

PTEN Level in Tumor Suppression: How Much Is Too Little?

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Abstract

The importance of PTEN (phosphatase and tensin homolog located on chromosome 10) in cancer has surpassed all predictions and expectations from the time it was discovered and has qualified this gene as one of the most commonly mutated and deleted tumor suppressors in human cancer. PTEN levels are frequently found downregulated in cancer, even in the absence of genetic loss or mutation. *PTEN* is heavily regulated by transcription factors, microRNAs, competitive endogenous RNAs (such as the PTEN pseudogene), and methylation, whereas the tumor suppressive activity of the PTEN protein can be altered at multiple levels through aberrant phosphorylation, ubiquitination, and acetylation. These regulatory cues are presumed to play a key role in tumorigenesis through the alteration of the appropriate levels, localization, and activity of PTEN. The identification of all these levels of PTEN regulation raises, in turn, a key corollary question: How low should PTEN level(s) or activity drop in order to confer cancer susceptibility at the organismal level? Our laboratory and others have approached this question through the genetic manipulation of *Pten* in the mouse. This work has highlighted the exquisite and tissue-specific sensitivity to subtle reductions in *Pten* levels toward tumor initiation and progression with important implications for cancer prevention and therapy. *Cancer Res*; 71(3): 629–33. ©2011 AACR.

Introduction

PTEN was originally discovered as the tumor suppressor gene frequently lost on chromosome 10q23 (1, 2). Soon after, the relevance of PTEN in cancer was addressed through the generation of germline knockout *Pten* mice by several independent laboratories (3–5). These studies revealed the requirement of PTEN for embryonic development. Importantly, heterozygous loss of this tumor suppressor in the mouse resulted in the development of cancer of multiple origins (3–6), as well as in a lethal lymphoproliferative disease (3–5). These studies provided the first evidence for a haploinsufficient tumor suppressive activity of PTEN, because a fraction of cancers arising in *Pten* heterozygous mice would not exhibit loss of the wild-type allele (4, 5). In humans, germline loss and mutation of PTEN is observed in a group of autosomal dominant syndromes [PTEN hamartoma tumor syndromes (PHTS)], which are characterized by neurologic disorders, multiple hamartomas, and cancer susceptibility (7). Studies from the mouse have recapitulated a fraction of the

features observed in PHTS patients; however, the cooperative genetic or environmental factors contributing to the full symptomatic spectrum in this group of syndromes remain to be defined. In addition, analysis of cancer biopsies suggests that the notion that PTEN is a haploinsufficient tumor suppressor, formally proven in the mouse, can also be translated to humans (8).

How Does PTEN Exert Its Powerful Tumor Suppressive Activity?

PTEN functions as a lipid phosphatase, dephosphorylating the 3' position of phosphoinositide 3,4,5-triphosphate (PIP3). This lipid second messenger is the product of a potent proto-oncogenic kinase, phosphoinositide 3-kinase (PI3K), and the trigger for activation of the PI3K pathway. The relevance of the PI3K pathway in cancer is highlighted by the elevated number of components within the cascade whose level or activity is found altered, and it represents one of the main targets for cancer therapy (9). However, despite the main role of PTEN as a negative regulator of the PI3K pathway, recent studies report a number of tumor suppressive activities for PTEN that are exerted from within the nucleus (10–14), where catalysis of PIP3 does not seem to represent a central function of this enzyme (15). These nuclear PTEN activities include the regulation of genomic stability, cell cycle progression, differentiation, and gene expression (10–14). On the basis of these studies, it is apparent that we have only begun to understand the multiple other functions of PTEN that occur in the nucleus or other cellular compartments, and to elucidate the activities of PTEN for which the phosphatase domain might not be required.

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What Are the Consequences of Progressive PTEN Dose Reduction in Tumorigenesis?

PTEN is one of the most mutated and deleted tumor suppressors in human cancer but, importantly, it is also found partially downregulated in cancer in the absence of genetic loss or mutation (10). Mouse genetic engineering has allowed us to study the impact of fractional *PTEN* level reduction in cancer. *Pten* heterozygous mice develop a number of cancers, including mammary, prostate, uterine, and pheochromocytoma (3–5). However, a lethal lymphoproliferative disease in the *Pten* heterozygous model has limited the study of cancers that might develop with longer latencies. In this respect, the generation and use of conditional *Pten* knockout mice has contributed to a better understanding of its tumor suppressive activity in specific cells and/or tissues *in vivo*, in a partial or complete loss setting (16). Moreover, conditional *Pten* knockout mouse models have uncovered unexpected and seemingly paradoxical consequences of complete *Pten* loss. These findings include the activation of potent fail-safe mechanisms such as a novel cellular senescence program (17), or the exhaustion of the hematopoietic stem cell compartment prior to leukemia development (18, 19). These findings, however, did not address the impact of subtle or incremental reductions in PTEN levels.

In an effort to study the impact of subtle *Pten* reduction in a nonallelic manner [i.e., wild-type (100%) versus heterozygous (50%) versus null (0%)], our laboratory generated a hypomorphic *Pten* mouse allelic series taking advantage of genetic expression interference. These mice harbor a neomycin cassette in one allele of *Pten*, which interferes with *Pten* transcription and reduces mature mRNA levels (20). In this manner, we at first generated and characterized mice in which the levels of *Pten* lie between the heterozygous and knockout [by combining the hypomorphic allele with a knockout allele, which resulted in a 70 to 80% decrease in *Pten* levels, termed *Pten* "hypo" mice (*Pten*^{hy/-})]. Although the *Pten* heterozygous mutants develop high-grade prostate intraepithelial neoplasia, an *in situ* form of prostate cancer, at incomplete penetrance, the *Pten hypo* mice develop prostate cancer lesions at full penetrance. Importantly, a fraction of cancers in these mutants now progresses toward invasive prostate carcinoma in the absence of loss of heterozygosity of the wild-type allele (20). This study provided the first experimental evidence showing that a subtle decrement in *Pten* expression can have dramatic consequences in cancer progression.

Because complete loss of *Pten* elicits the activation of a fail-safe response in the prostate (17), and possibly in other tissues, tumors with loss of one copy of PTEN might benefit from "hypomorphing," or further reducing PTEN levels, without losing the remaining functional allele, at least until the fail-safe pathway (i.e., cellular senescence) has been bypassed (for instance through p53 mutation or loss; ref. 17).

As for how PTEN reduction below heterozygosity can occur, it is plausible that environmental factors (diet and chemicals, such as zinc; ref. 21) or genetic events [i.e., microRNAs and loss of competitive endogenous RNAs (ceRNA), such as the PTEN pseudogene; ref. 22] might contribute in leading to more

aggressive cancer (Fig. 1). Among the mechanisms that induce PTEN downregulation in cancer as way to favor tumor progression, the effect of the mitogen-activated protein kinase (MAPK) pathway on the transcription of this tumor suppressor is remarkable. Expression of an oncogenic version of the small GTPase Ras (in which glycine 12 is mutated to valine, G12V) leads to the reduction of PTEN levels. PTEN downregulation is transcriptional and is driven by the induction of c-Jun downstream of MAPK signaling (23). On the other hand, a recent study from our laboratory has uncovered a novel type of regulatory mechanism on the basis of the existence of ceRNAs. As a proof of principle, we have shown that a noncoding RNA, the PTEN pseudogene, exerts a tumor suppressive activity on the basis of the homology of its 3' untranslated region with PTEN, which allows it to quench PTEN-targeting miRNAs and, in turn, to regulate PTEN expression level (22). These studies exemplify the complexity of PTEN regulatory networks and, therefore, the importance of fine tuning the expression of this tumor suppressor. Of interest, the promoter region of PTEN exhibits regulatory elements not only for c-Jun, but also for other survival signals (e.g., NFκB; ref. 24), therefore, suggesting that additional cancer-promoting factors may act through PTEN downregulation, leading to cancer progression or resistance to therapy.

Is There Evidence That a Subtle Reduction of PTEN Expression Level Is Sufficient to Promote Cancer Susceptibility?

We have addressed this question by generating the *Pten* "hyper" mouse model. Taking advantage of the *Pten* hypomorphic allele, we generated mice with one *Pten* hypomorphic allele over a wild-type allele (*Pten*^{hy/+}). These mice, as predicted, express 80% of normal *Pten* mRNA and protein levels (25). Surprisingly, *hyper* mice exhibit many of the features present in *Pten* heterozygous mice, including early lethality, signs of a lymphoproliferative disease, and cancer susceptibility. More importantly, they develop tumors. Interestingly, the spectrum of tumors is distinct from that observed in heterozygous mice. Mammary tumors are most prevalent, whereas lung tumors (which were not observed in the *Pten* heterozygous cohort analyzed) are also detected. Interestingly, tumors arising in *hyper* mice retain the wild-type *Pten* allele and gene expression, and do not harbor mutation at the remaining PTEN locus. In addition, *Pten hyper* mice do not show signs of lesions in the prostate epithelium, unlike heterozygous and *hypo* mice. These data suggest that there is an exquisite tissue-specific sensitivity to the reduction of *Pten* levels (Fig. 1), with tissues that require a more profound downregulation in *Pten* for cancer progression to occur (prostate) and others more sensitive to small decreases (lymph nodes, mammary epithelium). In the mammary gland, we can readily observe signs of hyperproliferation in the epithelium of 2-month-old females, indicating that the reduction of *Pten* has immediate cell autonomous consequences in the mammary tissue. The fact that 80% of total levels are not sufficient for *Pten* to suppress cancer revisits the idea that *Pten* is solely a haploinsufficient tumor suppressor, suggesting that levels

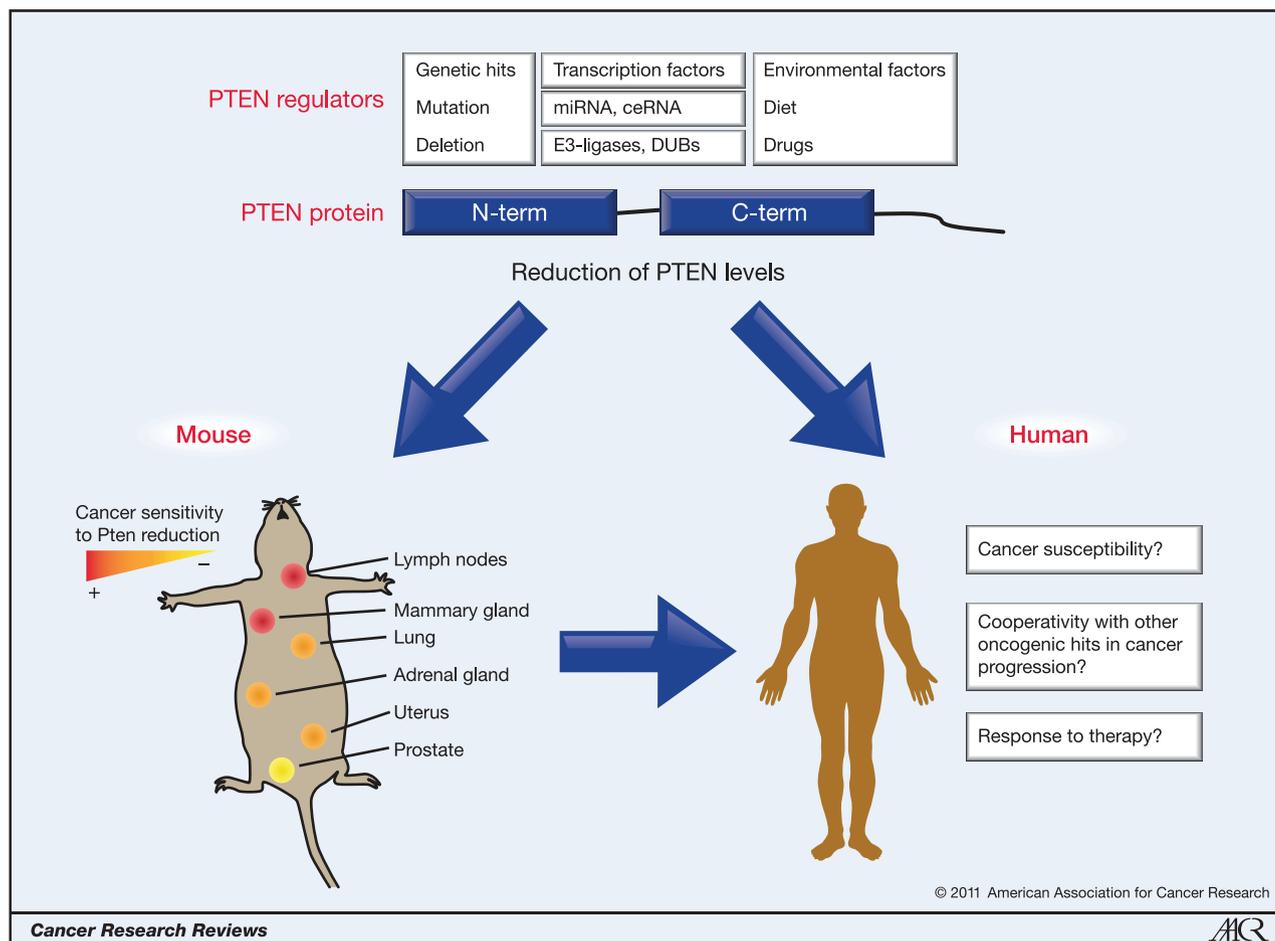


Figure 1. Impact of PTEN regulatory cues in tumorigenesis. Schematic of the tissue-specific cancer sensitivity to PTEN downregulation and the regulatory factors that could lead to the reduction of PTEN levels. PTEN expression is regulated at multiple levels, genomic (mutation and deletions), transcriptional (transcription factors), post-transcriptional (miRNAs, ceRNAs, such as the PTEN pseudogene), and post-translational [drugs, E3-ligases, deubiquitinating enzymes (DUB)]. In turn, the reduction of PTEN levels has a gradual and tissue-specific impact on cancer initiation in the mouse, with the mammary tissue being among the most sensitive tissues to PTEN level reduction. These data suggest that, in humans, subtle reduction of PTEN levels may lead to cancer susceptibility, cooperate with other oncogenic events in cancer progression, or affect the response to therapy.

above 50% still do not suffice to keep cancer development at bay, a phenomenon that we have termed tumor suppressor "quasi-insufficiency."

Can We Find Evidence of PTEN Quasi-insufficiency in Human Cancer?

We define "quasi-insufficiency" as the inability to sustain adequate biological functions upon a subtle reduction in the protein levels of a given gene (below homozygosity but above heterozygosity). Microarray analysis of normal breast tissue allowed us to define the normal average PTEN mRNA level and, in turn, to evaluate the level of PTEN in breast tumors (25). PTEN levels in cancer biopsies followed a linear distribution in the cohort analyzed, ranking from levels of expression equivalent to those observed in tissue from normal subjects to levels of expression below 20%. Notably, 20% of tumors displayed levels of PTEN slightly below the average levels of the normal tissue (above 65% of the normal average PTEN

levels are *hyper* tumors) and protein levels by immunohistochemistry that excluded a robust decrease in PTEN expression. Interestingly, these tumors presented a gene expression profile similar to the one detected in cells from our *hyper* mouse, suggesting that PTEN levels are associated with a defined gene signature related to cell proliferation. We find particularly interesting that PTEN levels in the normal breast tissue also show a linear distribution, with a 15% standard deviation in our small cohort. These data suggest that PTEN levels may vary by a small fraction of expression among healthy individuals, and according to our data, these patients might present cancer susceptibility and should, therefore, be followed up to closely monitor the development of tumors. Moreover, the idea that subtle changes in the expression of a gene can have profound effects on tumorigenesis adds complexity to the "two-hit hypothesis" postulated by Alfred Knudson (26). On the basis of the information obtained from our *hyper* mice, alterations in the protein levels of a tumor suppressor gene, even in the absence of a "genetic hit," could

be sufficient to trigger a cellular response resulting in cellular transformation and cancer. Further studies will identify "cancer quasi-insufficient genes" and the deregulated pathways relevant for cancer development.

The other important implication derived from years of study on the role of PTEN in tumor suppression is the tissue-specific sensitivity to PTEN level reduction and its tissue-specific cooperativity with various oncogenes and tumor suppressors. In fact, mouse modeling has uncovered that loss of *Pten* cooperates with a diverse array of genetic hits in tumor development and progression. An exemplifying case is the study of cellular senescence evoked by *Pten* inactivation (17). Upon PTEN loss in the prostate epithelium, the tumor suppressor p53 is potently induced to elicit senescence and oppose cancer progression. In turn, compound loss of *Pten* and *Tp53* in this tissue results in aggressive and lethal prostate cancer development. Additionally, *Pten* loss in the prostate cooperates with *Tsc2* and *p27* loss as well as *Erg* overexpression (16), among others. In the breast, data showing that *BRCA1* loss results in increased mutations and microdeletions in the *PTEN* locus (27) also suggest a possible cooperativity between PTEN and *BRCA1*.

On the basis of our data, it is tempting to speculate that the sensitivity to PTEN-dosage reduction of a given tissue could be determined by its gene expression signature. Specifically, the sensitivity of the mammary gland to subtle PTEN downregulation could be due to a gene expression network that makes it less tolerant to variations in PTEN expression. Therefore, identifying the *PTEN* loss-cooperating events (or susceptibility gene signatures) in specific tissues could contribute to define the network of functions exerted by PTEN in tumor suppression and the relevance of each PTEN function in a given tissue.

Future Directions

More than 10 years of PTEN research has led to a new perspective on this critical tumor suppressor. From the phosphatase that opposes PI3K signaling in the cytoplasm, PTEN

has evolved into a protein that exerts a variety of tumor suppressive activities in the nucleus, beyond the dephosphorylation of PIP3. These studies have contributed to the understanding of how sensitive tissues are to even subtle reduction of PTEN levels and, in turn, have raised further questions on how PTEN functions. Does the subtle reduction of PTEN drive cancer susceptibility because of the reduction of PIP3 hydrolysis or because of loss of nuclear functions? Conversely, is elevation of PTEN level(s) a safe and effective approach to prevent or treat cancer? Can we quantitatively monitor PTEN levels in patients in order to predict cancer susceptibility? Our laboratory and others are focusing on providing definitive answers to these questions. Addressing these questions will, in turn, bring our understanding of the rules and mechanisms of tumor suppression and oncogenesis to a new exciting level. On the basis of these new notions, a journey of discovery that started at the bedside through the discovery of PTEN in a human cancer specimen, which subsequently was translated to cell culture and faithful mouse models of cancer, will next be brought back to the bedside in the form of effective diagnostic, prognostic, and therapeutic tools for cancer prevention and therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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