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Review

Inherited *PTEN* mutations and the prediction of phenotype

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ABSTRACT

PTEN has been heavily studied due to its role as a tumour suppressor and as a core inhibitory component of the phosphoinositide 3-kinase (PI3K) signalling network. It is a broadly expressed phosphatase which displays complexity and diversity in both its functions and regulation and accordingly, in the laboratory numerous classes of functionally distinct mutations have been generated. Inherited loss of function mutations in the *PTEN* gene were originally identified in sufferers of Cowden disease, but later shown to associate with more diverse human pathologies, mostly relating to cell and tissue overgrowth, leading to the use of the broader term, PTEN Hamartoma Tumour Syndrome. Recent phenotypic analysis of clinical cohorts of *PTEN* mutation carriers, combined with laboratory studies of the consequences of these mutations implies that stable catalytically inactive PTEN mutants may lead to the most severe phenotypes, and conversely, that mutants retaining partial function associate more frequently with a milder phenotype, with autism spectrum disorder often being diagnosed. Future work will be needed to confirm and to refine these genotype–phenotype relationships and convert this developing knowledge into improved patient management and potentially treatment with emerging drugs which target the PI3K pathway.

Contents

1. Introduction

In 1997, PTEN, was first identified as a tumor suppressor gene and phosphatase mutated in multiple cancer types [1–3]. Soon after and based on gene localization, [4] germline PTEN mutations were shown to be causative of Cowden disease, a phenotypically complex cancer prone syndrome (OMIM: 158350) [5] and its pediatric presentation, the Bannayan Riley Ruvalcaba Syndrome (OMIM: 153480) [6]. The diversity of phenotypes now observed in PTEN mutation carriers and the numerous organs and cell types affected provide important evidence for the multiple actions of the PI3K/PTEN signalling network in the regulation of many cellular processes and sit well alongside many studies conducted in cultured cells and animal models. Here we discuss how studies of

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patient phenotypes have linked to our understanding of PTEN function at the molecular and cellular level and conversely whether this may allow clinically useful prediction of the phenotype of PTEN mutation carriers.

The PI3K (class I phosphoinositide 3-kinase) signalling system is activated by diverse extracellular stimuli, including many growth factors, hormones including insulin, cytokines, chemokines and extracellular matrix components, which drive PI3K-dependent synthesis of phosphatidylinositol 3,4,5-trisphosphate (PIP_3) in the plasma membrane. In turn, increased PI3K activity and PIP_3 levels promote the growth, proliferation and survival of many cell types, as well as influencing cell metabolism, polarity and movement, all through effects on a large and diverse set of PIP_3-binding proteins which include the AKT protein kinases [7]. PTEN is a lipid phosphatase which directly opposes the function of the PI3Ks by dephosphorylating PIP_3. Accordingly, loss of PTEN function has been shown experimentally to cause many disturbances in cell and organism physiology, commonly linked to increases in cell growth and proliferation [8–10] and clinically PTEN loss has been identified as a driver event in the development of many sporadic cancers [11,12]. Functions for PTEN independent from its action on PIP_3 have been proposed [13–16], including protein substrates for its phosphatase activity [17–19]. The discovery of a PTEN mutation in two Cowden disease families, PTEN G129E, and characterisation showing that this mutant enzyme lacks lipid phosphatase activity, yet retains protein phosphatase activity was a key factor connecting PTEN lipid phosphatase activity with tumour suppressor function [20,21]. However, the significance of these alternative functions including protein phosphatase activity is currently unclear and the development of tumours both in these PTEN G129E carrying patients and in PTEN G129E knockin mice indicates that PIP_3-independent functions of PTEN are not independently responsible for its tumour suppressor functions in many organ systems [21–23]. The frequent loss of PTEN function and the activation of PI3K signalling observed in many, probably most, tumours, has motivated the development of a range of drugs targeting different points within the PI3K signalling network, most notably the PI3Ks themselves, AKT and further downstream, the growth promoting TOR kinase, which is activated in part by AKT [24,25] (Fig. 1). These efforts, involving most of the world's major pharmaceutical companies, have provided a range of drugs that relatively selectively inhibit their targets in the clinic, but although there have been some notable successes [26,27], response rates for these drugs as monotherapies against solid tumours have generally been disappointing [28,29].

2. The PTEN protein and the functional consequences of mutation

2.1. The PTEN protein and its post-translational regulation

The *PTEN* gene encodes a 403 amino acid cytosolic protein [1–3,10], here termed PTEN, and also a recently described 576 amino acid protein which includes a 173 amino acid N-terminal extension. This longer protein is termed PTEN-L or PTEN-Long, and its function is currently unclear: it has been proposed to be secreted and potentially enter other cells or alternatively play a role in mitochondria [30–32]. Almost all functional studies of PTEN have used the originally isolated 403 amino acid protein, although approaches interfering with the function of endogenous PTEN would generally interfere with both PTEN and PTEN-L proteins, making the functional distinctions between the two currently hard to judge. Here we will use 'PTEN' to refer to the 403 amino acid form.

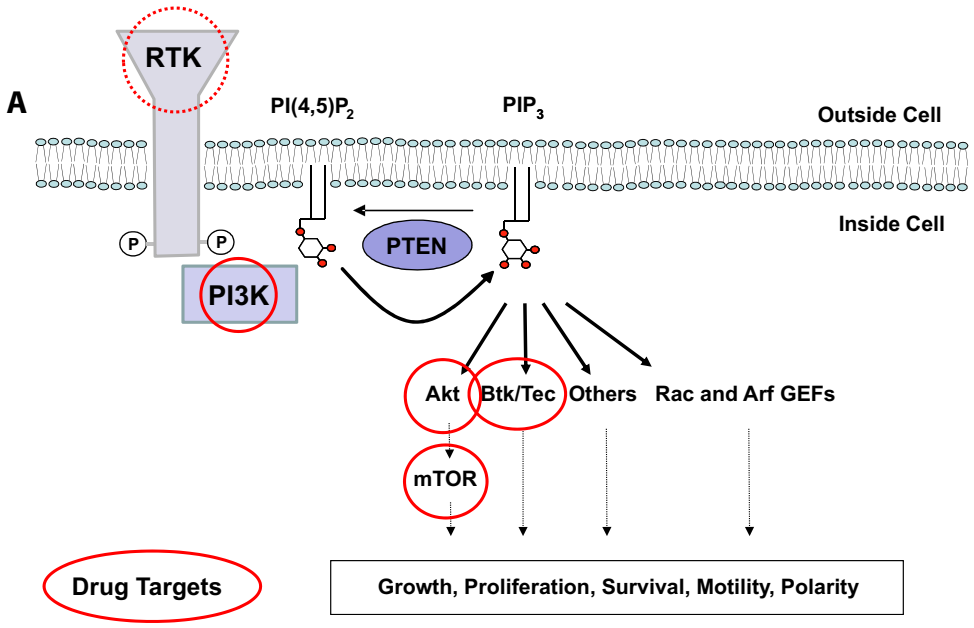
Most of PTEN is made up of an N-terminal phosphatase domain (amino acids 7–185) and a tightly associated C2 domain (186–351) which are required together for protein stability and catalysis [33–35] and also both contain basic residues required for transient membrane association and co-localisation with its phosphoinositide substrate [34,36–38] (Fig. 2). The less tightly structured C-terminal tail of PTEN (352–403) appears to play regulatory roles, containing two clusters of phosphorylation sites. Four phosphorylatable residues in an acidic Ser-Asp-Thr-Thr-Asp-Ser cluster at 380–385 appear to be phosphorylated by CK2 to relatively high stoichiometry in analysed cells and tissues and a further more N-terminal group, commonly displaying lower stoichiometry, encompass phosphorylation at Ser 370, perhaps also by CK2, priming for phosphorylation at Thr 366 and perhaps Ser 362 by GSK3 [39–42]. Phosphorylation at the 380–385 cluster is generally inhibitory to function. A series of consistent studies support a model in which these phosphorylations lead to a closed conformation through interaction of this phosphorylated acidic tail with the basic Phosphatase-C2 core, greatly reduced interaction with membranes and less biological activity and additionally increased protein stability, apparently as a secondary consequence of reduced membrane localisation [38,41–45]. Further sites of phosphorylation including Ser, Thr and Tyr residues have been identified, which appear to be more cell-type selective in their phosphorylation, or at least exhibit lower stoichiometry. Sites have also been identified at which PTEN is oxidised and/or nitrosylated (C71, C124, C83), ubiquitinated (including Lys13, Lys289), SUMOylated (Lys254, Lys266) and acetylated (Lys 125, Lys128, Lys163, Lys402) although in most of these examples of post-translational regulation, a clear picture is yet to emerge of how these modifications of PTEN alter its function and integrate into mechanisms of cellular regulation and this area has been reviewed elsewhere [46–48].

2.2. Experimental analysis of the functional consequences of PTEN mutations

One consequence of the requirement for both the PTEN phosphatase and C2 domains to form a minimal stable catalytic unit is that any truncation or frameshift mutation leads to a complete loss of stability and activity, other than mutations in the sequences encoding the C-terminal tail (amino acids 352–403). In addition, truncating mutations located within the first eight exons lead to mono allelic expression by nonsense mediated decay [49]. Conversely, any mutation in the C-terminal tail encoding by the ninth exon, seems very unlikely to directly disrupt catalytic activity, and more likely instead to influence protein stability and regulation. In light of this, it is notable that although across many tumour types there seems to be an underrepresentation of mutations in sequences encoding the C-terminal tail, a significant number of mutations have been described there from sporadic gliomas (15 C-terminal mutations/734 total mutations in PTEN) and colorectal cancers (10/387, omitting 11 mutations at aa 352 and 5 silent mutations), but never in endometrial cancer (0/978) and very few indeed in any germline cases (2/454) [50–53].

The PTEN protein fulfils a complex array of functions which have been reviewed elsewhere [9–11]. Most notably, it regulates cell growth and proliferation, in part via influences on the AKT group of kinases [54] and also influences processes including chemotaxis and epithelial cell and neuronal polarisation through mechanisms involving localised PTEN activity and downstream mediators [43,55–57]. PTEN displays unusually high protein sequence conservation. For example, the human and murine PTEN proteins have only one conservative Ser-Thr amino acid difference, 99.75% identity, whereas the genomic average human-murine ortholog conservation is around 85% amino acid identity [58]. Recent data from the 1000 Genomes project (www.1000genomes.org) gives us a picture of variation within human populations, revealing very little variation within PTEN. This database lists 54 single nucleotide

A



B

Drug Target	Drug Class	Drug examples	Furthest Trial progression
PI3K	Pan-Class I PI3K inhibitors	Pictilisib / GDC0941, Buparlisib / BKM120, Pilaralisib / XL147	Phase III
PI3K	Isoform-selective PI3K inhibitors	Idelalisib / CAL101 (p110d)	Approved
		BYL719 (p110a)	Phase II
		GSK2636771 (p110b)	Phase I/II
PI3K/TOR	Class I PI3K and TOR	BEZ235, GDC0980	Phase II
AKT	Allosteric	MK2206 (allosteric)	Phase II
	ATP competitive	GSK690693, GSK2141795	Phase II
TOR	Allosteric TORC1	Rapamycin / Sirolimus, RAD001	Approved
	TORC1/2	AZD8055	Phase I/II
BTK	Covalent	Ibrutinib	Approved

Fig. 1. Drug targets within the PI3K signalling network. (A) A model for the action of the class I phosphoinositide 3-kinase (PI3K) signalling network is shown. Activation of PI3K by diverse cell surface receptors, in particular receptor tyrosine kinases (RTKs), catalyses the phosphorylation of PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3 (PIP_3) using ATP as a phosphate donor. PTEN is a PI 3-phosphatase, which catalyses the reverse conversion of PIP_3 to PtdIns(4,5)P_2. Downstream effects are driven by PIP_3 binding proteins, including the Akt and Btk/Tec groups of kinases, activating Guanine Nucleotide Exchange Factors (GEFs) for the Rac and Arf families of GTPases and others. Direct effects are indicated by solid lines, indirect effects by dashed lines. Red circles indicate drug targets, in this case all kinases, for which inhibitors have progressed into clinical trials or entered widespread use. (B) Drug targets within the pathway are identified along with inhibitor classes, examples and their stage of progression through clinical trials. Almost all of these agents have been trialled extensively in patients with sporadic tumours including the approved drugs. Only in a few cases (e.g. Sirolimus) are trials being conducted specifically in Cowden/PHTS patients. Further details can be found in recent reviews [29,115–117,121,132].

variants as candidate SNPs in the PTEN coding sequence of which approximately half appear to be included due to recorded association with pathology. Only 3 of the candidate SNPs have been identified in more than 1 unrelated individual and current data show none to have an apparent allele frequency in human populations above 0.001. Perhaps related to this, PTEN contains many different sites of post-translational modification, many different amino acids contribute to its interactions with membrane lipids and a large number of other proteins have been shown to interact with different binding sites on PTEN [11,37,43,59].

Accordingly, numerous mutations designed in the laboratory have been shown to disrupt specific regulatory features such as protein–protein interactions and post-translational modifications, without greatly impairing the catalytic activity of PTEN or its ability

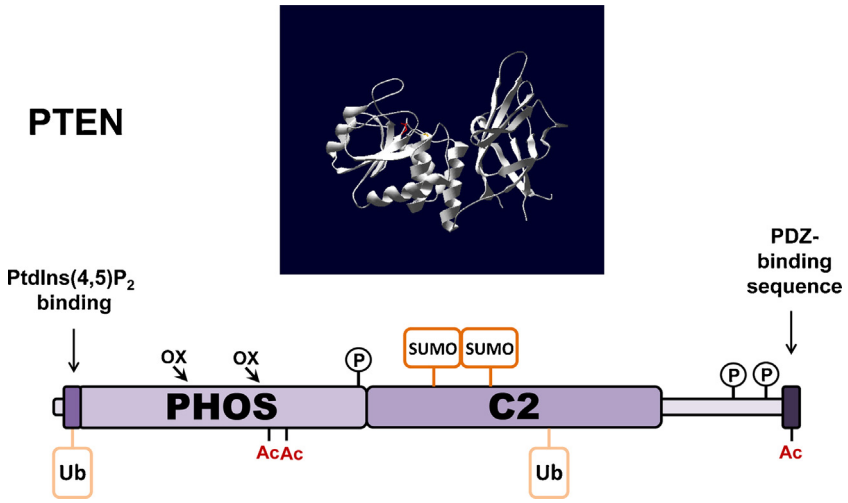


Fig. 2. The PTEN protein. The 48 kDa, 403 amino acid (aa) PTEN protein is shown. PTEN has an N-terminal protein tyrosine phosphatase (Phos) and more C-terminal C2 domain. The upper panel shows the 3D crystal structure of the minimal catalytic unit (7–353), lacking a less structured loop within the C2 domain (286–309) with the phosphatase domain on the left. The domain structure and post-translational modifications are shown in the lower panel. The positions of the N-terminal PtdIns(4,5)P_2 binding motif and the extreme C-terminal PDZ-binding sequence are shown in darker shades of purple. Post-translational sites of modification by phosphorylation (P—for clarity only a small subset of probable sites is shown), Ubiquitin, (Ub), SUMO, oxidation (Ox) and acetylation (Ac) are indicated.

to reduce cellular PIP_3 levels and AKT activity, and the phenotypes of these mutants are diverse and generally differ from those of full null mutants [13,22,30,59–65]. The likelihood is therefore, that such functionally selective mutations arise in the human population and will contribute similar phenotypic diversity. In silico structural analysis approaches have been developed with some power to predict the phenotypic consequences of mutations, based on evolutionary conservation and other features, e.g. Mutation Assessor [66]. By these methods, mutations disrupting core structural and catalytic features are likely to be correctly identified. However, while some classes of PTEN mutation have predictable outcomes (e.g. truncations in all exons except the last), it remains that from only sequence information, predicting the functional consequences of many missense mutations is challenging.

There have been several attempts to investigate experimentally the functional consequences of large numbers of clinically isolated *PTEN* missense mutations on function. Notably, Han et al. assayed the phosphatase activity in vitro of 42 mutations identified in sporadic tumours or PHTS, finding 31 lacked all detectable catalytic activity, 7 had only partial catalytic activity and 4 appeared fully active [67]. Later, Rodriguez-Escudero et al. used an in vivo yeast assay to study the cellular activity of 68 disease associated PTEN mutants, finding rather more mutants displayed activity in this setting, possibly due to greater protein stability in the cellular environment [68]. Almost 50% of the mutants in this study showed some detectable activity, with 16 appearing similar to WT PTEN. These authors also identified important associations with specific phenotypes which are discussed more below [68]. This key finding that some mutant enzymes retaining substantial cellular activity are associated with sporadic and familial tumour formation is in line with the apparent dose-dependency of PTEN's actions as a tumour suppressor [69] and the known diversity of tumour related processes controlled by PI3K/PTEN with very different activity/output dose relationships.

3. *PTEN* Hamartoma Tumour Syndrome and the phenotype of *PTEN* mutation carriers

3.1. *Human germline* PTEN *mutation*

Soon after the identification of PTEN as a tumour suppressor, heterozygous mutations in the *PTEN* gene were identified in patients suffering from the familial multi-system cancer syndromes, Cowden disease and its pediatric presentation, Bannayan–Riley–Ruvalcaba syndrome [5,70]. Later, the discovery of inherited *PTEN* mutations associated with clinical manifestations as Lhermitte Duclos syndrome (or cerebellum dysplastic hamartoma) [71] juvenile polyposis of infancy [72], segmental overgrowth [73] or autism spectrum disorder with macrocephaly [74,75] highlighted the complex relationship between genetic changes which impair the functions of the PTEN protein and patient phenotype. More recently, the umbrella term *PTEN* Hamartoma Tumour Syndrome (PHTS) has been used to encompass the range of symptoms identified in *PTEN* mutation carriers and broader diagnostic criteria have been proposed [76,77].

Several groups have studied the phenotypes of large cohorts of *PTEN* mutation carriers, revealing the high lifetime cancer risk in these patients and also the diversity of their other symptoms [78–80]. These studies indicate a lifetime cancer risk of over 80%, higher in women than men, with the highest risks being female breast cancer (>75%), thyroid, kidney and endometrial cancers [78–80]. The most common features of these patients are macrocephaly, gastrointestinal polyps with various histological patterns and dermatological lesions including trichilemmoma and oral papillomatosis which are each found in around 90% of these *PTEN* mutation carriers [78,79]. Notably, while polyps and dermatological features are common in *PTEN*-mutation negative cases of Cowden syndrome, rates of macrocephaly are much lower in these cases (<40%) and it may represent a usable distinguishing characteristic to identify *PTEN* mutation carriers [81]. Many other features are identified in these patients, mainly: complex benign mastopathy, and multi-nodular thyroid lesions, of various intensity but also, lipomas, invasive angiolipomas, ovarian cysts and uterine fibroids to cite the more frequently observed [78,79,82]. Because these clinical manifestations are inconsistent from one patient to another and, taken alone are common in patients without Cowden disease, several teams have defined phenotypically based scores predicting of *PTEN* mutation [77,82,83]. It should be noted however that these studies have understandably relied upon patient groups selected for *PTEN* sequencing after diagnosis with previously defined symptoms of PHTS and might therefore under-represent phenotypes caused by specific functionally distinct mutation classes. The possibility that some *PTEN* mutation carriers, perhaps with a functionally

distinct class of PTEN mutation, display phenotypes reliably distinguishable from PHTS has been investigated particularly in the case of individuals with ASD, as there are several reports of *PTEN* mutations being identified in individuals with ASD and macrocephaly without diagnosed additional characteristics of PHTS [74,84–87] and in a small number of individuals with sporadic ASD [88]. This phenotypic variability is also highlighted by other studies in which patients presenting with individual PHTS phenotypes [89,90] or malignancy without a prior PHTS diagnosis [62,91] are shown to carry germline *PTEN* mutations yet fail to fulfil diagnostic criteria for PHTS.

3.2. Somatic mosaicism

Homogenous germline *PTEN* mutation are mainly found in Cowden disease but recently some cases with a somatic mosaicism of *PTEN* heterozygous mutation have been reported without major phenotype specificities [92]. More intriguing was the description of a type 2 mosaicism of *PTEN* combining two loss of function *PTEN* mutations: the first one in homogeneous state and the second one, involving the second allele in a mosaic state [93]. The associated phenotype henceforth named type 2 segmental Cowden disease [94] or SOLAMEN syndrome [73] was initially confused with the Proteus syndrome. Interestingly, Proteus syndrome itself was subsequently linked to a mosaic gain of function mutation of the *AKT1* gene [95] encoding the PTEN regulated protein kinase, AKT1 (Table 1). Moreover, several overgrowth syndromes including macrodactyly, CLOVE syndrome, megalencephaly-capillary malformation or fibroadipose hyperplasia have been related to heterozygous gain of function *PIK3CA* mutation in a mosaic state [96]. These various segmental overgrowth syndromes show important phenotypic overlaps probably because activating mutation of PIK3CA or AKT1 and biallelic loss of function mutation of PTEN have similar functional consequences on the PI3K–AKT–TOR signalling network. These observations argue that the main phenotypic consequences of PTEN mutations are related to its role in the PIK3–AKT pathway, at least for the cells able to support constitutive activation of this pathway.

4. Phenotypic variability in human *PTEN* mutation carriers and genotype–phenotype relationships

4.1. Clinical studies

As discussed in Section 2, individual mutations would be expected to have different effects on PTEN function and this has been confirmed in many studies [37,60,61,63,67,68,102]. However, sufficient understanding of the PTEN protein relating to common mutations inherited in the human population has taken time to develop, and reliable genotype–phenotype relationships which might help in patient management have not been established. One difficulty in achieving this aim is that the phenotype of *PTEN* mutation carriers is strongly affected by other factors, including genetic background and environmental factors. For example, individual mutations have been found to lead to very different phenotypes in different individuals [78] even within the same family [103,104]. Such variability of the intra familial expression can be observed for the autism spectrum manifestations which involve frequently only one or a few members of a family, the others mutation carriers showing a more classical phenotype. Interestingly, the only correlation that can be drawn from the molecular diagnosis of the disease relates to the patients showing the association macrocephaly–autism, in which a predominance of missense mutation is detected. For example in a group of ten mutations found for the clinical indication macrocephaly–autism, eight are missense mutation (M Longy, unpublished) while in other indications the frequency of missense mutation is usually about 30% of the detected mutations.

4.2. Modelling human disease in Pten mutant mice

Studies in mice genetically modified at the *Pten* locus have been used to probe genotype phenotype relationships. Several studies have generated whole animal and tissue-specific mouse lines carrying mutant *Pten* deletion alleles which remove all detectable expression of the PTEN protein, leading to a range of tumours with some overlap with those observed in PHTS [9,105,106] and additional phenotypes including behavioural changes similar to ASD [107]. The full spectrum of phenotypes observed in these mice and their overlap with human subjects has been reviewed elsewhere [8,9,76,108]. Comparison of two apparent 'null' alleles indicates that differences in the phenotypes observed in different mouse colonies, and by extension, probably by different research groups, are caused by different genetic backgrounds [109]. Accordingly, rigourous analysis of mice expressing unstable mutant PTEN proteins also reveals phenotypes very similar to the null setting [23,110].

In contrast, studies of knockin mice expressing characterised stable mutant PTEN proteins which lack lipid phosphatase activity has led to very different conclusions [22,23]. Heterozygous mice expressing both normal PTEN and also either PTEN C124S, which lacks all detectable phosphatase activity, or PTEN G129E, which selectively lacks activity against lipid substrates, have a significantly higher tumour burden than similar mice carrying one wild-type allele along with one full null allele which expresses no protein. This difference seemed particularly clear in the aggressiveness of observed breast tumours [22,23]. This provides evidence for a dominant negative mechanism by which stable inactive protein disturbs the function of co-expressed wild-type protein and perhaps aggravates normal cellular behaviour independently; a paradigm that has been established with other tumour suppressors including p53 [111]. The implications for human patients are that those expressing stable yet inactive PTEN proteins may have a worse prognosis than those whose mutations simply cause a loss of normal PTEN.

This hypothesis that inactive stable PTEN mutants aggravate phenotype fits with further laboratory studies of PTEN mutant function. Recent studies of small numbers of PTEN mutations identified in patients with severe cases of PHTS showed that the encoded mutant proteins lacked all detectable activity in cell based assays, even when over-expressed [112]. This was in notable contrast to mutant proteins identified in patients with ASD and macrocephaly lacking other symptoms of PHTS, which, although most were unstable, all displayed normal activity in their ability to control P-AKT if they were ever-expressed and those tested retained efficacy in the regulation of soma size in hippocampal neurons [112]. This latter finding supports the earlier conclusion of Rodriguez-Escudero et al., who found ASD associated mutations were more likely to have higher activity in a yeast based PTEN activity assay than mutants associated with PHTS [68]. Finally, studies of ASD associated *PTEN* alleles in cortical neurons showed an inability to rescue the selective loss of somatostatin positive interneurons caused by *PTEN* loss and supported the conclusion that these alleles retain partial activity [113].

Together these studies support the important hypothesis that *PTEN* mutations which lead to a partial loss of function of the encoded protein are less likely to cause the more severe developmental features associated with PHTS and are more likely to be diagnosed in individuals with ASD and macrocephaly. These data also support the converse conclusion that *PTEN* mutations which cause the accumulation of stable inactive PTEN protein are more likely to lead to severe developmental symptoms and malignancy

Table 1
Human genetic diseases with symptoms related to PHTS.

Syndrome	Gene(s)	Comments	References
PHTS	*PTEN*		See text
Juvenile polyposis syndrome	*SMAD4, BMPR1A*		[97]
Proteus syndrome	*AKT1*	Mosaic/somatic	[95]
PIK3CA-related overgrowth spectrum (PROS)	*PIK3CA, AKT3*	Mosaic/somatic	[90,96,98,99]
Tuberous sclerosis	*TSC1, TSC2*		[100,101]
Peutz-Jeghers syndrome	*LKB1/STK11*		[97]

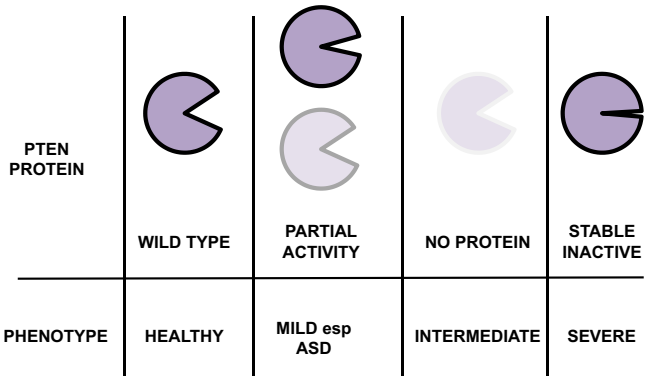


Fig. 3. A hypothesis linking PTEN mutations with phenotype. The PTEN mutant protein phenotype is shown above, with partial activity representing either an unstable active enzyme (feint enzyme cartoon) or a stable enzyme with reduced activity (partially closed active site). A stable inactive enzyme is represented by an enzyme cartoon with a closed active site. Mutations leading to partially active PTEN protein have been phenotypically linked to autism spectrum disorder (ASD) and fully inactive mutations have been linked to more severe PHTS phenotypes.

(Fig. 3). This illustrates the difficulty of phenotype prediction, as both partially functional proteins and stable inactive proteins are most likely to be encoded by missense mutations with only limited grounds for predicting the consequences of each mutation on protein function (see Section—2.2). In this regard, building up and collating the body of data covering mutations with recognised consequences would be of great benefit, as the robust experimental determination of PTEN activities and stability in appropriate assays currently remains a significant task even in a specialist laboratory. It is worth noting therefore that genuine null alleles, which disrupt the accumulation of PTEN, appear to have intermediate severity [22,23,68,112,113] and regardless, the clear influence of other factors on phenotype makes patient management challenging [77,114].

5. Therapeutic implications

The management of PHTS sufferers and other PTEN mutation carriers is complex and has been reviewed elsewhere [114] generally involving surveillance, screening for malignancies and surgery. However, as previously mentioned, the implication of PTEN in the negative control of the PI3K–AKT pathway led the pharmaceutical industry to design many new inhibitors of this signalling network that provide the promise of well-tolerated targeted therapies (Fig. 1). Such therapies targeting the PI3K–TOR signalling network and receptors that activate it have been extensively reviewed as have the prospects for their application to Cowden and other related syndromes [25,115–117]. The pharmacological agent rapamycin, known as an inhibitor of mTOR was previously used in preclinical studies on $Pten^{+/-}$ mice showing a cytostatic effect on endometrium hyperplasia and adrenal medullary neoplasia [118] and both chemoprevention and improved survival as well as regression of multiple lesions in an epithelial specific PTEN deletion model of Cowden disease [119]. The same agent showed a clinically improvement and a reduction of soft tissue mass in a young patient with SOLAMEN and invasive angiolipoma [120]. Currently, the results of clinical trials with TORC1 and PI3K inhibitors in *PTEN* germline mutation carriers are yet pending (for review: [121]). It should be noted that many other prognostic and predictive biomarkers are being developed to inform PI3K-pathway targeted cancer therapies, some of which could be considered alongside *PTEN* genotype in planning the treatment of PHTS sufferers. However the significance of these markers is currently unclear [122,123].

A further finding that has motivated therapeutic testing is the apparent reliance of PTEN null tumours on PI3K signalling specifically activated through its PIK3CB-encoded p110β catalytic isoform, observed in the prostate and other tumour types [124–126]. However, this connection is not consistent for all tumour types [127,128]. This point also highlights the challenges of targeting a signalling network with multiple mechanisms to modulate signal sensitivity via feedback and crosstalk, particularly induced by PTEN loss [129,130]. Selective inhibition of p110β in PTEN null tumours has been shown to lead in many cases to feedback activation of p110α (PIK3CA) dependent signalling and drug resistance, diversifying the PI3K isoforms upon which these tumors rely [128,131].

6. Concluding remarks

Understanding is beginning to develop of genotype–phenotype relationships in *PTEN* mutation carriers with the potential to generate insight that is reliable and has utility in terms of prognosis and therapeutic selection. However, the emergence of this application would seem to require progress on several fronts. We still understand little about the downstream mechanisms driving PTEN mutant pathology other than the AKT–TOR axis. Many other downstream PIP_3-binding proteins and linked PI3K-responsive signalling mechanism have been identified, yet their significance in PTEN- and PI3K-driven pathology is unknown and the same is true of the many PI3K-independent processes which have been proposed to be targets of PTEN regulation. Since it seems unlikely that PTEN itself can be re-activated therapeutically, most treatments aim to inhibit opposing signal activators and without improved knowledge of the

importance of these targets, it is hard to judge the likelihood of success and, as has occurred recently in most cases, interpret poor results in clinical trials.

Other areas requiring research progress include the development of large maintained and accessible datasets recording *PTEN* carrier phenotype and the experimentally determined functional consequences of PTEN mutations, the latter feature also potentially supported by simpler, more cost effective and reliable technologies to functionally characterise novel PTEN mutations at discovery. Ultimately, this protein-specific data should also provide a framework supporting greatly improved ways to predict PTEN mutation severity beyond existing algorithms applicable to all proteins. In the long term, and in advance of most genetic diseases, the large body of research providing solid understanding of PTEN function and mutation carriers, allied with the availability of several classes of drugs targeting components of the PI3K–PTEN pathway should mean that genuinely personalised medicine may well be possible.

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