PTEN in Hereditary and Sporadic Cancer

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Germline pathogenic phosphatase and tensin homolog (*PTEN*) mutations cause *PTEN* hamartoma tumor syndrome (PHTS), characterized by various benign and malignant tumors of the thyroid, breast, endometrium, and other organs. Patients with PHTS may present with other clinical features such as macrocephaly, intestinal polyposis, cognitive changes, and pathognomonic skin changes. Clinically, deregulation of *PTEN* function is implicated in other human diseases in addition to many types of human cancer. *PTEN* is an important phosphatase that counteracts one of the most critical cancer pathways: the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathways. Although *PTEN* can dephosphorylate lipids and proteins, it also has functions independent of phosphatase activity in normal and pathological states. It is positively and negatively regulated at the transcriptional level as well as posttranslationally by phosphorylation, ubiquitylation, oxidation, and acetylation. Although most of its tumor-suppressor activity is likely to be caused by lipid dephosphorylation at the plasma membrane, *PTEN* also resides in the cytoplasm and nucleus, and its subcellular distribution is under strict control. In this review, we highlight our current knowledge of *PTEN* function and recent discoveries in understanding *PTEN* function regulation and how this can be exploited therapeutically for cancer treatment.

The tumor-suppressor gene *phosphatase and tensin homolog (PTEN)* (OMIM 601728) was originally recognized as mutated somatically in multiple sporadic cancers (Li et al. 1997; Steck et al. 1997), as well as mutated in the germline of patients with Cowden syndrome (CS; OMIM 158350), a hereditary overgrowth and cancer predisposition disorder (Nelen et al. 1996;

Liaw et al. 1997). *PTEN* is a dual specificity phosphatase that canonically negatively regulates the phosphatidylinositol 3-kinase (PI3K) signaling cascade. Through its lipid phosphatase activity, *PTEN* dephosphorylates phosphatidylinositol 3,4,5-trisphosphate (PIP₃) to phosphatidylinositol 4,5-bisphosphate (PIP₂), thereby dampening downstream protein kinase B

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(PKB/AKT) signaling (Maehama and Dixon 1998; Stambolic et al. 1998). Left unchecked, such as through *PTEN* mutation or inactivation, elevated PIP₃ levels cause constitutive activation of AKT and downstream cascades such as mammalian target of rapamycin (mTOR) signaling (Inoki et al. 2002). This ultimately leads to cell survival, growth, proliferation, and decreased apoptosis (Furnari et al. 1997; Di Cristofano et al. 1998; Weng et al. 1999). Although originally believed to be an exclusively cytoplasmic phosphatase, *PTEN* is now known to also function within the nucleus to regulate the cell cycle, double-strand break repair, genomic stability, and chromatin remodeling (Wu et al. 1998; Weng et al. 2001a,b; Baker 2007; Shen et al. 2007; Chen et al. 2014). Therefore, *PTEN* is characterized by various phosphatase-dependent and -independent cellular functions (Fig. 1; Lee et al. 2018; for additional information on *PTEN* signaling, see Shen 2019; Lee and Trotman 2019; Yang and Yin 2019).

Germline *PTEN* mutations have been identified in patients with different clinical syndromes, and the mutation-positive subset is termed *PTEN* hamartoma tumor syndrome (PHTS) (Marsh et al. 1999; Eng 2016; for review, see Yehia et al. 2019). Besides *PTEN* mutation-positive CS, PHTS also encompasses individuals with Bannayan–Riley–Ruvalcaba syndrome (BRRS) and Proteus syndrome (PS) and Proteus-like syndrome who have germline *PTEN* mutations (Zhou et al. 2001; Smith et al. 2002; Kirk et al. 2004; Loffeld et al. 2006; Eng 2016). BRRS (OMIM 153480) is a rare congenital disorder classically characterized by macrocephaly in combination with intestinal hamartomatous polyposis, vascular malformations, lipomas, hemangiomas, and genital freckling (Ruvalcaba et al. 1980; Gorlin et al. 1992). PS (OMIM 176920) is a rare, complex, and highly variable disorder characterized by progressive, postnatal overgrowth of multiple tissues derived from different cell lineages (Cohen and Hayden 1979). Relatedly, germline and somatic mosaic mutations in other genes encoding components of the PI3K/AKT/mTOR signaling pathway downstream of *PTEN* predispose patients to partially overlapping sets of clinical manifestations rem-

iniscent of PHTS, referred to as *PTEN*-opathies (Yehia et al. 2019).

PTEN DYSFUNCTION AND HERITABLE CANCER

The identification of germline *PTEN* mutations allowed for the comprehensive elucidation of component cancers and associated lifetime risks in individuals with PHTS (Tan et al. 2012). Three independent studies revealed elevated risks for breast, thyroid, endometrial, kidney, and colon cancers and melanoma in PHTS (Table 1; Tan et al. 2012; Bubien et al. 2013; Nieuwenhuis et al. 2014). Similar to other hereditary cancer syndromes, the risk for bilateral and multifocal cancer is elevated (Eng 2016). Relatedly, individuals with PHTS have a sevenfold increased risk of developing second malignant primary neoplasms (Ngeow et al. 2014). Collectively, these cancer risk-assessment studies inform clinical surveillance recommendations and medical management of individuals with germline *PTEN* mutations (Tan et al. 2012), with the aim of detecting malignancies at the earliest, most manageable stages (Table 2).

PTEN comprises nine exons encoding a 403-amino acid protein (Fig. 2A; Li et al. 1997; Li and Sun 1997). Broadly, *PTEN* mutations could impact the abundance of *PTEN* protein resulting in haploinsufficiency, result in reduced or lost phosphatase activity, act in a dominant-negative manner, and/or result in aberrant localization and function (Lee et al. 2018). The germline mutation spectrum in PHTS is broad, with mutations affecting all nine exons of *PTEN* (Fig. 2B; Tan et al. 2011, 2012; Mester and Eng 2013; Ngeow et al. 2014). Approximately two-thirds of germline *PTEN* mutations occur in exons 5, 7, and 8, encoding the phosphatase domain (Tan et al. 2011). Interestingly, up to 40% of all germline *PTEN* mutations are located in exon 5, encoding the core catalytic motif, although this exon represents only 20% of the coding sequence (Waite and Eng 2002; Eng 2003; Tan et al. 2011). Relatedly, two distinct Alu elements have been reported in two unrelated CS patients with identical breakpoints within exon 5, suggesting that this exon is a possible retrotranspo-

Figure 1. Cytoplasmic and nuclear PTEN signaling. In the cytoplasm, PTEN canonically functions to regulate the phosphatidylinositol 3-phosphate kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway. Under growth factor stimulation, PI3K is activated and catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP₂) to phosphatidylinositol 3,4,5-trisphosphate (PIP₃). PIP₃ recruits PDK1 to the plasma membrane, which then contributes to the activation of AKT. AKT regulates a myriad of downstream cellular processes such as cell growth, proliferation, and decreased apoptosis. The lipid phosphatase activity of PTEN counteracts PI3K by dephosphorylating PIP₃ to PIP₂, thereby dampening AKT activation. In the nucleus, PTEN plays a vital role in maintaining genomic stability, chromosomal architecture, cell-cycle control, and the regulation of ribosome biogenesis within nucleoli.

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Table 1. Component cancer risks in PHTS patients

Cancer	Population risk (SEER) (%)	Lifetime risk in PHTS (%)
Breast (female)	12	67–85
Thyroid	1	6–38
Endometrial	2.6	21–28
Renal cell	1.6	2–34
Colon	5	9–17
Melanoma	2	2–6

Table created from data in Tan et al. (2012), Bubien et al. (2013), and Nieuwenhuis et al. (2014). Lifetime risks calculated to age 70 by Tan et al. (2012) and Bubien et al. (2013), and to age 60 by Nieuwenhuis et al. (2014). Cancer risk percent ranges reflect lowest and highest frequencies reported in all three studies.

(SEER) surveillance, epidemiology, and end results, (PHTS) *PTEN* hamartoma tumor syndrome.

sition hotspot (Crivelli et al. 2017). Mutations within the core catalytic motif typically abrogate pan-phosphatase (lipid and protein) activity such as mutations affecting p.Cys124, but, rarely, mutations such as p.Gly129Glu result in abrogation of lipid phosphatase activity only (Liaw et al. 1997; Myers et al. 1998; Davidson et al. 2010).

PTEN protein domains have distinct functions (Fig. 2A). For example, the amino-terminal phosphatase domain contains two ATP-binding motifs, critical for regulating PTEN exit from the nucleus (Lobo et al. 2008). ATP-binding motif mutants (e.g., p.K62R, p.Y65C, p.K125E) do not bind ATP efficiently, resulting in nuclear PTEN mislocalization. This subsequently leads to increased cellular proliferation, reduced/abrogated apoptosis, and increased anchorage-independent growth, leading to carcinogenesis (Lobo et al. 2009; He et al. 2011a). Mechanistically, nuclear mislocalization of PTEN results in both overexpression of p-AKT due to compromised cytoplasmic lipid phosphatase activity and loss of control over cyclin D1 levels, an important regulator of G₁ to S-phase cell-cycle transition downstream of PTEN (Weng et al. 2001a; He et al. 2011a). Additionally, PTEN ATP-binding mutations result in decreased nuclear p53 protein levels and transcriptional activity, as well as accumulation of oxidative DNA damage due to elevated reactive oxygen species (ROS) production. Indeed, mutant cells exhibit decreased transcript levels of *TP53INP1*, a major mediator of p53-mediated antioxidant function (Cano et al. 2009). Finally, compared to wild-type PTEN, subsets of ATP-

binding mutants have reduced PTEN protein stability, both in vitro and in vivo (He et al. 2011a).

Other mutations affecting amino acids critical for PTEN posttranslational regulation also offer important insights. For example, catalytically active mutant PTEN p.Lys289Glu is characterized by a nuclear import defect due to loss of monoubiquitination at p.Lys289 (Trotman et al. 2007). Hence, the p.Lys289Glu protein is reminiscent of a cytoplasmic-predominant phenotype and compromised nuclear PTEN tumor-suppressive function (Fig. 1). Nuclear PTEN is thought to be protected from polyubiquitination and subsequent proteasome-mediated degradation in the cytoplasm and is able to dampen AKT signaling and induce p53-independent apoptosis (Trotman et al. 2007). In support of these observations, nuclear exclusion of PTEN has been associated with more aggressive advanced-stage sporadic cancers (Gimm et al. 2000; Zhou et al. 2000, 2002; Tachibana et al. 2002; Whiteman et al. 2002).

Aside from intragenic mutations, ~10% of CS patients harbor germline *PTEN* promoter mutations (Zhou et al. 2003b). Pathogenic promoter mutations result in decreased *PTEN* transcription and translation, the latter due to altered mRNA secondary structure (Zhou et al. 2003b; Teresi et al. 2007). More recently, some unsuspected *PTEN* intronic variants were shown to result in pathogenic exon skipping, alternative splicing, or the use of cryptic splice sites (Chen et al. 2017b). These splicing changes correlate with significantly lower PTEN protein

Table 2. Screening and management recommendations for PHTS

	Screening/surgical guidelines ^a	Age to start	Frequency
Breast	Breast awareness and self-exam: report changes to health care provider	18	Consistent
	Clinical breast exam	25 ^b	Every 6–12 months
	Mammogram with consideration of tomosynthesis and breast MRI with contrast	30–35 ^b	Every 12 months
	Discuss mastectomy	Personalized	As needed
Thyroid	Thyroid ultrasound	Time of PHTS diagnosis, including childhood	Every 12 months
Kidney	Consider renal ultrasound	40	Every 1–2 years
Endometrium	Encourage patient education and prompt response to symptoms (e.g., abnormal bleeding)	Not applicable	Not applicable
	Consider screening via endometrial biopsy	Not applicable	Every 1–2 years
	Transvaginal ultrasound in postmenopausal women at the clinician's discretion	Not applicable	As needed
	Discuss hysterectomy with completion of childbearing	Personalized	As needed
Colon	Colonoscopy	35 ^b unless symptomatic	Every 5 years or more frequently depending on whether patient is symptomatic or polyps are found
Dermatologic	Dermatologic exam	Personalized	Clinician's recommendation
Developmental	Consider psychomotor assessment in children	Time of PHTS diagnosis	Clinician's recommendation
	Brain MRI if symptomatic	Time of PHTS diagnosis	Clinician's recommendation

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(PHTS) PTEN hamartoma tumor syndrome, (MRI) magnetic resonance imaging.

^aAnnual Comprehensive physical exam starting at age 18 or 5 years before the youngest age of diagnosis of a component cancer in the family (whichever comes first), with particular attention to thyroid exam. Encourage patient education regarding the signs and symptoms of cancer.

^bCancer screening should begin 5–10 years before the earliest known component cancer in the family or according to the ages listed in the above table, whichever comes first.

levels and elevated p-AKT in patients with splicing changes compared to those without aberrant splicing. Finally, large *PTEN* deletions occur in ~3%–10% of PHTS patients and can be found over the entire coding sequence (Zhou et al. 2003b; Tan et al. 2011; Mester and Eng 2013).

GENOTYPE-PHENOTYPE CORRELATIONS AND MODIFIERS OF CANCER RISKS IN PHTS

As with other inherited cancer syndromes, although it is possible to risk-assess increased organ-specific cancer probabilities, it is still impos-

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Figure 2. PTEN structure and gene mutation spectrum in PTEN hamartoma tumor syndrome (PHTS). (A) Three-dimensional structure of the PTEN protein. PTEN is a 403-amino acid (aa) protein that comprises an amino-terminal region including a phosphatidylinositol 4,5-bisphosphate (PIP₂)-binding domain (PBD) and a phosphatase domain, and a carboxy-terminal region including a C2 domain and a PDZ-binding domain. The active site is included between amino acid residues 123 and 130. The carboxy (C) tail contributes to PTEN stability and activity. The PDZ domain is important for protein–protein interactions that play a vital role in cellular signaling transduction. WPD loop, residues 88–98; P loop, residues 123–131; T1 loop, residues 160–171; CBR3 loop, residues 260–269; domain linker, residues 185–191. (B) *PTEN* germline mutation spectrum from 291 PHTS probands. *PTEN* consists of nine exons that encode a 403 amino acid protein. Different types of mutations are depicted in the lollipop plot overlaying the *PTEN* protein structure. The frequency of mutations correlates with the heights of the vertical lines representing each lollipop. The different types of mutations are color-coded as depicted in the key at the top of the figure. (From Yehia et al. 2019, with permission, from Elsevier © 2017.)

sible to predict at an individual level who will go on to develop any particular component cancer during his/her lifetime. Hence, multiple studies have attempted to find predictive *PTEN* genotype–phenotype correlations. Earlier studies revealed an association between *PTEN* germline mutations and malignant breast disease (Marsh et al. 1998, 1999). Missense mutations and mutations within and 5′ of the phosphatase core motif appear to be associated with multiorgan manifestations, serving as a surrogate of disease severity (Marsh et al. 1998). Other groups could not find such genotype–phenotype correlations (Nelen et al. 1999), although their sample size of

studied PHTS patients is small ($n = 13$), compared to the 44 families and 43 probands of the preceding studies (Marsh et al. 1998, 1999). More recently, germline *PTEN* frameshift mutations have been found to be overrepresented, but not absolute, in thyroid cancer (Ngeow et al. 2011), nonsense mutations in colorectal cancer (Tan et al. 2012), promoter mutations in breast cancer (Tan et al. 2012), and missense mutations overrepresented in individuals with autism spectrum disorder (ASD) (Frazier et al. 2015). Interestingly, a theoretical computational approach revealed global three-dimensional *PTEN* structural instability and inactive conformation in

cancer-associated *PTEN* mutations, whereas ASD-associated *PTEN* mutations revealed localized destabilization contributing to partial opening of the active site (Smith et al. 2019). Such effects cannot be extrapolated from *PTEN* secondary structure alone and indeed provide an important dimension to consider for assessing *PTEN*-genotype–PHTS-phenotype associations. Additionally, studies suggest that ASD-associated mutations tend to be less severe relative to non-ASD-associated mutations (Spinelli et al. 2015; Leslie and Longy 2016; Mighell et al. 2018). Finally, imbalances in *PTEN* subcellular localization could impact PHTS phenotypic manifestations. A murine model of germline-mislocalized cytoplasm-predominant Pten exhibits macrocephaly and a neurocognitive profile reminiscent of high-functioning ASD (Tilot et al. 2014, 2016). Intriguingly, germline-mislocalized nuclear-predominant *PTEN* can exist in patients with either cancer or ASD (He et al. 2011a; Mingo et al. 2018). In this context, it is tempting to speculate whether mutant nuclear *PTEN* plays distinct roles in an organ-specific manner related to the seemingly disparate phenotypes.

The lack of absolute *PTEN* genotype–phenotype correlations suggest that additional factors act as phenotypic modifiers in PHTS. A proof-of-principle study showed that ~6% of *PTEN* mutation–positive CS/CS-like individuals also harbor germline variants in genes encoding three of the four subunits of mitochondrial complex II (*SDHB*, *SDHC*, and *SDHD*), originally discovered as alternative susceptibility genes in *PTEN* wild-type CS/CS-like patients (Ni et al. 2008, 2012). Individuals carrying *SDHx* variants show an increased risk of breast and thyroid cancers that surpass the risks mediated by mutant *PTEN* alone (Ni et al. 2012). Interestingly, although individuals with *SDHx* variants alone show the highest prevalence of thyroid cancer, the coexistence of a *PTEN* mutation was associated with a 77% snapshot prevalence of breast cancer, as compared to 32% with *PTEN* mutations alone and 57% with *SDHx* variants alone. Although the prevalence of thyroid cancer was not significantly elevated in individuals with both *PTEN* mutations and *SDHx* variants, the histology was papillary for all tumors

versus the notable enrichment in follicular thyroid tumors in individuals with only *PTEN* mutations. Mechanistically, *SDHx* variants result in ROS-mediated stabilization of HIF-1 α , destabilization and decreased protein expression of p53 due to defective interaction with NQO1, and resistance to apoptosis (Ni et al. 2012). These data also reveal how mitochondrial dysfunction leads to tumorigenesis subsequent to elevated flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD⁺), the cofactor and product of NQO1 enzymatic catalysis, respectively. Subsequent studies showed that *SDHD* p.G12S and p.H50R variants directly lead to impaired *PTEN* subcellular localization and function through SRC-induced oxidation, accompanied by apoptosis resistance and induction of cellular migration (Yu et al. 2015). Importantly, the specific SRC inhibitor bosutinib could rescue these tumorigenic phenotypes only when wild-type *PTEN* was present. Similarly, *SDHD* p.G12S and p.H50R variants result in reduced autophagy in a *PTEN*-dependent manner (Yu et al. 2017). These data indeed provide mechanistic insights regarding the clinically observed increased prevalence of thyroid cancer in CS patients with *SDHx* variants alone compared to those with *PTEN* mutations alone, yet the seemingly paradoxical decreased prevalence of thyroid cancer in the setting of coexisting *PTEN* mutations and *SDHx* variants.

Additionally, the type of germline *PTEN* mutation could also influence eventual cell fates. For example, germline *PTEN* mutations such as carboxy-terminal deletions that result in genomic instability could prime tissues that are particularly sensitive to DNA damage for progression to malignancy (Sun et al. 2014). Finally, the immune system has been recognized as a major determinant of cancer development (Cavallo et al. 2011; Michaud et al. 2015; Singh and Dorak 2017). *PTEN* loss promotes resistance to tumor immune cell infiltration through the production of inhibitory cytokines, hence resulting in immune escape (Peng et al. 2016). Interestingly, pregnant mice treated with low-dose lipopolysaccharide to induce maternal inflammation give offspring with brain overgrowth (Le Belle et al. 2014). This phenotype is

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more pronounced in *Pten* heterozygous mice compared to wild-type, indicating evident cross talk between genetic susceptibility and the inflamed microenvironment mediated through ROS signaling. Importantly, ROS cause oxidation and subsequent inactivation of PTEN, a mechanism observed in a subset of CS/CS-like patients (Kwon et al. 2004; Yu et al. 2015). Hence, the manifestation of a cancer phenotype does represent a complex interplay among predisposing factors, genetic and epigenetic confounders, tissue-specific signaling networks, oncogenic signaling pathways, and microenvironmental context (Schneider et al. 2017).

PTEN AND SPORADIC CANCERS

PTEN dysfunction in PHTS offers important biological insights in the context of common sporadic cancers. Indeed, *PTEN* is one of the most frequently somatically mutated genes in cancer (Cantley and Neel 1999; Simpson and Parsons 2001; Hollander et al. 2011). The Sanger Institute maintains a database of annotated *PTEN* mutations and 30 tumor types (see the Catalogue of Somatic Mutations in Cancer [COSMIC] website). From this database, it is clear that in sporadic tumors, mutations and small insertions and deletions occur throughout the length of *PTEN*. Intriguingly, despite the fact that germline *PTEN* mutations result in component cancers within a restricted set of organs (Tan et al. 2012), *PTEN* somatic driver mutations are enriched in multiple sporadic cancer types that are not components of the PHTS spectrum, including prostate cancer, glioblastoma multiforme (GBM), and others (Li et al. 1997).

ROLE OF PTEN IN SPORADIC PHTS-RELATED CANCERS

Breast Cancers

Sporadic breast tumors were found to have somatic *PTEN* mutations in <5% of cases despite loss of PTEN immunoreactivity in nearly 40% (Perren et al. 1999; Perez-Tenorio et al. 2007). Promoter methylation as well as loss of heterozygosity (LOH) at 10q23 was seen in 40%–50% of breast cancer samples (Singh et al. 1998; Gar-

cía et al. 2004). Further evidence of the role of PTEN in breast tumorigenesis is seen from mice models. *Pten*^{+/-} mice develop breast tumors at high frequencies depending on their genetic background (Stambolic et al. 2000). Deletion of both *Pten* alleles in the mammary epithelium leads to altered mammary development and high-frequency early-onset tumors in mice (Li et al. 2002). Loss of a single *Pten* allele accelerated tumorigenesis in a Wnt-induced mammary tumor model and most tumors lost the remaining *Pten* allele (Li et al. 2001). Decreases in PTEN expression also resulted in increased risk of breast tumor formation in the absence of other induced mutations (Alimonti et al. 2010; Shen-Li et al. 2010). These models suggest that reduced PTEN expression resulted in increased tumor formation. Attenuated PTEN expression either by gene mutation, LOH, or promoter methylation may indeed drive breast carcinogenesis, making PTEN and downstream signaling pathways potential targets for novel drug development.

Thyroid Tumors

Thyroid tumors were one of the first tumor types to be reported as associated with PHTS (Lloyd and Dennis 1963). A quarter of thyroid adenomas and several sporadic malignant thyroid cancers were found to have PTEN LOH with PTEN mutations occurring less frequently (Dahia et al. 1997; Halachmi et al. 1998). Loss of PTEN expression occurs in <10% of thyroid cancers, but are more frequently seen in anaplastic and undifferentiated thyroid cancers (Gimm et al. 2000; Frisk et al. 2002). PTEN promoter methylation was seen in more than 50% of thyroid tumors and especially in follicular thyroid subtype with loss of PTEN immunoreactivity correlating significantly with promoter methylation (Alvarez-Núñez et al. 2006).

In mice, thyroid lesions develop late and with low frequency in *Pten*^{+/-} mouse models. Homozygous deletion of *Pten* in mouse thyroid cells did develop into benign follicular adenomas in female mice (Yeager et al. 2007). Hemizygous deletion of *Pten* was seen to accelerate thyroid adenocarcinoma formation when addi-

tional genetic alterations were present: loss of p27 (Di Cristofano et al. 2001) and in the setting of mutant thyroid hormone receptor- β (Yeager et al. 2007). Based on these mouse studies, it does appear that decreased gene dosage of PTEN is indeed important for thyroid cancer progression in the presence of other genetic alterations.

Endometrial Tumors

Sporadic mutations in PTEN are seen in 35%–50% of endometrial carcinomas and also in endometrial hyperplasias, which is believed to be a precursor for endometrial carcinomas (Risinger et al. 1997, 1998; Tashiro et al. 1997; Maxwell et al. 1998a,b; Mutter et al. 2000). The majority of the tumors demonstrate insertion or deletion frameshift mutations that result in microsatellite instability, suggesting that PTEN's role in endometrial carcinogenesis may lie in its impact on DNA repair mechanisms. Interestingly, altered PTEN expression can be noted, and may act as a biomarker of endometrial precancers (Mutter et al. 2000) and even latent precancers in histologically normal-appearing endometrium (Mutter et al. 2001).

In mice, *Pten*^{+/-} mice can develop endometrial hyperplasia with high penetrance with clear progression into endometrial carcinomas (Stambolic et al. 2000). The role of DNA repair in the maintenance of PTEN integrity is also highlighted in mouse models of endometrial cancer. Deletion of *Mlh1* in *Pten*^{+/-} mice accelerates endometrial carcinoma formation and was associated with earlier LOH of remaining *Pten* allele (Wang et al. 2002). This is corroborated in microsatellite unstable (hallmark of mismatch repair deficiency) versus stable human colorectal cancers, whereby all the former tumors with somatic *PTEN* mutations occur in the two poly(A) coding mononucleotide tracts, suggesting that mismatch repair preceded and is etiologic for somatic *PTEN* mutations (Zhou et al. 2002).

Melanoma

Sporadic melanomas frequently have a loss of PTEN through LOH, deletion, and mutation

(Trotman and Pandolfi 2003). PTEN can also be epigenetically silenced in melanoma, as decreased PTEN transcript levels were associated with PTEN promoter methylation (Mirmohammadsadeh et al. 2006). In mice, *Pten* deletion in pigmented mouse cells does not lead to the development of melanomas but did increase the number of melanocytes that led to melanoma formation in half of the samples when topical carcinogen was added (Mirmohammadsadeh et al. 2006). In the presence of other genetic alterations such as BRAF and *Cdkn2a*, *Pten* can contribute to the malignant phenotype (You et al. 2002; Dankort et al. 2009).

OTHER CANCERS NOT CLASSICALLY ASSOCIATED WITH PHTS

Brain Tumors

PTEN loss is seen in both benign and malignant brain tumors. Lhermitte–Dulcos disease (LDD) is a rare benign tumor that occurs in CS (Marsh et al. 1999; Zhou et al. 2003a; Gustafson et al. 2007, but gliomas are never seen in PHTS. In GBMs, sporadic *PTEN* mutations are found in >40% of tumors and PTEN LOH in 70% (Wang et al. 1997). As seen with other cancers, the level of PTEN expression is again seen to impact on tumorigenesis and grade of tumors. Decreased PTEN expression is characteristic of tumor progression with lower-grade gliomas expressing higher PTEN expression (Sano et al. 1999; Schmidt et al. 1999). Higher PTEN expression also correlated with better survival (Sano et al. 1999). Recently, miR-26a, which targets PTEN mRNA for degradation, was amplified in glioma and found to be associated with PTEN LOH (Huse et al. 2009), further strengthening the case that attenuation of PTEN expression results if there is increased cell proliferation. *Pten*^{+/-} mice do not develop brain tumors but homozygous deletions of *Pten* have resulted in changes that resemble LDD (Backman et al. 2001; Kwon et al. 2001). *Pten* deletion in the face of other genetic alterations also accelerated glioma formation (Xiao et al. 2002; Wei et al. 2006). Indeed, inhibition of fibroblast growth factor receptor (FGFR)-mediated tyrosine (Y240)

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phosphorylation on nuclear PTEN has been shown to enhance radiation sensitivity in GBM by attenuating DNA repair via RAD51 (Ma et al. 2019).

Prostate Cancers

Prostate carcinoma is never seen in males with PHTS. One of the early cytogenetic abnormalities identified in prostate cancer was the deletion of chromosome 10q, and subsequently PTEN loss in primary prostate cancer was mapped to this region (Lundgren et al. 1988; Cairns et al. 1997). Homozygous deletion of *Pten* in the mouse prostate led to prostatic intraepithelial neoplasia (PIN) lesions at 6 weeks of age that progressed to invasive and metastatic prostate carcinoma within a few weeks (Wang et al. 2003). As we saw in other tumor types, concurrent *Pten* hemizygosity coupled with deletions in other genes accelerated tumorigenesis (Di Cristofano et al. 2001; Abate-Shen et al. 2003). *Tmprss2-Erg* translocation was seen in mice to cooperate with *Pten* hemizygosity to promote malignant progression (Carver et al. 2009; King et al. 2009). *Pten* hypomorphic alleles with lower PTEN expression levels correlated with increased progression of prostate cancer in the mouse, suggesting the *Pten* may be haploinsufficient for prostate tumorigenesis and/or prostate tumor progression (Trotman et al. 2003).

Lung Cancer

Lung cancer is not a feature of CS and sporadic PTEN mutations are not seen frequently. It appears that other mechanisms are affecting PTEN expression in lung cancer. PTEN expression is reduced in 24% of early non-small-cell lung cancer associated with promoter methylation (Sos et al. 2009). In another study, >70% of lung tumors were associated with low or aberrant TP53 staining (Sos et al. 2009). Levels of miR-21 were up-regulated in lung tumors compared with normal lung tissue in >70% of cases, which was correlated with decreased levels of PTEN mRNA levels and advanced tumor stage (Zhang et al. 2010). PTEN is also thought to modulate

lung cancer resistance to epidermal growth factor receptor (EGFR) inhibitors by activation of AKT and EGFR (Sos et al. 2009). *Pten*^{+/-} mice have not been reported to develop lung tumors. Homozygous deletions in *Pten* in alveolar cells led to adenocarcinoma in 87% of mice by 70 weeks of age (Yanagi et al. 2007).

Pancreatic Cancer

Pancreatic cancer is not associated with CS, and mutations in PTEN are rare in sporadic pancreatic cancers. In mice, heterozygous deletion of *Pten* in the pancreas leads to metaplasia that progresses to pancreatic cancer in ~20% of mice (Stanger et al. 2005). However, codeletion of *Smad4*, the common mediator of signal transduction, does lead to tumor formation, with increased AKT and mTOR signaling (Xu et al. 2010).

TARGETING PTEN FOR CANCER TREATMENT

Altered PI3K/AKT/mTOR signaling in the PTENopathies implies that PI3K, AKT, and mTOR inhibitors are germane targets for therapeutic intervention. Because PHTS patients harbor identifiable *PTEN* germline mutations, the PI3K/AKT/mTOR signaling pathway is no longer negatively regulated by PTEN. Therefore, these patients theoretically represent ideal candidates for molecularly targeted pathway inhibition. Proof-of-principle case reports demonstrate the utilization of the mTOR inhibitor rapamycin to alleviate the symptoms and overgrowth manifestations of a child suffering from *PTEN*-related Proteus syndrome (Marsh et al. 2008) and a PHTS infant suffering from Lhermitte-Duclos disease (Zak et al. 2017). Indeed, the mTOR inhibitor sirolimus has been used in a phase II open label clinical trial in individuals with PHTS (NCT00971789), with an ongoing collection of follow-up data and analysis. Because individuals with PHTS also have neurodevelopmental disorders, a double-blind drug-placebo, crossover trial with the mTOR inhibitor everolimus is currently accruing pedi-

atric, adolescent, and young adult PHTS patients with ASD (Eng 2016).

Upstream components of the PTEN signaling pathway, such as PI3K and AKT inhibitors, also serve as candidates for pharmacologic inhibition. As such, AKT and PIK3CA inhibitors have been utilized in PS and PIK3CA-related overgrowth spectrum (PROS) disorders (Lindhurst et al. 2015; Ranieri et al. 2018; Venot et al. 2018).

Another major caveat for molecularly targeting the PI3K/AKT/mTOR pathway is feedback activation of collateral oncogenic signaling pathways, causing resistance. This led to the investigation of combinatorial therapies that would, in theory, effectively target the growth-promoting signals, without loss of feedback controls. Indeed, inhibiting mTOR has been shown to result in feedback activation of upstream signaling components such as AKT through insulin receptor substrate 1 (IRS1) or through direct phosphorylation at Ser473 by mTORC2 (Mahalingam et al. 2009). However, experimental studies show promise in that the rebound up-regulation of AKT during mTOR inhibition can be abrogated by pretreatment or cotreatment with Resveratrol, at least in vitro (He et al. 2011b). Moreover, PI3K inhibition can result in therapeutic resistance in *PIK3CA* mutant cell lines as a result of a rebound insulin-dependent feedback mechanism (Hopkins et al. 2018) or failure to suppress CDK 4/6 as evidenced through persistent RB phosphorylation (Vora et al. 2014). In these contexts, the combination of various PI3K inhibitors with antiglycemic therapies or CDK 4/6 inhibitors, respectively, results in the attenuation of the progrowth feedback signaling cascades, hence overcoming resistance. Interestingly, NVP-BEZ235, a dual PI3K/mTOR inhibitor, has been shown to selectively inhibit the growth of a subset of androgen receptor-positive (AR⁺) breast cancer cell lines (Wang et al. 2014). AR is positively correlated with PTEN expression in breast cancer, resulting from direct *PTEN* transcription, mediated by an androgen response element (ARE) in the *PTEN* promoter (Wang et al. 2011, 2012). Mechanistically, the beneficial effect of AR activation in combination with PI3K/AKT/mTOR inhibition

in AR⁺/ER⁺ breast cancers can be explained, at least partially, through PTEN up-regulation and suppression (Wang et al. 2014).

A novel approach could be to target the vulnerabilities caused by patient-specific germline *PTEN* mutations. For example, because PTEN plays a vital role in maintaining genomic integrity in the nucleus, it is possible to therapeutically use PARP inhibitors (Dillon and Miller 2014). Moreover, patients with germline *PTEN* mutations within the carboxy-terminal region could benefit from proteasome inhibitors to mitigate PTEN degradation (Georgescu et al. 1999). Indeed, treatment with the proteasome inhibitor MG-132 can restore both nonsense and missense mutant PTEN protein levels in vitro (He et al. 2013). However, restoring missense mutant PTEN levels in the presence of the wild-type allele can result in dominant-negative effects and could worsen the condition (Papa et al. 2014). Predictably, in the era of precision medicine, cancer prevention is key and determining context-specific therapeutic indices will be vital for the effective management of PHTS.

IMMUNOTHERAPY

Immune checkpoint monoclonal antibody blockades, such as programmed cell death 1 (PD-1) inhibitors, have led to remarkable and durable antitumor effects in multiple cancer types, including melanoma and non-small-cell lung cancer (Cheng et al. 2019). The PI3K/AKT/mTOR pathway is an important regulator of immunity (Okkenhaug 2013; Chen et al. 2017a). Because PTEN is a master regulator of this pathway, it is therefore not surprising that PTEN disruption can result in immune dysregulation. The latter is germane in carcinogenesis, whereby immune surveillance, evasion of immune recognition, and a chronically inflamed microenvironment represent major immune hallmarks of cancer (Cavallo et al. 2011). However, GBM patients respond poorly to immunotherapy, and the molecular determinants in the resistance mechanisms to this immunotherapy are poorly characterized. A recent longitudinal study was performed comprising 66 GBM pa-

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tients during standard therapy and after anti-PD-1 immunotherapies, including 17 long-term responders who showed durable responses to therapy (Zhao et al. 2019). Via whole-exome sequencing analysis, they found that somatic *PTEN* mutations were significantly enriched in nonresponders after correcting the background mutation rate derived from The Cancer Genome Atlas (TCGA). By individual sample gene-set enrichment analysis, the authors showed significantly elevated PI3K-AKT pathway activity in nonresponsive tumors with *PTEN* somatic mutations (Zhao et al. 2019). To investigate the resistance mechanism of *PTEN* mutations further, they inspected the single-cell RNA-sequencing profiles across 9000 cells in three GBM patients, including one *PTEN*-mutant sample. They found that cells associated with immune signatures were significantly enriched in the *PTEN*-mutant tumor. They further identified three major types of cell subpopulations: tumor cells with migration markers (CD44⁺), actively proliferating tumor cells, and microglia. As expected, the immunosuppressive signature was significantly associated with the CD44⁺ tumor cell subpopulations in the *PTEN*-mutant tumor, indicating that *PTEN* mutations may play a crucial role in the immune microenvironment and influence resistance to immunotherapy. This study suggests that *PTEN* mutations are significantly enriched in nonresponders to anti-PD-1 inhibitors, offering a potential personalized immunotherapy approach for GBM. By integrating bulk RNA-seq and published single-cell RNA-seq data analysis, the authors further showed that *PTEN* mutations may lead to a distinct immunosuppressive microenvironment, which helps to explain the resistance mechanisms to immunotherapy.

Additionally, PI3K/AKT/mTOR pathway activation has been shown to modulate responses to immunotherapy. Loss of *PTEN* in the sporadic context has been associated with resistance to anti-PD-1 therapy in melanoma (Peng et al. 2016), a CS component cancer, and, more recently, a case study of metastatic uterine leiomyosarcoma (George et al. 2017). Interestingly, activation of the PI3K/AKT/mTOR pathway has been shown to drive expres-

sion of PD-1/PD-L1 in a subset of solid tumors, causing immunoresistance (Parsa et al. 2007; Crane et al. 2009; Lastwika et al. 2016). Indeed, because *PTEN* seems to be a major immunotherapeutic response predictor, multiple questions arise regarding the promising utility of immunotherapeutic agents in individuals with germline *PTEN* mutations and cancer. Studies have shown that a subset of individuals with PHTS have autoimmune phenotypes, and B- and T-cell-related immune dysfunctions (Heindl et al. 2012; Chen et al. 2017a; see Taylor et al. 2019). Importantly, reduction in peripheral lymphocyte numbers compared to control subjects, including decreased CD4⁺ cell numbers and hence absolute FOXP3⁺ Treg (regulatory T) cell numbers would suggest that these individuals will have a different response to immunotherapy compared to individuals with normal immune systems.

CONCLUSIONS

One of the most serious complications of PHTS is the increased lifetime risk for cancer. Although *PTEN*-enabled organ-specific cancer risk estimates and management guidelines are part of the routine clinical armamentarium of precision care, it remains virtually impossible to absolutely predict which individual (versus a probability) will develop which component malignancy. Identical germline *PTEN* mutations are observed in patients with these seemingly disparate phenotypes (e.g., cancer vs. ASD), indicating that additional factors may act as phenotypic modifiers in PHTS. Hence, future studies elucidating absolute modifiers of disease manifestations and associated signaling networks will be key to define more precise and effective preventative and therapeutic strategies for the individual at risk. Greater understanding of the different mechanisms underlying *PTEN* loss will undoubtedly refine the ability to more optimally treat patients. It will be exciting to see further studies examining the interaction of *PTEN* with other tumor suppressors or oncogenes as well as how we may exploit the phosphatase-dependent and phosphatase-independent functions of *PTEN* for treatment.

COMPETING INTEREST STATEMENT

The authors have declared that no relevant conflicts of interest exist.

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