Regulations of Tumor Suppressor Gene PTEN and Its Potential as Therapeutic Target

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Abstract. Phosphatase and Tensin homolog deleted from chromosome ten (PTEN) is a tumor suppressor gene which is found frequently mutated or deleted in many types of human cancer. By dephosphorylating the phosphatidylinositol (3,4,5) trisphosphate (PIP3) to phosphatidylinositol (4,5) bi-phosphosphate (PIP2), PTEN negatively regulates of the PI3K-AKT signaling pathway and causes the inhibition of cell survival, growth, and proliferation. In tumor cells, the expression and function of PTEN are dysregulated by numerous mechanisms, including epigenetic modification, transcriptional control, microRNA (miRNA) regulation, various post-translational modifications and also direct protein-protein interaction. This review summarized the mechanisms of PTEN protein regulation, ways in which perturbations in these regulations may lead to tumorigenesis and also the potential of PTEN as a drug target.

Introduction

PTEN (Phosphatase and Tensin homolog deleted from chromosome ten) was firstly identified by two independent groups through mapping the human chromosome 10q23, which is frequently deleted or mutant in various advanced cancers[1, 2]. Later, it was found that PTEN is a dual-specificity phosphatase with both protein and lipid phosphatase activity. PTEN functions as a tumor suppressor mainly through the lipid phosphatase activity. The primary target of PTEN is the lipid second messenger phosphatidylinositol (3,4,5) tri-phosphate (PIP3). PTEN removes the D3 phosphate from PIP3, producing phosphatidylinositol (4,5) bi-phosphate (PIP2)[3]; while phosphoinositide 3-kinase (PI3K) could phosphorylate PIP2 back to PIP3. PIP3 is a kind of phospholipid that resides on plasma membrane, recruiting the pleckstrin homology domain (PH domain) containing proteins, such as the most notable AKT and PDK1 kinase and promoting their activation. The AKT kinase regulates multiple important cellular functions, including cell growth, differentiation, proliferation, survival, motility, invasion and intracellular trafficking[4, 5]. Loss of PTEN leads to PIP3 accumulation, unrestrained activation of AKT and downstream signal molecules. Indeed, numerous studies demonstrated that dysfunction of PTEN occurs in a wide spectrum of human cancers through mutations, deletions, transcriptional silencing, or protein instability at a frequency that can even rival the famous p53 tumor suppressor gene in some cancer types[6]. Considering the importance of PTEN in suppressing tumor progression, it is being extensively studied worldwide, and drugs targeting PTEN/PI3K/AKT pathway are also being developed. In this Review, I summarize the recent progress on the multiple regulation mechanisms of PTEN and its potential as a therapeutic target.
Regulation of PTEN

Cellular expression and function of PTEN are tightly controlled by multilayered mechanisms, including epigenetic modification, transcriptional control, microRNA (miRNA) regulation and various post-translational modifications, such as enzyme-mediated covalent modifications, cellular localization and direct protein-protein interactions[6]. The following sections highlight our current understanding of the diverse regulatory mechanisms of PTEN.

Transcriptional Regulation of PTEN Expression.

Deletion of PTEN from chromosome and mutation in the coding sequence are the most common reasons for its loss of function in tumorigenesis. Besides that, downregulation of PTEN’s expression is also used by cancer cells to evade its tumor suppressor function. Aberrant epigenetic modification is a hallmark of cancer[7], and numerous studies reported that the PTEN promoter region become a closed chromatin state by hypermethylation on CpG islands and histone deacetylation, as is observed in many types of cancer. Methylation status of the 5' CpG islands of PTEN is closely associated with the silencing of PTEN in gastric carcinoma, gallbladder cancer and breast cancer[8-10], but Ten-Eleven Translocation 1 (TET1) inhibits gastric cancer growth and metastasis by demethylating PTEN promoter and inducing its re-expression[11]. Transcription factor SALL4 represses PTEN expression by recruiting histone deacetylase HDAC1 and HDAC2, and clinically, it is associated with downregulation of PTEN and tumor prognosis in hepatocellular carcinoma[12]. Other transcription factors c-Jun and polycomb group protein Bmi-1 were also reported to negatively regulate PTEN transcription[13, 14]. Overall, the downregulation of PTEN in numerous cancers is mediated by aberrant epigenetic remodeling and complex transcription network.

Regulation of PTEN at the mRNA Level

Non-coding RNAs (ncRNAs) are RNA molecules transcribed from genome but do not encode proteins, mainly including microRNA (miRNAs), Piwi-interacting RNAs (piRNAs), Small interfering RNAs (siRNAs) and Long non-coding RNAs (lncRNAs)[15]. miRNA can regulate gene expression at mRNA level by targeting to the 3' untranslated region (UTR) of mRNAs. Recently, numerous miRNAs were proved to promote tumorigenesis by downregulating PTEN expression. The miR-17~92 cluster, including five miRNAs (miR-17, miR-19a, miR-19b, miR-20a and miR-92), were reported to target PTEN[16]. Bahena-Ocampo reported that miR-10b negatively regulates PTEN expression in breast cancer stem cells and further promotes tumor metastasis[17]. miR-106a was found up-regulated in many NSCLC patients and PTEN was its direct target[18]. miR-155-5p, miR-101 and miR-92a were also demonstrated to indirectly modulate PTEN transcription via the PI3K/Akt pathway[19-21]. Long noncoding RNA(lncRNA) FER1L4 is also found to function as a competing endogenous RNA (ceRNA) to regulate the expression of PTEN by decoying miR-106a-5p in gastric cancer[22]. Recently, it was found in brain microenvironment, microRNA that is included in the astrocyte-derived exosomes downregulates PTEN expression and promotes brain metastasis in vivo[16]. Taken together, the regulations of PTEN through ncRNAs are being extensively investigated. These studies enrich our knowledge about PTEN modulation, and also provide new opportunities for effective treatment of tumor with PTEN dysfunction.
Regulation of PTEN in The Protein Level

Post-translational regulations through different direct modifications play an important role in modulating PTEN functions. Various modifications were found on PTEN protein, such as phosphorylation, acetylation, oxidation, ubiquitylation and SUMOylation. N-terminus of PTEN is the phosphatase active domain, while C-terminus contains a PEST sequence and a PDZ interaction motif.

**Phosphorylation.** PTEN’s C-terminal is heavily modified by phosphorylation and these phosphorylations in different amino acids render diverse functional consequences to PTEN[6]. As is well documented, casein kinase 2 (CK2) phosphorylates PTEN at Ser380, Thr382, Thr383 or Ser385 and inhibits PTEN’s phosphatase activity[23]. RhoA-associated kinase (Rock) phosphorylates PTEN at Ser229 and Thr321, inducing its membrane localization[24, 25]. PTEN can also be phosphorylated at Tyr336 by tumor suppressor RAK kinase, which will inhibit the binding of E3 ubiquitin ligase NEDD4-1 to PTEN, decrease the polyubiquitylation level, and maintain its stability[6, 26].

**Acetylation.** Studies showed that PTEN interacts with PCAF and can be acetylated by PCAF at Lys125, Lys128. Acetylation of PTEN inhibits its phosphatase activity and causes marked increase of Akt phosphorylation level[27]. The phosphatase activity of PTEN depends on membrane translocation (activation). It was reported that inhibition of HDAC6 can enhance PTEN acetylation at K163 and promote its membrane translocation[28].

**Oxidation.** As other protein tyrosine phosphatases (PTPs), two cysteines at the active site are susceptible to oxidation. Under certain conditions, Cys124 residue of PTEN specifically forms a disulfide with Cys71, which will result in the inhibition of the lipid phosphatase activity of PTEN and the activation of Akt pathway[3, 6]. Recently, one study reported that Apoptosis-inducing factor (AIF) directly interacts with and inhibits the oxidation of PTEN, prevents the oxidation-mediated inactivation of PTEN, leads to the inactivation of Akt kinase, dephosphorylation of the Akt substrate GSK-3β, and inhibits the metastasis of cancer cells[29].

**Ubiquitylation.** Ubiquitin is a highly conserved 76-aa long protein that can be covalently conjugated to other proteins, a process called ubiquitylation. Ubiquitylation is essential for the degradation of proteins and many other biological processes[30]. It was demonstrated that NEDD4-1 is an E3 ligase that ubiquitylates PTEN at Lys13 and Lys289. Monoubiquitination at Lys289 by NEDD4-1 is responsible for PTEN’s nuclear localization and the nuclear translocation is positively correlated with suppression of tumor progression in various cancers[26]. Besides NEDD4-1, RING domain E3 ligase XIAP and NEDD4-like E3 ligase WWP2 were also reported to be ubiquitin ligases for PTEN[31]. Recently, OTU domain-containing protein 3 (OTUD3) was found as a deubiquitylase of PTEN, which can up-regulate PTEN protein level. Loss function of OTUD3 in breast cancer cell line markedly accelerates cancer cell migration and metastasis[32]. Overall, ubiquitylation plays a vital role in regulating the functions of PTEN, and this process is tightly controlled by both ubiquitin ligases and deubiquitylase.

**Poly-ADP ribosylation.** Poly-ADP ribosylation is mediated by PAR polymerase enzymes, which is also known as PARylation. This enzyme can covalently attach negatively charged polymer ADP-ribose to proteins[33]. This unique modification regulates many biological processes, such as transcriptional regulation, energy metabolism, intracellular trafficking and so on. Researchers recently found that tankyrases, which are members of the poly (ADP-ribose)
polymerases (PARPs), can physically interact with and ribosylate PTEN. The Poly-ADP ribosylation can promote PTEN ubiquitination and degradation, which is mediated by a PAR-binding E3 ubiquitin ligase, RNF146[34].

**Protein–protein interactions.** Numerous proteins have been reported to interact with PTEN to positively or negatively modulate its tumor-suppressive activity by influencing its PPase activity, stability, subcellular localization and conformational change. Researchers found that β-arrestins, which function as dynamic scaffolds to influence many protein’s activity and subcellular localization, can physically interact with PTEN through the C2 domain and enhance its lipid phosphatase activity. The RhoA/ROCK signaling pathway further promotes the β-arrestin–PTEN interaction[35]. Phosphatidylinositol 3,4,5-trisphosphate RAC exchanger 2a (P-REX2a) is demonstrated to be a PTEN-interacting protein. It specifically inhibits PTEN’s lipid phosphatase activity and results in the stimulation of the AKT signaling pathway, and then further promotes tumor cell proliferation and transformation[36]. Importin-11 (Ipo11) is recently identified as a transport receptor for PTEN. It directly separates PTEN from protein degradation machinery and maintains its stability. Loss function of Ipo11 results in degradation of PTEN and tumor progression[37]. A lot of other proteins with diverse modulating functions are also reported to have interaction with PTEN, such as NHERF[38], MyosinV[39], MAN2C1[40].

**Cellular Localization**

The cellular localization of PTEN has been well studied. Results showed that it located in the cytoplasm, nucleus and even extracellular space[41] and activities of PTEN maybe differ at distinct locations. The cytoplasm and nucleus localization and activity have been well documented before[42]. Recently, PTEN was demonstrated that it can be exported outside by exosome and direct secretion. Putz, U., et al found that PTEN can be transferred to recipient cells by exosomes, leading to reduced phosphorylation level of Akt kinase and cellular proliferation[43]. Hopkins, B.D., et al found PTEN can be translated from an alternative initiation codon (CUG) within the 5’ region of PTEN mRNA. The resultant PTEN-Long variant contains an extra 173–amino acid at its N terminus followed by the classical 403 amino acids of PTEN. This PTEN-Long protein with normal phosphatase function can be secreted and taken up by other cells. Uptake of the “extended version” of phosphatase enzyme inhibits the AKT/mTOR pathway and induces tumor cell death[44].

**Drugs Targeting the PTEN/PI3K/AKT Pathway**

Dysfunction of PTEN results in overactivity of the downstream AKT/mTOR kinases, therefore, inhibition of the aberrant activity of these enzymes is the first choice to normalize the aberrant activity of PTEN/PI3K/AKT axis. The pharmaceuticals industry have developed many chemical drugs targeting this pathway, and most of them targeted PI3K, AKT and mTOR kinases. These inhibitors are divided into pan-PI3K inhibitors, isoform-specific PI3K inhibitors, dual PI3K/mTOR inhibitors, mTOR inhibitors and AKT inhibitors[45-47]. And many of them have already been approved by FDA, such as rapamycin analog temsirolimus and everolimus, PI3Kδ selective inhibitor idelalisib [48].

Along with the increasing knowledge on the regulatory mechanisms of PTEN, an alternative way is directly targeting PTEN itself. It was reported that recombinant
adenoviral-mediated PTEN gene therapy could rescue the loss function of PTEN and effectively inhibit tumor progression\cite{49, 50}. The PTEN-Long protein variant with signaling peptide can also be used directly as therapeutic drug candidate\cite{44, 51}. A hallmark of cancer is the aberrant epigenetic modifications\cite{7}. PTEN was found to be aberrantly silenced by inhibitory epigenetic modifications and this also serves as an important option for drug development\cite{9, 14}. As summarized above, many post-translational modifications cause PTEN dysfunction and enzymes that responsible for these aberrant modifications are also drugable, but it should be more cautious about the side-effects, for many enzymes are not cancer cell specific.

Concluding Remarks

Taken together, the mechanism in regulating of PTEN at different levels is complicated, though we have achieved great progress in understanding these regulatory pathways, further investigations are still needed to fully elucidate its regulation networks. Based on these studies, we could design better therapeutic drugs to cure cancer patients with PTEN dysfunction.

References


