Commentary

PTEN, a Protean Tumor Suppressor

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The molecular biology of the *PTEN* tumor suppressor gene is as multifaceted as the range of sporadic human malignancies in which it has been implicated. Multiple mechanisms can inactivate *PTEN* in glioblastoma, melanoma, and carcinomas of the thyroid, breast, prostate, endometrium, and ovary. Initial impressions, based on mutational analysis alone, that ovarian cancer *PTEN* inactivation is infrequent bear revision in light of a 27% frequency of lost *PTEN* protein expression reported by Kurose and colleagues in this issue of *The American Journal of Pathology*.¹ Only 8% of *PTEN* protein nonexpressing ovarian adenocarcinomas are explained by combined allelic imbalance (loss of heterozygosity) and mutation, suggesting that transcriptional silencing by epigenetic mechanisms may be yet an additional means of modifying *PTEN* activity. Although they show for ovarian carcinoma that *PTEN* function can be delimited by an identifiable and consistent repertoire of downstream effectors, such as the Akt pathway, the consequences of *PTEN* inactivation are nonuniform in different tissues. Thus, in the endometrium *PTEN* acts as a gatekeeper for initiation of carcinogenesis, yet in prostate cancer and melanoma it defines a much later event, metastasis. Tissue context determines the molecular events that inactivate *PTEN* and heavily modify the resultant phenotype.

A Multitude of Inactivating Mechanisms

Functional inactivation of the *PTEN* gene may occur through deletional and mutational mechanisms, and these are variably invoked between tumor types (Table 1) and hereditary/sporadic settings. Thus, it is necessary to have an integrated and comprehensive snapshot of different modalities of *PTEN* inactivation before an accurate model of changing *PTEN* function can be concluded for a specific tumor type. Patients with Cowden syndrome have a high incidence of breast cancer caused by heritable constitutive structural mutations of the *PTEN* gene,²⁻⁴ yet such mutations are rarely seen in sporadic breast cancer. Rather, the *PTEN* lesions of sporadic breast cancers are a deletion-induced hemizygous state.⁵⁻⁷ Deletion of the *PTEN* region at 10q23 are also

predominant findings in *PTEN*-deficient melanomas^{8,9} and glioblastomas.¹⁰⁻¹⁵ In contrast, both *PTEN* deletion and mutation are frequent events in sporadic endometrioid endometrial adenocarcinoma.¹⁶⁻²¹ It should be remembered that technical variation can confound reproducibility of loss of heterozygosity and mutational data between laboratories. Deletion and mutation detection is affected by the extent to which normal tissue contaminates isolated lesional DNA, a process that varies between investigators. For example, denaturing gradient gel electrophoresis (DGGE) is particularly suited for detecting as low as 1 to 10% mutant DNA contribution. These problems are not unique to *PTEN*, but are compounded when multiple mechanisms must be evaluated in a single tumor type. Many of the literature inconsistencies between reported involvement of the *PTEN* gene in specific tumor types can be attributed to the methodologies used. Ovarian carcinoma is an example where the impression of the extent of altered *PTEN* genotype has been highly dependent on whether mutation alone,²² or deletion and mutation^{1,23} are evaluated.

The mechanism of *PTEN* inactivation seems to be conserved in a given histological subtype of adenocarcinoma irrespective of the primary site. For example, *PTEN* inactivation in endometrioid adenocarcinomas of the ovary^{1,24} and endometrium^{18,21} have similar patterns of deletion and mutation. In contrast, *PTEN* mutation is quite rare in carcinomas with papillary serous differentiation irrespective of whether they occur in the ovary or endometrium. We can thus identify the histological mix²⁴ of tumors as another variable that must be considered in comparing results from series of cases derived from a common site.

Loss of *PTEN* function in endometrial,²¹ breast,⁷ prostate,²⁵ ovarian,¹ and melanocytic⁹ tumors is more frequent than can be adequately explained by structural genomic changes alone. Careful exclusion of deletional and mutational mechanisms in these cases has led to the prediction of epigenetic mechanisms as yet another means by which this tumor suppressor can be silenced. Proving an epigenetic mechanism of *PTEN* silencing is technically nontrivial because of the large size (>250 kb)

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Table 1. PTEN Lesions (Deletion and/or Mutation) in Sporadic Human Malignancies

Site	Tumor type	PTEN inactivation		Comment	Reference no.
		Range	Average		
Brain	Glioblastoma	17–70%	48% (107/224)	Mostly LOH	10–15
Breast	Ductal carcinoma	15–48%	37% (37/100)	Mostly LOH	6, 7
Endometrium	Endometrioid carcinoma	34–83%	42% (139/334)	LOH and mutation	16–21
Prostate	Adenocarcinoma	17–41%	33% (49/149)	Mostly LOH	43, 45, 46
Ovary	Cystadenocarcinoma	6–45%	33% (65/198)	LOH and mutation	1, 22–24, 47
Skin	Melanoma	32–33%	33% (18/55)	Mostly LOH	8, 9
Thyroid	Carcinoma	37%	37% (19/51)	Mostly LOH	44

LOH, loss of heterozygosity.

of its upstream regulatory region, the existence of a highly conserved processed pseudogene²⁶ with homology maintained up to 1 kb upstream of the translational start site, and technical challenges in linking epigenetic events with expression level. Recent reports of PTEN promoter methylation²⁷ in endometrial cancer, and reacquisition of PTEN expression on treatment of prostate cancer cells with the demethylating agent 5-azadeoxycytidine²⁵ are consistent with epigenetic mechanisms at work in these models.

Implication in Diverse Regulatory Pathways

In vitro cell line data has suggested that the tumor suppressor functions of PTEN, including G₁ arrest and enabling of apoptosis, are mediated by a cascade that maintains the putative downstream factor Akt in a dephosphorylated state.^{28,29} Thus, the prediction that PTEN protein and phospho-Akt have an inverse quantitative relationship. The article by Kurose and colleagues¹ (page xx, this issue) uses a series of primary sporadic ovarian cancers to confirm this inverse relationship between PTEN protein and phospho-Akt. This is an important extrapolation from a cell culture model, which is necessarily limited in representing the range of genetic variation and heterogeneity present in sporadic human tumors. Because the majority of ovarian cancers with elevated phospho-Akt levels are accompanied by demonstrable changes in PTEN function, PTEN presents itself as a major determinant of Akt-mediated apoptosis and G₁ arrest in ovarian cancer. Mitotic arrest and cell death are, however, basic cellular functions controlled by a complex web of regulatory pathways that probably include elements outside the PTEN-Akt axis. The finding that p27 and cyclin D1 do not necessarily behave according to a simple linear model aligned with PTEN and Akt¹ suggests that these downstream events are indirect or subject to modification.

Additional functions of PTEN outside the Akt pathway have been proposed, and are still under active experimental consideration. These include control of cell adhesion and migration by dephosphorylation of focal adhesion kinases.^{30,31} Although this might explain some of the altered functions expected in neoplastic transformation, the effect of PTEN on adhesion is probably a complex one that likely involves other intermediary molecules.³²

Nongenetic Modifiers

There must be as yet unidentified factors that are capable of significantly modifying the phenotypic presentation of cells with altered PTEN function. The constellation of sporadic human tumors characterized by PTEN inactivation (Table 1) only partly overlaps with that of the heritable cancer syndrome caused by constitutive PTEN inactivation, Cowden syndrome. Cowden syndrome is often accompanied by thyroid (mainly follicular histology), breast, and endometrial cancers and benign hamartomatous lesions of the skin and brain.³³ Surprisingly, they have not been reported to have heightened incidences of ovarian and prostate adenocarcinomas or glioblastoma, sporadic tumor types that often have PTEN inactivation. One possible explanation is that existing series of Cowden syndrome patients are too small to precisely measure even significant changes in incidences of these tumors beyond their already low sporadic occurrence rates. There is also divergence of tumor spectrum between murine *pten*-deficient mice and human neoplasia. *pten* knockout mice develop papillary thyroid, breast, prostate, and endometrial tumors, but not glioblastomas or ovarian carcinoma.^{34,35} One constitutively *pten*-deficient mouse model³⁶ resulted in lymphoproliferative lesions that are not seen in human Cowden syndrome and have not been otherwise associated with prominent somatic PTEN mutation. It is reasonable to postulate that a germline PTEN mutation such as knock-out murine models or Cowden patients may result in permanent compensatory changes during early development that in turn results in a different spectrum of tumors compared to sporadic somatic mutations. Thus, although heritable PTEN-deficient mice and Cowden patients have been useful in defining the tumor suppressor activity of PTEN, caution must be used in extrapolation between species, and to a sporadic setting.

Hormonal environment is one systemic factor that may modulate physiological demand for PTEN protein, thereby defining a shifting normal baseline against which the functional implications of PTEN loss must be measured. Normal PTEN expression increases in endometrial glands during the estrogenic follicular phase of the menstrual cycle,³⁷ and declines dramatically on introduction of the antiestrogenic hormone progesterone. It is unknown whether estrogen-associated increases in endo-

metrial PTEN expression is part of a general mitogenic response, or whether there is a direct upstream link between the estrogen response pathway and the *PTEN* gene. Whatever the case, it seems that a rapidly dividing estrogen-stimulated endometrial gland has a greater PTEN requirement than a quiescent progesterone-exposed nonmitotic gland, and it is reasonable to conclude that these settings would respond differently to loss of PTEN function. Consistent with this notion is the fact that the primary epidemiological risk factor for PTEN-deficient endometrial carcinomas (endometrioid histological subtype) is protracted estrogen exposure.³⁸ The possibility of hormonal regulation of PTEN protein in other tissue types is largely unexplored. There is, however, enticing preliminary data that suggests that PTEN may inhibit cell growth through the MAP kinase-dependent insulin response pathway in an *in vitro* breast cancer model.³⁹

Early and Late Effects: Tumor Initiation Versus Metastasis

It is remarkable that PTEN inactivation may affect quite different stages of tumor evolution, being highly consistent in multiple examples of tumors at one site. In the case of endometrioid endometrial adenocarcinoma, loss of PTEN expression is usually invoked quite early on, in the manner of a gene that performs a gatekeeper function.⁴⁰ PTEN mutation, deletion, and loss of expression are seen in the premalignant phases of endometrial carcinogenesis, and appear at highest frequency (83%) in those adenocarcinomas that are preceded by a histologically evident premalignant hyperplasia (EIN, endometrial intraepithelial neoplasia)²¹ and have an indolent, nonaggressive clinical course. Loss of PTEN function may even precede acquisition of cytological atypia, a histopathological feature long thought to distinguish the threshold between benign and premalignant endometrial disease.²¹ Clear cell and endometrioid ovarian adenocarcinomas may also share deletion of the PTEN locus^{41,42} with premalignant (endometriosis) tissues.⁴²

In contrast, PTEN inactivation in melanoma,⁸ prostate carcinoma,⁴³ and glioblastoma,^{11,13} is a marker for an aggressive subset of tumors likely to metastasize. Although metastasis is considered to be a late event in tumor progression, PTEN inactivation in these cases probably occurs earlier in tumor evolution. Even for those prostatic carcinomas that have metastasized, PTEN deletions are already widely present at the primary site.⁴³ Aggressive glioblastomas with PTEN loss of function are primarily high grade throughout, and PTEN inactivation does not often occur in tumors that undergo a progressive increase in grade during their growth.¹⁵ The full manifestation of a complex phenotype such as metastasis and acquisition of a contributing single mutation (such as PTEN) may be separated by a lag period, during which other mutations required for the full manifestation of this phenotype are accumulated. As a consequence, despite being an early event, PTEN mutations in some tumors may become important in later stages, such as metastasis and aggressive behavior.

Future Challenges

Compromise of PTEN function is widespread in human cancers, occurring in fully a third of some of the most common human malignancies. This has caught the keen attention of clinicians and scientists alike, and highlights the need to reconcile data garnered from a variety of settings. Enticing as it is to combine these in a sweeping model centered on a single gene, the truth is in the details. The basis of lost PTEN function is not yet completely explained, and investigation of whether epigenetic events are in fact a common mechanism of inactivation is a priority. Recent availability of antibody reagents applicable to paraffin-embedded tissues^{7,21} will greatly facilitate continued use of primary human material in those experiments intended to relate *PTEN* gene expression to epigenetic modification. The effects of post-translational factors on PTEN action have not yet been systematically studied. Cell-type-specific shifts in cellular compartmentalization of PTEN protein have been seen in conjunction with neoplastic transformation and cellular differentiation,^{1,37,44} but the functional impact of these changes is unknown. Lastly, observed idiosyncrasies of tissue-specific PTEN inactivation mechanism and their resultant phenotype will be a useful tool for unraveling those factors that modify the functional requirements for PTEN protein, and the biological implications of their loss.

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