PTEN: Its Deregulation and Tumorigenesis

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The tumor suppressor phosphatase and tensin homolog (PTEN) functions as a phosphoinositide 3-phosphatase, that antagonizes phosphatidylinositol 3-kinase action, and negatively regulates cell proliferation and survival signals. Inactivation of PTEN by loss-of-function mutations gives rise to deregulated hyperproliferation of cells, leading to oncogenic transformation. Recent studies have identified a number of upstream regulatory factors for PTEN and unveiled that the impairment in the PTEN regulatory system potentially becomes a causal factor for oncogenic transformation of cells. This article will review the PTEN inactivation mechanism which is linked to human tumorigenesis, particularly focusing on recent research progress in PTEN regulators.

Key words phosphoinositide; tumor suppressor; phosphatase

1. INTRODUCTION

Phosphoinositides (PIs) are multiply phosphorylated derivatives of phosphatidylinositol, a glycerophospholipid, and seven PI species exist in higher eukaryotes. Each PI species is produced and converted to another PI species by strictly regulated phosphorylation and dephosphorylation on the inositol ring through PI-specific kinases and phosphatases, respectively.1–4) PIs function as intracellular signaling molecules and regulate diverse cellular functions including proliferation, apoptosis and motility; therefore impaired PI turnover due to the malfunction of responsible PI kinase(s)/phosphatase(s) gives severe impacts on a wide variety of intracellular signaling systems and potentially becomes causal factors for certain human diseases.5–10)

Phosphatase and tensin homolog (PTEN) exhibits PI phosphatase activity towards phosphatidylinositol 3,4,5-trisphosphate (PIP3),4,11,12) which is produced by phosphatidylinositol 3-kinase (PI3K) upon growth factor stimulation and displays robust effects on multiple cellular events including proliferation and apoptosis (Fig. 1).1,10) As expected from its biochemical properties, PTEN antagonizes PI3K actions to negatively regulate cell proliferation and survival (Fig. 1).4,5,13–18) The PTEN gene was initially identified as a tumor suppressor candidate in 1997,19,20) thereafter over 2500 papers about PTEN have been published. Most of these studies have focused on its tumor suppressor function, and an accumulating body of evidence has established the conclusion that impaired PTEN function induces PIP3 accumulation in cells followed by the hyperactivation of its downstream signals, leading to oncogenic transformation of cells.4,5,15–18) Since several review articles have already summarized and discussed PTEN functions extensively; this review will focus on the mechanisms for tumorigenesis-directed PTEN inactivation, particularly in the causal action of impaired regulatory systems for PTEN function.

2. INACTIVATION OF PTEN BY GENETICAL LESIONS IN THE PTEN GENE

The PTEN gene displays the characteristics that are expected of a tumor suppressor gene. Specifically, numerous mutations and/or deletions in the PTEN gene have been found in tumor tissues and human cancer cell lines,4,21,22) indicating a strong correlation between loss of PTEN function and human cancer. In addition, germline mutations in the PTEN gene have been associated with Cowden syndrome and related diseases in which patients suffer from a significantly increased risk of certain tumors, including breast and thyroid carcinomas.23)

Human PTEN protein comprises four functional modules; (from N-terminus to C-terminus) phosphatase domain, C2 domain, extended C-terminal tail (C-tail), and PSD-95/Dlg/ZO-1 homolog (PDZ)-binding motif. Most of missense mutations associated with human tumors and Cowden syndrome has been found in the phosphatase domain.4,21,22)
and biochemical analyses have confirmed that most of them result in robust decrease in the phosphatase activity.\textsuperscript{11,12,24—27} The phosphatase domain also frequently receives a number of tumor-associated nonsense and frameshift mutations.\textsuperscript{21,22} These mutations induce early termination of the translation, resulting in the production of unstable messages and/or immature gene products, which are no longer able to form functional phosphatase domain and absolutely lose the phosphatase activity. In contrast to these easy-to-imagine inactivation mechanisms, nonsense and frameshift mutations occurred near the C2 domain–C-tail junction (from Thr-319 to Arg-335), another hot spot for tumor-associated mutations, employ relatively complicated mechanism for the PTEN inactivation. These mutants retain the intact phosphatase domain and most of the C2 domain, implying these truncated forms of PTEN would be biochemically active. However, when expressed in cells, these truncated PTEN proteins completely lose their abilities to suppress PI3K/PIP3 signals presumably due to the lack of functional modules downstream of the C2 domain; the C-tail and the PDZ-binding motif.\textsuperscript{27—30} A number of studies demonstrated that the C-tail encompassed multiple phosphorylation sites (Ser-370, Ser-380, Thr-382, Thr-383, and Ser-385) that were indispensable for PTEN protein stability.\textsuperscript{31—34} In fact, C-tail deletion and phosphorylation-resistant mutants are known to become quite unstable and undergo rapid degradation in cells.\textsuperscript{31—34} In addition, the PDZ-binding motif is believed to recruit PTEN protein to the plasma membrane, where PTEN meets the lipid substrate PIP3, through the binding towards several membrane-anchored PDZ proteins.\textsuperscript{35—41} Therefore PTEN gene products which lack the C2 domain and the PDZ-binding motif, due to nonsense and frameshift mutations within the C2 domain–C-tail junction, are no longer able to exist in cells as stable and functional protein.

3. PTEN REGULATORS INVOLVED IN HUMAN TUMORIGENESIS

During the decade of research on PTEN genetics and biochemistry, a number of research groups has also attempted to discover PTEN-associated proteins that potentially regulate PTEN function. As the results of these extensive studies using yeast two-hybrid screening and affinity pull-down method, several PTEN-binding proteins have been identified so far.\textsuperscript{35—53} Some of these binding proteins recruit PTEN to specific subcellular compartment; others function as regulators for PTEN protein stability in cells. All of these binding proteins could potentially participate in PTEN-dependent tumorigenesis; because disruption in the interaction of PTEN with these binding partners may cause mislocalization and/or deregulated turnover of PTEN protein in cells. However, only a few case has been studied to link tumorigenesis so far.\textsuperscript{53—55} Protein interacting to the C-terminus-1 (PICT-1)\textsuperscript{52,55} and neural precursor cell expressed, developmentally downregulated 4-1 (NEDD4-1)\textsuperscript{53} will be particularly important for their correlation with human tumors.

PICT-1, which was encoded by GLTSCR2 gene, was originally identified as a candidate tumor suppressor gene located at human 19q13.3 locus.\textsuperscript{56—58} An accumulating body of evidence has revealed that the 19q13.3 locus is frequently altered in certain types of human tumors, such as glioma and neuroblastoma, indicating that a yet unidentified tumor suppressor gene(s) specifically encoded in this region may participate in the tumorigenesis.\textsuperscript{56—59} Our group has demonstrated that PICT-1 facilitates the C-tail phosphorylation and plays an essential role in stabilizing PTEN protein in cells.\textsuperscript{52,55,60} Forced downregulation of PICT-1 in cells by the RNA interference decreased both PTEN C-tail phosphorylation and PTEN protein levels, leading to the hyperactivation of PI3K/PIP3 signals.\textsuperscript{52,55} Therefore, as resulted from the activation of diverse signaling molecules, the PICT-1 knockdown in HeLa and NIH3T3 cells robustly promoted both anchorage-dependent growth and anchorage-independent growth; the latter is a hallmark of tumorigenic transformation.\textsuperscript{55} Strikingly, PICT-1 expression was frequently abrogated in human neuroblastoma specimens and impaired PICT-1 expression was associated with downregulation of PTEN protein expression.\textsuperscript{55} These observations prompt us that PICT-1 may participate in human tumorigenesis as a novel tumor suppressor. More genetical analyses to unveil the certain relationship between PICT-1 inactivation and human tumors will be expected; in addition, underlying biochemistry of PICT-1 action, particularly in the C-tail phosphorylation, should be elucidated to understand the molecular basis of PICT-1—PTEN regulatory system.

While PICT-1 may participate in the initial step for the regulation of PTEN protein turnover by facilitating the C-tail phosphorylation, NEDD4-1 might govern the execution of the degradation process. Unphosphorylated and C-tail-deleted forms of PTEN will be polyubiquitinated and readily degraded in a proteasome-dependent manner.\textsuperscript{31—34} In the most recent research by Jiang and colleagues,\textsuperscript{53} the ubiquitin ligase (E3) activity for PTEN was purified from HeLa cell lysate through seven consecutive column chromatograpy; then this research group successfully identified NEDD4-1 as the E3 ligase. Isolated NEDD4-1 absolutely induced robust polyubiquitination of PTEN in the reconstituted cell-free assay system.\textsuperscript{53} In addition, NEDD4-1 expression promoted the polyubiquitination and degradation of PTEN protein in intact cells; while NEDD4-1 knockdown upregulated PTEN protein expression.\textsuperscript{53} Intriguingly, NEDD4-1 promoted Ras-
induced cell transformation, presumably due to the downregulation of PTEN, when expressed in mouse embryonic fibroblasts. Most strikingly, NEDD4-1 expression was inversely correlated with PTEN protein expression in mouse prostate cancer model. Although it is still controversial how NEDD4-1 expression/activity is regulated, this report provides fresh insight into the relationship between PTEN deregulation and tumorigenesis. More studies on NEDD4-1-dependent PTEN regulation using human tumor specimens will be expected.

In addition to these biochemically-characterized regulators (PICT-1 and NEDD4-1) for PTEN, genetic analysis in a model animal has suggested that DJ-1 would be another candidate that regulates PTEN function and may participate in human diseases. DJ-1 was originally isolated as a putative oncogene which promoted Ras-induced cell transformation. Another study independently identified DJ-1 as a gene associated with autosomal early-onset Parkinson’s disease. Recently, using Drosophila model system, Mak and colleagues demonstrated that DJ-1 genetically interacted with PTEN as an upstream regulator and suppressed PTEN function. Although the molecular mechanism of how DJ-1 regulates PTEN remains elusive, DJ-1 expression unequivocally activated PI3K/PIP3 downstream signals also in mammalian cells and subsequently promoted oncogenic transformation. Moreover, in human breast cancer specimens, the DJ-1 expression exhibited positive correlation with protein kinase B activation, a downstream effector of PIP3, as well as inverse correlation with PTEN protein expression.

4. IMPAIRED PTEN EXPRESSION BY DeregulatEd TRANSCRIPTION

In addition to the posttranslational regulation as described above, some transcription factors are implicated in transcriptional regulation of PTEN and affect PTEN expression level. Early growth response-1 (EGR-1) peroxisome proliferator-activated receptor γ (PPARγ) and p53 have been shown to directly bind to the promoter region of the PTEN gene and induce the transactivation; in contrast, nuclear factor κB (NFκB) is known to suppress the PTEN expression. Extensive studies have been performed in order to confirm the relationship between PTEN and these famous transcription factors. Both PTEN and p53 affected their activity and protein stability reciprocally each other; mouse model system confirmed their genetical interaction. Pharmacological activation of PPARγ induced PTEN expression, in contrast, cytokine-induced NFκB activation resulted in PTEN downregulation. All these results, taken together, prompt that PTEN transcription could be regulated by quite complicated system. On the other hand, a number of expression analyses, such as Northern blot and microarray analysis, has shown that PTEN is expressed ubiquitously and invariant in most cases. These observations lead us to the notion that neither of these transcriptional regulations could solely become critical master regulatory system for the PTEN expression. In fact, no clear cooperation of PTEN and these transcription factors in human tumorigenesis has been yet established. Only EGR-1 was shown to be positively correlated with the expression of PTEN in certain human tumors. In contrast to trans-factors as above, cis-acting epigenetic alteration, such as the gene-silencing by DNA methylation, has been found in a number of human tumors. Although the molecular mechanism for the PTEN gene silencing remains unknown; an accumulating results from methylation analyses indicated that the methylation-mediated gene silencing largely accounted for the PTEN downregulation observed in human tumors.

5. CONCLUSION

As well as the other biologically important signaling molecules, PTEN seems to be protected by multiple systems to prevent accidental deregulation. However, once severe defect occurs on the system and overcomes the protection mechanisms, PTEN inactivation will have enormous impact on diverse intracellular signaling systems. This review article briefly described molecular actions of PTEN and the relationship between PTEN deregulation and tumorigenesis from some aspects; however, more detailed discussion will be absolutely required. Recent researches have pointed out that there may be more regulatory mechanisms for PTEN; direct control of the phosphatase activity by phosphorylation and by phosphatidylinositol 4,5-bisphosphate; reversible inactivation of the phosphatase activity by oxidation; that we did not include in this review due to space limitation. Thus the regulatory mechanisms for PTEN appear to be quite complex, and these regulatory processes are likely to affect each other. Further study will be required to gain a more complete understanding of the molecular basis of the regulatory mechanism for PTEN.
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