signal-Seeker™ kits to provide researchers with the necesssary tools to study PTMs targeting their proteins of interest. For example, using Cytoskeleton's Signal-Seeker™ kits, we have identified a novel mono-ubiquitination of PD-L1 in A431 cells following EGF treatment, research recently published in *Neoplasia*[18] (http://www.neoplasia.com/article/S1476-5586(17)30029-5/pdf). To learn more about Cytoskeleton's Signal-Seeker™ product line, please visit our website (www.cytoskeleton.com) or contact one of our technical support scientists (tservice@cytoskeleton.com).

References

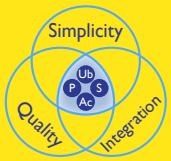
1. Shi Y. et al. 2012. PTEN at a glance. *J. Cell Sci.* **125**, 4687–4692.
2. Lee J.O. et al. 1999. Crystal structure of the PTEN tumor suppressor: Implications for its phosphoinositide phosphatase activity and membrane association. *Cell.* **99**, 323-324.
3. Song M.S. et al. 2012. The functions and regulation of the PTEN tumor suppressor. *Nat. Rev. Mol. Cell Biol.* **13**, 283-296.
4. Al-Khouri A.M. et al. 2005. Cooperative phosphorylation of the tumor suppressor phosphatase tensin homologye (PTEN) by casein kinases and glycogen synthase kinase 3beta. *J. Biol. Chem.* **280**, 35195-35202.
5. Torres J. and Pulido R. 2001. The tumor suppresspr 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.
6. Rahdar M. et al. 2009. A phosphorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN. *Proc. Natl. Acad. Sci. USA.* **106**, 480-485.
7. Yim E.K. et al. 2009. Rak functions as a tumor suppressor by regulating PTEN protein stability and function. *Cancer Cell.* **15**, 304-314.
8. Maddika S. et al. 2011. WWP2 is an E3 ubiquitin ligase for PTEN. *Nat. Cell Biol.* **13**, 728-733.
9. Li Z. et al. 2005. Regulation of PTEN by Rho small GTPases. *Nat. Cell Biol.* **7**, 399-404.
10. Trotman L.C. et al. 2007. Ubiquitination regulates PTEN nuclear import and tumor suppression. *Cell.* **128**, 141-156.
11. González-Santamaría J. et al. 2012. Regulation of the tumor suppressor PTEN by SUMO. *Cell Death Dis.* **3**, e393.
12. Huang J. et al. 2012. SUMO1 modification of PTEN regulates tumorigenesis by controlling its association with the plasma membrane. *Nat. Commun.* **3**, 911.
13. Bassi C. et al. 2013. Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. *Science.* **341**, 395-399.
14. Okumura K. et al. 2006. PCAF modulates PTEN activity. *J. Biol. Chem.* **281**, 26562-26568.
15. Ikenoue T. et al. 2008. PTEN acetylation modulates its interaction with PDZ domain. *Cancer Res.* **68**, 6908-6912.
16. Cho S.H. et al. 2004. Redox regulation of PTEN and protein tyrosine phosphatases in H2O2-mediated cell signaling. *FEBS Lett.* **560**, 7-13.
17. Cao J. et al. 2009. Prdx1 inhibits tumorigenesis via regulating PTEN/AKT activity. *EMBO J.* **28**, 1505-1517.
18. Horita H. et al. 2017. Identifying regulatory posttranslational modifications of PD-L1: A focus on monoubiquitinaton. *Neoplasia.* **19**, 346-353.
19. Xu W. et al. 2014. Posttranslational regulation of phosphatase and tensin homolog (PTEN) and its functional impact on cancer behaviors. *Drug Des. Devel. Ther.* **8**, 1745-1751.

Signal Seeker™ Kits

Kit or Affinity Beads	Type	Reactions	Cat. #
Signal-Seeker™ Phosphotyrosine Enrichment Kit	Kit	30 10	BK160 BK160-S
Signal-Seeker™ Ubiquitin Enrichment Kit	Kit	30 10	BK161 BK161-S
Signal-Seeker™ SUMO 2/3 Enrichment Kit	Kit	30 10	BK162 BK162-S

PTM antibodies, beads, etc

Kit or Affinity Beads	Type	Reactions	Cat. #
Phosphotyrosine Affinity Beads	Beads	40-80	APY03-beads
SUMO 2/3 Affinity Beads	Beads	20-40	ASM24-beads
Ubiquitin Affinity Beads	Beads	40	UBA01-beads
Control for Ippt IgG Beads	Beads	10	CIG01-beads
Control for Ubiquitin Affinity Beads	Beads	10	CUB02



Learn more about Signal-Seeker™
PTM Detection Kits, Antibodies, and Reagents
www.cytoskeleton.com/signal-seeker