Molecular Pathways: Intercellular PTEN and the Potential of PTEN Restoration Therapy

Benjamin D. Hopkins and Ramon E. Parsons

Abstract

Phosphatase and Tensin homolog deleted on chromosome Ten (PTEN) acts as a tumor suppressor through both PI3K-dependent and -independent mechanisms. Reduced PTEN activity has been shown to affect not only tumor cell proliferation and survival but also the microenvironmental context in which nascent tumors develop. As a result of the multifaceted tumor-suppressive roles of PTEN, tumors evolve by selecting for clones in which PTEN activity is lost. PTEN activity within tumors can be modulated in numerous ways, including direct mutation, epigenetic regulation, and amplification or mutation of other proteins that can regulate or degrade PTEN. These events functionally prevent PTEN protein from acting within tumor cells. Paracrine roles for PTEN gene products (exosomal PTEN and PTEN-L) have recently been identified, through which PTEN gene products produced in one cell are able to enter recipient cells and contribute to PTEN functions. In preclinical models purified PTEN-L protein was able to enter tumor xenografts and downregulate PI3K signaling as well as cause tumor cell death. Here, we review the role of PTEN as a multifaceted tumor suppressor and reflect upon the potential for PTEN restoration therapy.

Clin Cancer Res; 20(21); 5379–83. ©2014 AACR.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Editor's Disclosure
The following editor(s) reported relevant financial relationships: P.S. Steeg reports receiving commercial research grants from Geron, GlaxoSmithKline, and Sanofi.

CME Staff Planners' Disclosures
The members of the planning committee have no real or apparent conflicts of interest to disclose.

Learning Objectives
Upon completion of this activity, the participant should have a better understanding of PTEN's multifaceted tumor-suppressive functions, the mechanisms that tumors develop to circumvent these functions, and the rationale for restoring lost tumor-suppressive functions through the use of novel protein-based therapeutic strategies.

Acknowledgment of Financial or Other Support
This activity does not receive commercial support.

Background

Phosphatase and Tensin homolog deleted on chromosome Ten (PTEN) was originally identified as a tumor suppressor, frequently lost from chromosome 10q23 in multiple cancers (1, 2). Though it was identified as a dual specificity protein phosphatase, PTEN’s major cellular substrate is the lipid second messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3), from which PTEN facilitates the removal of a phosphate, preferentially from the 3 position of the inositol ring. Thus, PTEN’s primary enzymatic activity directly opposes that of phosphoinositide 3 kinase (PI3K), and thereby negatively regulates the PI3K/AKT signaling axis (3–5). Although PTEN is most well known for its effects on PI3K signaling, PTEN also has PI3K pathway-independent functions. For example, PTEN acts through PI3K-independent mechanisms to inhibit cell migration and to regulate genomic stability (6–11). PTEN has also been implicated in regulation of the MAPK signaling pathway (12). The tumor-suppressive roles of PTEN are
highlighted by the observation that germline mutations in PTEN give rise to the PTEN hereditary tumor syndromes (PHSs), for example, Cowden disease, Bannayan–Riley–Ruvalcaba syndrome, Proteus syndrome, and Proteus-like syndrome. Individuals with PHSs are predisposed to develop benign hamartomas in a wide array of tissues and cancers of the thyroid, breast, and other organs (13–16). The tumor-suppressive functions of PTEN are further supported by the vast array of global and tissue-specific Pten knockout murine models, which develop a variety of hyperplastic and tumorigenic phenotypes (17). Murine models have also demonstrated that subtle changes in the dose of PTEN are sufficient to allow for tumor progression (18, 19). An array of somatic mutations in PTEN has been identified in sporadic human tumors from numerous tissues, including the brain, ovary, and prostate, and in some tissues such as the pancreas the loss of a single copy of PTEN can influence tumor progression, indicating that it acts as a haplo-insufficient tumor suppressor (20, 21).

The tumor-suppressive role of PTEN is not limited to tumor cells. PTEN loss in tumor cells increases tumor angiogenesis (22, 23). Wen and colleagues demonstrated that reexpression of PTEN in U87MG xenografts affected the ability of the xenografted tumors to develop a blood supply that was not related to their rate of proliferation (24). Alternatively, deletion of PTEN from the fibroblasts of the tumor microenvironment resulted in accelerated tumor development in Erbb2-driven mouse models of breast cancer, suggesting that under normal conditions PTEN is playing a tumor-suppressive role by acting in the cells of the tumor microenvironment to regulate the tumor cells themselves (25).

Taken together, such findings suggest that in addition to its role in controlling tumor cell proliferation and survival, and thereby acting as a "gatekeeper tumor suppressor," PTEN is also acting to regulate the tumor microenvironment, and thereby acting as a "landscaper tumor suppressor" (26, 27). This understanding of PTEN as a landscaping tumor suppressor is supported by the observation that loss of PTEN is associated with the development of hamartomas, which are characterized by an imbalance in the proportion of individual cell types within a tissue (28). The late occurrence of PTEN mutations in tumor development supports the idea that PTEN is a landscaping tumor suppressor as well, as it underscores the importance of limiting PTEN activity in late stages of tumor development when continued tumor progression shifts from being about deregulation of cell proliferation and becomes increasingly about acquiring mutations that allow the tumor to modulate its microenvironment. Thus, more advanced tumors that require these modulations to the tumor microenvironment may be under greater selective pressure to ablate PTEN activity than earlier-stage tumors that are still largely driven by selective pressure to proliferate.

PTEN mutation is only one of a variety of mechanisms by which tumors decrease PTEN activity as they evolve. Studies have shown that PTEN is frequently silenced through a variety of different mechanisms, including hypermethylation of its promoter, and interaction with a variety of microRNAs (10, 29, 30). The functional dose of PTEN can also be affected by an array of PTEN-interacting proteins that have been demonstrated to affect PTEN activity. Some proteins, such as PREX2a, are thought to affect PTEN activity through direct binding events that inhibit the enzymatic activity of PTEN (31). Other proteins affect PTEN activity through posttranslational modifications such as ubiquitination, SUMOylation, acetylation, oxidation, and phosphorylation (32). Although the mechanisms for the down-regulation of PTEN activity caused by these modifications vary, from changing the subcellular localization or stability of PTEN to affecting its conformation, they are all similar in that they are selected for by tumors as they evolve because they functionally downregulate PTEN activity. As tumors develop, the acquisition of traits that reduce PTEN activity, even in modest ways, may be sufficient to confer a growth advantage to a clone within a developing tumor. As we begin to engage the idea that PTEN gene products may not only be acting within their cells of origin but may also be acting in a paracrine manner, some of the above regulatory mechanisms may affect the function of extracellular forms of PTEN once inside cells.

**Intercellular PTEN**

Two recent publications identified mechanisms by which PTEN can be generated in one cell and then act in another cell (Fig. 1). PTEN-L (previously termed PTEN-Long; ref. 33), a recently identified translational variant of PTEN, contains all of the domains of PTEN with an N-terminal alternately translated region (ATR) that has an additional 173 N-terminal amino acids (34). The PTEN-L ATR contains a signal sequence with a putative cleavage site, which allows PTEN-L to be secreted. Consistent with this, PTEN-L is detectable in culture medium from human cells as well as both human plasma and serum. The PTEN-L ATR also contains a polyarginine motif that is similar to the polybasic residues of cell permeable peptides. Purified PTEN-L protein is able to enter cells by a mechanism that depends on its polyarginine motif. Thus, PTEN-L is able to enter cells as an exogenous agent. Remarkably, exogenous PTEN-L is capable of downregulating PI3K in a manner similar to intracellular PTEN, and as a result is similarly able to affect cell survival in vitro. Another demonstration of PTEN’s intercellular functions comes from Putz and colleagues (35), who show that PTEN is packaged into exosomes. PTEN-containing exosomes travel from a producing cell to a recipient cell in which PTEN affects PI3K signaling (35). Because functional PTEN gene products are able to exit the cell in which they were generated and act in recipient cells, the PTEN status of the stromal cells in the tumor microenvironment may contribute to the experienced level of active PTEN in tumor cells. In this manner, it is possible that these extracellular forms of PTEN may be playing a tumor-suppressive role in a paracrine manner. This
The hypothesis is supported by the observation that PTEN-L protein levels are high in macrophage-like populations surrounding a subset (∼10%) of PTEN-deficient tumors (34). The discovery of these paracrine functions of PTEN also may explain some of the previously identified phenomena in which PTEN loss had significant effects upon tumor growth and the development of the tumor microenvironment into a hospitable environment for tumor growth without necessarily affecting tumor cell proliferation (24).
The Next Cell Hypothesis

Tumors do not initiate and develop in isolation. To grow, tumors must acquire traits that allow them to co-opt nutrients and other resources from their environment. These traits may be detrimental to neighboring cells. Communal selective pressure may have been at work during eukaryote evolution, such that clones that used more than their share of resources were selected against for the good of the group. Secreted PTEN is an example of a mechanism through which cells can respond to aberrant growth. This hypothesis is supported by the observation that PTEN-L protein is elevated in histiocytes directly adjacent to tumors (34). These “next” cells may be responding to tumor initiation and may be acting to inhibit the aberrant proliferation of the neighboring cell. This type of response may act as a first line of defense against tumor development. From this perspective, the secretion of factors by the next cell could be useful as biomarkers of early tumor development. If we can understand why some tumors elicit an endogenous response from their neighbors, it may be possible to use this type of response for therapeutic benefit. It is likely that some tumors will evolve mechanisms to evade these types of intercellular regulation of their growth. Further exploration of this type of innate response may also lend insights about how to effectively manipulate tumor microenvironments to keep them in a tumor-suppressive rather than tumor-promoting state.

Clinical–Translational Advances

Traditional clinical efforts to restore PTEN functionality have focused on the role of PTEN in antagonizing PI3K signaling. These efforts have targeted kinases throughout the PI3K pathway from receptor tyrosine kinases to PI3K, AKT, and mTOR. Recently, some attention has been paid to targeting the effects of PTEN loss on genomic stability, and specifically targeting PTEN-deficient tumors with PARP inhibitors in the hopes of exploiting these tumors’ defect in DNA repair (36). It has been observed in a wide variety of preclinical systems, both in vitro and in vivo, that genetic restoration of PTEN is able to induce apoptosis in some tumor cell lines and can have a profound effect on tumor progression in a variety of models (37–40). However, such models have relied on genetically reintroducing the PTEN gene, which is not currently possible in the clinic. In preliminary studies using purified PTEN-L protein as an antitumor agent in preclinical murine models, the protein was able to enter the mouse blood stream and was detectable at levels that greatly exceed those of endogenous PTEN in tumor xenografts. Treatment with PTEN-L caused dose-dependent reductions in PI3K signaling and induction of apoptosis in tumor cells in vitro, and led to frank tumor regressions in a variety of PTEN-null xenografted tumors (34). Although exogenous PTEN-L is still subject to some of the regulatory mechanisms that have been selected for by tumors to target and functionally downregulate the activity of the protein (such as the upregulation of PREX2, or the E3 ubiquitin ligase WWP2), the protein-based restoration of PTEN through PTEN-L therapy is not subject to events that occur on the genomic and message levels (41). Thus, this therapy evades the alterations that downregulate endogenous PTEN mRNA. Furthermore, it is possible that the superphysiologic levels that are observed in tumor xenografts that have been treated with PTEN-L could swamp the tumor’s evolved mechanism(s) for downregulating endogenous PTEN activity (as the tumors would have only needed to evolve mechanisms capable of coping with the levels of PTEN achievable in the endogenous state).

As we learn more about PTEN and how its loss affects tumor development and evolution, both in the context of the nascent tumor as well as in clinical settings in which PTEN loss seems to have profound effects upon drug efficacy, we will be able to target a subset of tumors for PTEN restoration therapy (42–44). It will be interesting to determine how PTEN-L performs versus PI3K inhibitors and in combination with other targeted treatments, because PTEN has functions beyond regulation of the PI3K pathway.

Conclusions

PTEN activity plays critical tumor-suppressive roles by acting both as a gatekeeper and landscaper tumor suppressor. Although small-molecule inhibitors have been successful in compensating for specific detriments caused by PTEN deficiency, the multifaceted nature of the protein makes it difficult to completely compensate for the loss of PTEN activity. The recent identification of extracellular variants of PTEN that can restore PTEN to tumor cells is exciting in that it offers the possibility that the full complement of PTEN activities could be restored in a straightforward manner.

Authors’ Contributions

Writing, review, and/or revision of the manuscript: B.D. Hopkins, R.E. Parsons

Acknowledgments

The authors thank Deepti Mathur for help in the preparation of the article.

Grant Support

This work is supported by NCI R01CA184016 and R01CA082783 (to B.D. Hopkins and R.E. Parsons).

Received May 23, 2014; accepted June 24, 2014; published online October 31, 2014.

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Inter cellular PTEN


