The functions and regulation of the PTEN tumour suppressor: new modes and prospects

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Abstract | PTEN is a potent tumour suppressor, and its loss of function is frequently observed in both heritable and sporadic cancers. PTEN has phosphatase-dependent and phosphatase-independent (scaffold) activities in the cell and governs a variety of biological processes, including maintenance of genomic stability, cell survival, migration, proliferation and metabolism. Even a subtle decrease in PTEN levels and activity results in cancer susceptibility and favours tumour progression. Regulation of PTEN has therefore emerged as a subject of intense research in tumour biology. Recent discoveries, including the existence of distinct PTEN isoforms and the ability of PTEN to form dimers, have brought to light new modes of PTEN function and regulation. These milestone findings have in turn opened new therapeutic avenues for cancer prevention and treatment through restoration of PTEN tumour suppressor activity.

PTEN hamartoma tumour syndromes
(PHTS). Encompass a spectrum of disorders caused by germline mutations of the *PTEN* gene. These disorders are characterized by multiple hamartomas that can affect various organs. Hamartoma is a general term for a benign tumour-like malformation composed of mature cells in a tissue that has grown in a disorganized manner as a result of developmental defects.

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PTEN is one of the most frequently mutated tumour suppressor genes in human cancer. It was identified simultaneously by three groups in 1997 as a candidate tumour suppressor gene[1,2] and a novel protein tyrosine phosphatase[3]. Shortly thereafter, *PTEN* was found to be targeted by germline mutations in a group of rare autosomal dominant syndromes that are collectively referred to as the PTEN hamartoma tumour syndromes (PHTS)[4,5], which provided direct evidence of the deregulation of PTEN in tumorigenesis. Subsequently, genetic ablation of *Pten* in mice reaffirmed its tumour-suppressive role in multiple tumour types[6–8]. Further genetic analyses also demonstrated that *Pten* acts as a haploinsufficient tumour suppressor gene in some tissues[9]. Additionally, studies of both human samples and hypomorphic *Pten* mice showed that even partial loss of PTEN function is sufficient to promote some cancer types and that a reduction in PTEN levels below 50% further accelerates cancer progression[10], suggesting that a continuum model of PTEN tumour suppression, rather than a stepwise alteration of PTEN levels, is key to tumour development[11]. Nevertheless, it should also be noted that complete loss of *Pten* (*Pten*-null) can impede tumour growth by inducing a potent fail-safe mechanism, cellular senescence, in the prostate[11,12] and that *Pten* inactivation also paradoxically increases apoptosis in the liver owing to fatty acid accumulation, which leads to non-alcoholic fatty liver disease and long-latent liver tumorigenesis[13].

In line with the profound effects of subtle downregulation of PTEN on tumour development, a plethora of mechanisms regulating PTEN expression and function are often found to be altered in cancer. Apart from mutations, the suppression of PTEN function by either repression of *PTEN* gene expression or aberrant PTEN subcellular localization is tightly related to tumorigenesis and disease progression[14]. Moreover, PTEN-interacting proteins may have important roles in cancer progression by perturbing the fine-tuning of PTEN activity[14,15]. Recently, PTEN was reported to be active in its dimer configuration within membrane compartments[16], suggesting that yet unknown mechanisms underlying PTEN dimerization are deregulated in cancer, leading to its inactivation. Intriguingly, two studies recently reported that PTEN is secreted into the extracellular environment for uptake by recipient cells, thus, also functioning as a tumour suppressor in a cell non-autonomous manner[17,18]. Taken together, these findings shed new light on PTEN biology and function and open up avenues for the establishment of novel effective therapies for cancer prevention and treatment. While several excellent reviews cover the molecular details of PTEN regulation of the PI3K–AKT–mTOR pathway and its role in human disease[14,15,19,20], here, we highlight recent advances in our understanding of the functions of PTEN and discuss novel modes of PTEN regulation emerging from current research. We also examine ways and opportunities to restore and improve PTEN function for cancer therapy.

Biochemical functions of PTEN

PTEN was initially identified as a protein tyrosine phosphatase (PTP) on the basis of its sequence homology in the catalytic domain to members of the PTP family[1–3].

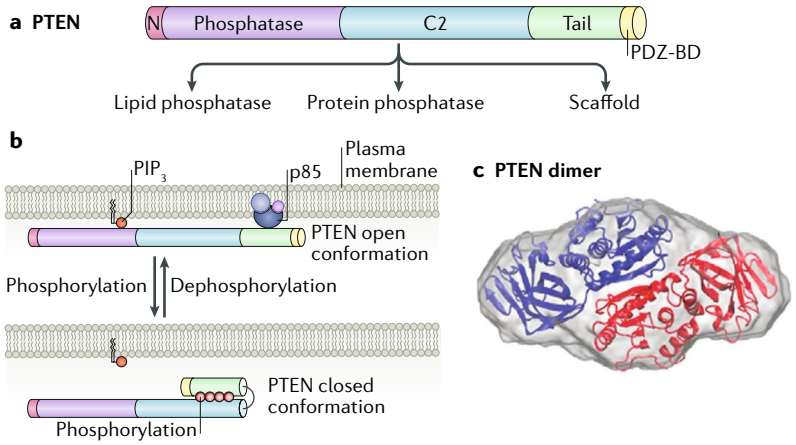

Fig. 1 | **Structure and activity of PTEN. a** | PTEN is a multi-domain protein that exerts tumour-suppressive functions in a lipid phosphatase-dependent, protein phosphatase-dependent or scaffold-dependent manner. PTEN is composed of five functional domains: a short N-terminal phosphatidylinositol (PtdIns)(4,5)P_2-binding domain (PBD), a catalytic phosphatase domain, a C2 lipid/membrane-binding domain, a C-terminal tail containing Pro, Glu, Ser and Thr (PEST) sequences and a class I PDZ-binding (PDZ-BD) motif. PEST sequences determine short intracellular half-lives and have been linked to targeting proteins for proteasomal degradation, while the PDZ-BD acts as a protein–protein interaction motif. **b** | Model for the conformational regulation of PTEN. Phosphorylation of the C-terminal of PTEN promotes an interaction between the acidic tail and C2 domain (closed conformation), which in turn masks the membrane binding of PTEN. In the open conformation, dephosphorylation of PTEN reverses this closed conformation to an open conformation, allowing PTEN to bind to the membrane and PDZ domain-containing proteins. **c** | Structure modelling of PTEN dimerization. PTEN forms a homodimer in vitro. The PTEN dimer is superimposed as a ribbon structure and determined by small-angle X-ray scattering (SAXS) analysis (figure adapted from REF.[181], Elsevier). PIP_3, phosphatidylinositol (PtdIns)-3,4,5-trisphosphate; p85, PI3K regulatory subunit alpha.

However, it is now known that it majorly functions as a phosphatase for lipids, mainly phosphatidylinositol (PtdIns)-3,4,5-trisphosphate (PIP_3). It also displays non-enzymatic (scaffold) functions.

Lipid and protein phosphatase function. The crystal structure of PTEN has revealed that the PTEN protein consists of a short N-terminal PtdIns(4,5) P_2 (PIP_2)-binding domain (PBD), a catalytic phosphatase domain, a C2 lipid or membrane-binding domain and a C-terminal tail containing PEST (Pro, Glu, Ser, Thr) sequences, as well as a class I PDZ domain-binding motif[21] (FIG. 1). A cornerstone of PTEN biology was laid with the discovery that its main substrate is a component of the lipid membrane PIP_3, by which it opposes the activation of the proto-oncogenic PI3K–AKT–mTOR signalling pathway[22,23], which is a key pathway that coordinates cell proliferation, growth, survival and metabolism[14,24]. PIP_3 can also act as a second-ary messenger regulating cell polarity and migration[25–27]. Even though the biological effects of PTEN are dominated by its ability to dephosphorylate lipid substrates, PTEN displays intrinsic phosphatase activity towards the poly-peptides phosphorylated on Tyr, Ser and Thr in vitro, establishing it as a dual-specificity protein phosphatase[3,28]. Additionally, PTEN has been reported to directly dephosphorylate residues on itself and several other pro-tein substrates, such as focal adhesion kinase 1 (FAK), cAMP-responsive element-binding protein 1 (CREB1), proto-oncogene tyrosine-protein kinase SRC, insulin receptor substrate 1 (IRS1) and others, to exert its tumour-suppressive function[29–33]. Identification of mutant forms of PTEN, in particular G129E and Y138L, in which lipid and protein phosphatase activities are selectively abolished[34,35], has allowed the field to characterize the protein phosphatase function of PTEN in many experi-mental systems; however, the physiological relevance of protein dephosphorylation by PTEN has yet to be fully established. A thorough genetic analysis of PTEN G129E and Y138L mutants in mice is warranted to understand the role of PTEN protein phosphatase in vivo.

Scaffold function. Many studies have revealed that PTEN exerts part of its tumour-suppressive function independently of PIP_3 and the PI3K–AKT axis by acting as a scaffold protein in both the nucleus and cytoplasm, which is as relevant as modulation of the PI3K–AKT signalling to tumour suppression[14,15]. Accordingly, PTEN loss is not synonymous with AKT overexpression, as revealed by in vivo genetic analyses in mouse mod-els[36]. In the nucleus, PTEN regulates diverse processes, including cell proliferation, transcription and main-tenance of genomic stability (see examples in the fol-lowing section). In the cytoplasm, scaffolding by PTEN modulates the activity of inositol 1,4,5-trisphosphate receptors (IP3Rs) and, consequently, Ca^{2+}-mediated apoptosis. Both wild-type PTEN and a catalytically dead PTEN mutant can compete with the F-box/LRR-repeat protein 2 (FBXL2) for IP3R3 binding in the cytosol, thereby preventing FBXL2-mediated IP3R3 degrada-tion, which in turn induces persistent Ca^{2+} mobilization and apoptosis[37]. Moreover, a recent study has demon-strated that cytosolic PTEN stimulates chromodomain-helicase-DNA-binding protein 1 (CHD1) proteasomal degradation through the recruitment of F-box/WD repeat-containing protein 1A (β-TRCP) E3 ubiquitin ligases, thereby suppressing CHD1-induced trimethyl lysine-4 histone H3 modification, which leads to tran-scriptional activation of the oncogenic tumour necrosis factor (TNF)–nuclear factor-κB (NF-κB) pathway[38]. It will be important to consider these non-canonical, scaf-folding functions of PTEN when developing treatments for effective management of *PTEN*-deficient cancers.

Physiological role of PTEN

PTEN has a key role in a variety of biological processes through regulation of both PIP_3-dependent and PIP_3-independent pathways, as highlighted by a number of recent studies.

PTEN and cell metabolism. Cancer cells undergo meta-bolic reprogramming to support their rapid proliferation and growth. In particular, they switch from mitochon-drial oxidative phosphorylation to glycolysis as an energy source despite the presence of oxygen (a phenomenon termed 'the Warburg effect')[39]. By doing so, cancer cells generate a large number of intermediates for macromol-ecule biosynthesis that allow the accumulation of bio-mass for cell growth and proliferation[40]. Additionally, glutamine uptake and glutaminolysis are also critical for cancer cell growth by fuelling the tricarboxylic acid

Haploinsufficient
A situation in a diploid organism when one copy of a gene is inactivated due to mutation or deletion but the product of the remaining normal allele is not sufficient for normal cellular functional output (for example, suppression of tumorigenesis), resulting in an abnormal phenotype or diseased state (for example, tumour initiation).

PI3K–AKT–mTOR pathway
A central signalling pathway that integrates both extracellular and intracellular signals to control cellular metabolism, growth, proliferation, cancer and longevity. Its activation contributes to the pathogenesis of many tumour types. This pathway is antagonized by various factors and, notably, by PTEN.

cycle intermediates, NAPDH, as well as phospholipid and nucleotide synthesis[41]. We now know that metabolic reprogramming in cancer cells is driven by activation of oncogenes or loss of tumour suppressors[42]. In this regard, through a variety of downstream targets, mostly through the activation of the PI3K–AKT–mTORC1 pathway, PTEN deficiency influences a plethora of metabolic processes that sustain cancer cell growth and proliferation[43].

One well-known impact of PTEN loss on cell metabolism is increased protein synthesis through modulation of two important components of the protein synthesis machinery, eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and p70S6 kinase, two downstream effects of mTORC1 (REF.[44]). Additionally, because insulin-mediated metabolic responses are primarily achieved through PI3K[45], PTEN has a critical role in modulating insulin-induced glucose uptake. Indeed, heterozygous *Pten* inactivation increases glucose uptake and promotes insulin hypersensitivity in mice, presumably owing to increased translocation of the glucose transporter type 4, insulin responsive (GLUT4) to the plasma membrane resulting from inhibition of TBC1 domain family member 4 (TBC1D4; also known as AS160), which is an AKT substrate[46–48]. Moreover, through upregulation of ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5) in the endoplasmic reticulum, *PTEN* loss and AKT activation promote ATP hydrolysis, leading to a compensatory increase in the Warburg effect[49]. In addition, by blocking the activities of forkhead box protein O1 (FOXO1) and peroxisome proliferator-activated receptor-γ (PPARγ) co-activator 1α (PGC1α)[50,51], the PI3K–AKT pathway overactivated by PTEN deficiency regulates insulin-induced suppression of hepatic gluconeogenesis. Similarly, the expression of most lipogenic enzymes involved in lipid biosynthesis is controlled by the sterol regulatory element-binding proteins (SREBPs) and can be activated by the PI3K–AKT pathway[52–55]. Consequently, hepatic-specific *Pten*-null mice display pronounced hepatomegaly and steatohepatitis with triglyceride accumulation[56]. Recently, integrative metabolomics has also revealed that in prostate-specific *Pten*-null mice there is an increase in metabolites of polyamine synthesis resulting from mTORC1-dependent stabilization of S-adenosylmethionine decarboxylase proenzyme (SAMDC), and that this metabolic pathway that is essential for oncogenic transformation[57].

Interestingly, owing to metabolic rewiring, *Pten*-null cells seem to be dependent on de novo pyrimidine synthesis from glutamine to sustain increased cell growth and proliferation, which creates sensitivity to the inhibition of dihydroorotate dehydrogenase (quinone), mitochondrial (DHODH), a rate-limiting enzyme for pyrimidine ring synthesis in this de novo pathway[58]. Therefore, targeting DHODH could open a therapeutic window for patients with *PTEN*-null cancers. Finally, two studies have reported that the systemic elevation of *Pten* in mice (super-*Pten*) induces a tumour-suppressive anti-Warburg state, characterized by decreased glycolysis but increased oxidative phosphorylation. Thus, super-*Pten* mice display increased energy expenditure and healthy metabolism and are protected from metabolic pathologies (for example, obesity) and cancer[59,60], an observation that is fully consistent with the tumour-suppressive function of PTEN.

PTEN and cell motility and polarity. Cell migration is an essential process in embryogenesis, morphogenesis, angiogenesis, immune response and metastasis. By regulating the level of PIP_3, PTEN activity at the plasma membrane is a key factor not only influencing the amounts of PIP_3 and the activity of its downstream effectors, such as Rho GTPases RAC1) and CDC42, but also contributing to the establishment of a PIP_3–PIP_2 gradient that has been shown to regulate cell motility and polarity in diverse species and cell types[25–27,61]. Additionally, PTEN could inhibit cell migration through its C2 domain and protein phosphatase activity, independent of its effect on the PI3K–AKT pathway (for example, by dephosphorylation of FAK)[31,62]. It has also been reported that PTEN and PIP_2 regulate annexin 2 (ANXA2), CDC42 and atypical PKC (aPKC) to generate the apical plasma membrane domain and maintain apico-basal polarity during epithelial morphogenesis[63]. Thus, the inactivation of PTEN may lead to the loss of apico-basal polarity and tight junctions, which are hallmarks of the epithelial–mesenchymal transition (EMT), which in turn promotes cell dissemination. Hence, loss of epithelial polarization, along with increased cell migration, may promote the invasiveness of *PTEN*-deficient cancer cells.

PTEN and genome maintenance. PTEN can be found both in the cytoplasm and in the nucleus. The absence of nuclear PTEN is associated with more aggressive cancers, suggesting that the nuclear pool of PTEN importantly contributes to its tumour-suppressive functions and that the analysis of PTEN subcellular localization is a useful cancer prognostic marker[64–66] (FIG. 2a). Accordingly, *PTEN* depletion has been associated with loss of genomic integrity[67]. PTEN lacks a canonical nuclear localization signal or nuclear export sequences, and numerous mechanisms for PTEN nucleocytosolic shuttling have been proposed, including simple diffusion, major vault protein (MVP)-mediated nuclear import and active shuttling by RAN-GTPase, as well as post-translational modifications (PTMs), including nuclear importing dependent on phosphorylation, sumoylation or monoubiquitylation[14] (FIG. 2a). Because the cytoplasmic, but not nuclear, pools of PIP_3 are sensitive to the lipid phosphatase activity of PTEN, it is generally believed that the function of nuclear PTEN is lipid phosphatase independent[65] and that nuclear PTEN exerts its role in the control of genomic stability through a variety of mechanisms (FIG. 2a). First, PTEN maintains centromere stability by interacting with centromere protein C (CENPC). PTEN also positively regulates DNA repair through induction of DNA repair protein RAD51, a crucial regulator involved in double-strand break (DSB) repair[67]. In this context, a recent study reported that PTEN nuclear localization is regulated by sumoylation at Lys254, which induces its nuclear retention. This is reversed by phosphorylation mediated by the DSB kinase ataxia telangiectasia mutated (ATM). Sumoylated nuclear PTEN controls DNA repair, and accordingly, *PTEN*-deficient cells were found to be hypersensitive to DNA damage, and, in the presence of

Small-angle X-ray scattering (SAXS) analysis
A powerful technique used to study protein structure and interactions that is capable of delivering structural information in dimensions between 1 and 100 nm.

PEST (Pro, Glu, Ser, Thr) sequences
A peptide sequence that is rich in proline (P), glutamic acid (E), serine (S) and threonine (T). Proteins possessing this sequence display a short intracellular half-life. Therefore, it is hypothesized that this sequence acts as a signal peptide for protein degradation.

PDZ domain
A common structural domain of 80–90 amino acids that serves as a protein–protein interaction motif and is found in signalling proteins in bacteria, yeast, plants, viruses and animals.

Dual-specificity protein phosphatase
A phosphatase that can act on both tyrosine and serine/threonine residues.

Annexin 2
A Ca^{2+}-regulated membrane protein and an F-actin-binding protein enriched at actin assembly sites on the plasma membrane and on endosomal vesicles. It is involved in diverse cellular functions, including cell motility and endocytosis.

Major vault protein
(MVP). Large multi-subunit ribonucleoprotein particle, which is considered to be a general carrier for nucleocytoplasmic transport.

RAN-GTPase
A GTP-binding protein involved in nucleocytoplasmic transport. It is required for the import of proteins into the nucleus and for RNA export.

Centromere
The part of a chromosome that links sister chromatids. The physical role of the centromere is to act as the site of assembly of the kinetochores — a highly complex multiprotein structure that is responsible for chromosome segregation.

the PI3K inhibitor (which blocks the pro-survival effects of the PI3K–AKT pathway, which is concomitantly activated by the loss of PTEN), they were susceptible to death owing to genotoxic stress[68]. This hypersensitivity offers an opportunity for therapeutic interventions in cancers associated with the loss of the nuclear PTEN pool[68].

PTEN and cell proliferation and survival. The role of PTEN in regulating cell proliferation by inducing cell cycle arrest and its negative influence on cell survival is well documented and largely attributed to its cytoplasmic activity against the PI3K–AKT pathway[14]. However, PTEN also has other activities in this context.

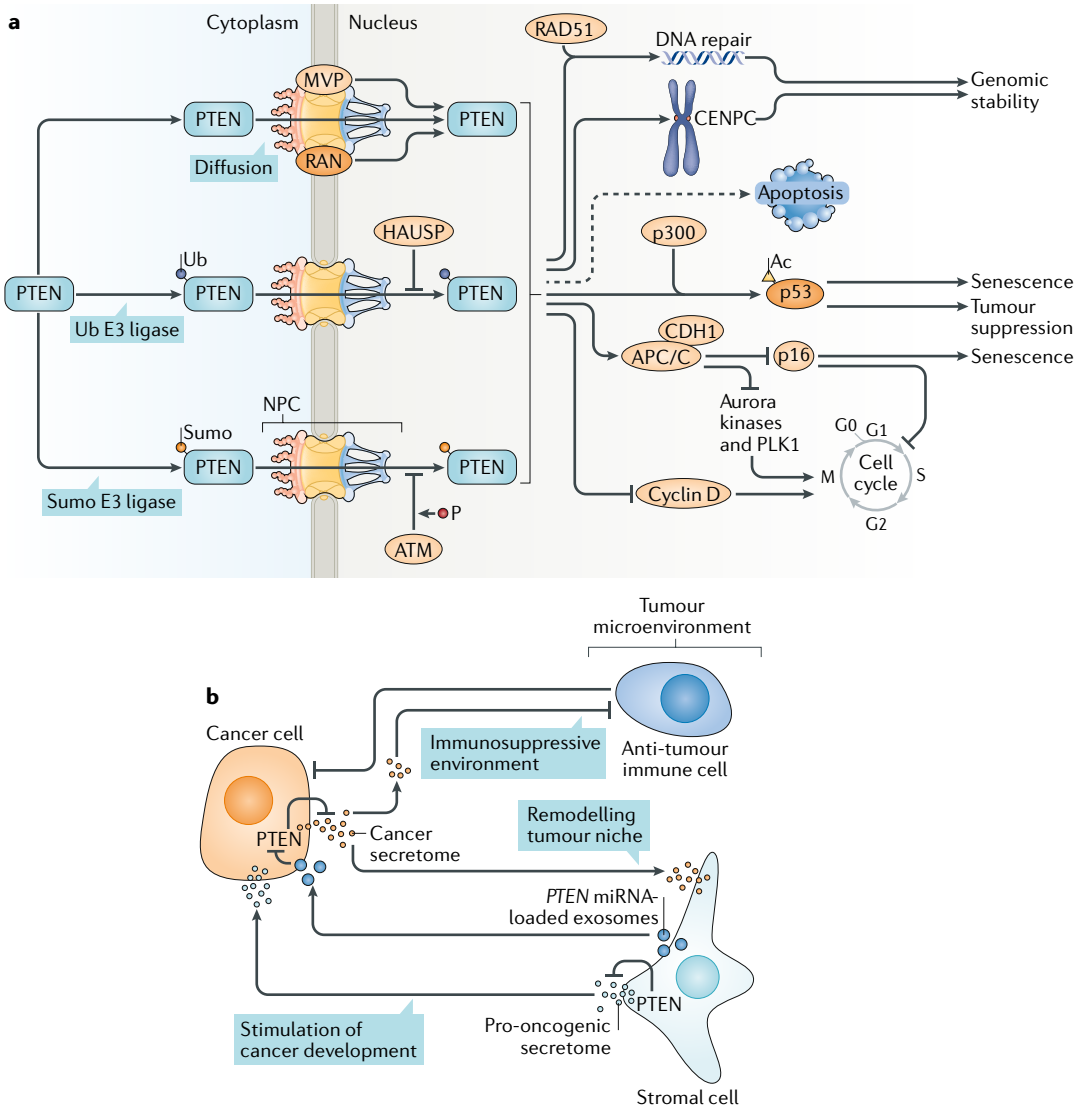

Fig. 2 | **PTEN functions in the nucleus and the tumour microenvironment. a** | PTEN exerts its many tumour-suppressive functions in the nucleus. PTEN protein can translocate into the nucleus through various mechanisms, including passive diffusion, RAS-related nuclear protein (RAN)- or major vault protein (MVP)-mediated import, and through a mechanism that depends on PTEN being monoubiquitylated or sumoylated. PTEN nuclear translocation induced by PTEN monoubiquitylation and sumoylation can be reversed by the deubiquitylating enzyme (DUB) herpesvirus-associated ubiquitin-specific protease (HAUSP) and ataxia telangiectasia mutated (ATM) kinase-mediated phosphorylation, respectively. In the nucleus, PTEN performs numerous cellular functions largely in a lipid-phosphatase-independent manner, including regulation of genomic integrity and induction of apoptosis, cell cycle arrest and senescence. **b** | PTEN also exerts its tumour-suppressive functions in the tumour microenvironment. PTEN in tumour cells may regulate the cancer cell secretome to prevent secretion of immunosuppressive chemokines and, in consequence, favouring the establishment of an immune-permissive tumour microenvironment, which would improve antitumour immune responses. Tumour-derived PTEN may also prevent the formation of reactive stroma with pro-tumorigenic activity, whereas PTEN in stromal cells may suppress tumorigenesis through inhibition of a pro-oncogenic secretome that reprogrammes cancer cells. Interestingly, cells in the tumour microenvironment may secret exosomes that contain PTEN-targeting microRNA (miRNA) to downregulate the expression of PTEN in cancer cells, thereby counteracting the tumour-suppressive effects of PTEN. Ac, acetylation; APC/C, anaphase-promoting complex; CDH1, CDC20-like protein 1; CENPC, centromere protein C; NPC, nuclear pore complex; PLK1, polo-like kinase 1; sumo, sumoylation; Ub, ubiquitylation.

For example, nuclear PTEN has been shown to regulate the cell cycle through the suppression of cyclin D1 activity by transcriptional downregulation or by limiting its nuclear accumulation. Moreover, nuclear PTEN can form a complex with histone acetyltransferase p300, and p300 was shown to be essential for PTEN-mediated cell cycle arrest[69]. Mechanistically, formation of this complex supports high levels of p53 acetylation, which regulates p53 stability and transcriptional activity (FIG. 2a). Additionally, nuclear PTEN was also found to regulate the cell cycle by directly binding to the anaphase-promoting complex (APC/C) E3 ligase. This binding facilitates the association of APC/C with its activator CDC20-like protein 1 (CDH1; also known as FZR) and in turn increases the tumour-suppressive activity exerted by the CDH1–APC/C complex through the degradation of its oncogenic substrates, such as polo-like kinase 1 (PLK1) and Aurora kinases (AURKs)[70] (FIG. 2a). Nuclear PTEN may also act as a pro-apoptotic factor by unknown mechanisms, as suggested by the observation that PTEN localizes to the nucleus in cells exposed to pro-apoptotic stimuli[71] (FIG. 2a).

PTEN and normal versus cancer stem cell maintenance. Normal stem cells are characterized by their intrinsic capacity to self-renew and differentiate into specialized cell types. Cancer stem cells (also known as cancer initiating cells), while retaining these properties, have lost the homeostatic mechanisms that maintain normal stem cell numbers. Understanding the mechanistic difference between normal and cancer stem cells is thus crucial to develop targeting therapies that selectively target cancer stem cells but spare normal stem cells.

In stem and progenitor cell homeostasis, PTEN displays profound tissue-specific and cell lineage-specific effects. In embryonic neural stem cells, for example, *Pten* deletion leads to increased self-renewal, cell cycle entry (G0 to G1 transition) and decreased growth factor dependency[72,73]. Likewise, in prostate tissues, *PTEN* deletion leads to an expansion of the stem and progenitor cell subpopulation and ultimately tumour initiation[74]. By contrast, in quiescent neural progenitors in the adult mouse dentate gyrus, *Pten* deletion leads to progenitor cell depletion[75]. Similarly, differential responses to *Pten* depletion have been observed in normal haematopoietic stem cells (HSCs) and leukaemic stem cells (LSCs). *Pten* deletion in HSCs promotes excessive proliferation and subsequent exhaustion of the HSCs, which is followed by the generation and expansion of LSCs and the development of myeloproliferative disease and acute leukaemia[76,77]. Importantly, both HSC depletion and LSC expansion are mediated by mTOR, and inhibition of mTOR signalling with rapamycin restores HSC function but considerably impairs the generation and maintenance of LSCs, demonstrating that it is possible to identify therapies that eliminate LSCs without damaging normal HSCs[76]. In follow-up studies, it was shown that mTOR activation leads to the upregulation of a tumour suppressor network that prevents maintenance of HSCs. Notably, this network is attenuated by secondary mutations in LSCs, which can expand upon *Pten* deletion[78]. In addition, genomic instability induced by *Pten* loss (see above) can drive further alteration in the HSC compartment, such as activation of β-catenin or overexpression of the oncogene *Myc*, which promotes *Pten*-null proliferation and leukaemia development[79]. Additionally, two studies have shown that the activities of both mTOR complexes, mTORC1 and mTORC2, contribute to the expansion of LSCs upon *Pten* loss[80,81], which explains why the effect of *PTEN* deletion on LSCs cannot be completely reversed by rapamycin, which primarily targets mTORC1 (REF.[76]).

PTEN and cellular senescence. Cellular senescence is a stress-induced, irreversible growth arrest that is established and maintained by at least two major tumour suppressor pathways — p53–p21 and Rb–p16^{INK4A}. Senescence is now well recognized as a fundamental mechanism that contributes to a variety of physiological and pathological processes, including embryogenesis, wound healing, ageing and tumour suppression[82–84]. Many stimuli that induce senescence are aberrant mitogen and proliferation-associated signals, such as the activation of oncogenes or the loss of tumour suppressor genes, which is consistent with the tumour-suppressive role of senescence.

Although partial loss of *Pten* increases cellular growth and proliferation[10,11,85], biallelic inactivation of *Pten* in the prostatic epithelium leads to cellular senescence both in vitro and in vivo[12,86]. In this context, cellular senescence acts as a potent fail-safe mechanism and a barrier that profoundly restricts tumorigenesis. These functions provide an explanation as to why complete inactivation of *Pten* alone in mouse prostate leads to indolent tumours with minimally invasive features after a long latency and why complete *Pten* loss is not frequently observed at cancer presentation but is prevalent in advanced and metastatic prostate cancer where the pathways responsible for senescence are often misregulated. *PTEN* loss-induced cellular senescence (PICS) is dependent on upregulation of both p53 and p16 (REFS[12,70,86]) (FIG. 2a). Unlike oncogene-induced senescence (OIS), where p53 is upregulated through protein phosphorylation and stabilization, in PICS, p53 is mainly upregulated through mTORC1-mediated increase in translation[86]. In addition, loss of nuclear PTEN impairs the E3 ligase activity of the APC/C–CDH1 complex (see also above), leading to stabilization of protein C-ets-2 (ETS2) and subsequent p16^{INK4a} upregulation[70]. Notably, this PTEN nuclear function towards APC/C–CDH1 in senescence is independent of its phosphatase activity (FIG. 2a), which explains the remnant tumour-suppressive activity associated with many phosphatase-inactive *PTEN* mutants[70]. Moreover, in contrast to OIS, which is invariably preceded by hyperproliferation and activation of a DNA damage response, PICS does not depend on hyperproliferation or DNA damage and can also be elicited in non-proliferating cells, such as the quiescent cancer stem cell pool, which renders PICS a more appealing pro-senescence therapeutic intervention than other senescence-inducing therapies[86,87]. However, it has recently been shown that PICS can be counteracted in a non-cell autonomous manner by a subpopulation of myeloid cells, which can protect a fraction of proliferating *Pten*-null mouse prostate cells from senescence,

Anaphase-promoting complex
(APC/C). Also known as the cyclosome. An E3 ubiquitin ligase that has a crucial function in the regulation of the mitotic cell cycle through targeting key mitotic regulators for degradation by the 26S proteasome.

Dentate gyrus
A part of the hippocampus that is thought to contribute to the formation of new episodic memories, the spontaneous exploration of unknown environments and other functions.

β-catenin
A subunit of the cadherin protein complex that constitutes adherens junctions and a key downstream effector in the canonical WNT signalling pathway.

ETS2
A transcription factor that binds specifically to the DNA GGAA/T core motif in gene promoters and stimulates transcription of genes involved in development and apoptosis.

thereby sustaining tumour growth. Therefore, targeting this non-cell-autonomous network for therapy has the potential to reinforce the action of PICS towards tumours and enhance its anticancer efficacy[88].

Despite the promise of pro-senescence therapy as a novel therapeutic approach to treat cancer, it is worth noting that a persistent senescent cell burden could also create a pro-inflammatory tumour environment via the senescence-associated secretory phenotype (SASP), which could otherwise promote malignant phenotypes and tumour growth[84]. Therefore, combining pro-senescence therapy with interventions aimed at clearing senescent cells could improve long-term therapeutic benefits of senescence induction[89,90].

PTEN and the tumour microenvironment. In addition to harbouring tumour cells, the tumour microenvironment is composed of multiple distinct cell types that have well-established roles in all stages of tumorigenesis. Importantly, the reciprocal crosstalk between tumour cells and the tumour microenvironment contributes to the development of cancer[91]. It has been previously suggested that PTEN contributes to regulating the tumour microenvironment, and this hypothesis has recently been validated and extended (FIG. 2b). In addition to its cell autonomous effect on cancer cells, *Pten*-null senescent cells in prostate tumours have been shown to establish an immunosuppressive tumour microenvironment, which contributes to *Pten*-null tumour growth and chemoresistance through the activation of the Janus kinase 2 (JAK2)–signal transducer and activator of transcription 3 (STAT3) pathway and the subsequent secretion of immunosuppressive chemokines that trigger the recruitment of myeloid-derived suppressor cells[92]. Additionally, *Pten* inactivation in hepatocytes causes hepatomegaly and steatohepatitis with fatty acid accumulation, a phenotype similar to human non-alcoholic steatohepatitis (NASH), which is characterized by increased hepatocyte death and hepatic injury. This chronic damage reshapes the hepatic microenvironment, resulting in infiltration of inflammatory cells and secretion of cytokines and chemokines that drive the transformation of tumour-initiating cells and, in consequence, fuel hepatocarcinogenesis[13]. Indeed, while recent studies have shown that ablating the proto-oncogenic *Shp2* suppresses the myeloproliferative effect driven by *Pten* loss, the concomitant loss of *Shp2* and *Pten* in hepatocytes cooperatively promotes hepatocarcinogenesis owing to early-onset NASH further promoted by *Shp2* loss and enhanced systematic inflammatory responses in the hepatic microenvironment[93,94], further highlighting the crucial role of hepatic injury and associated changes to tissue microenvironment — to which PTEN loss contributes — in liver tumorigenesis. Moreover, the deletion of *Pten* in fibroblasts of the mouse mammary gland creates a tumour-permissive stroma, which is shown to accelerate the initiation, progression and malignant transformation of mammary epithelial tumours. These effects are due to repression of the PTEN–miR320–ETS2 axis, which is associated with changes in gene expression and has been linked to proto-oncogenic alterations in the secretome of *Pten*-null fibroblasts, including secretion of extracellular matrix remodelling proteins[95,96]. These studies suggest that, to counteract tumorigenic processes, PTEN not only acts on pre-malignant cells but also exerts important functions in the stromal compartment.

Interestingly, it has been recently shown that stromal cells can modulate *PTEN* loss-driven tumorigenesis by secreting factors that enhance tumorigenic potential of cells or even by inhibiting tumoural PTEN expression[97,98]. In the latter case, it has been shown that stromal cells in metastatic tumours can secrete exosomes containing anti-PTEN microRNAs (miRNAs), which suppress PTEN expression in the tumour cells. These adaptive *PTEN*-deficient metastatic tumour cells were in turn stimulated to secrete CC-chemokine ligand 2 (CCL2), leading to the recruitment of a subset of myeloid cells, which further increased the outgrowth of metastatic tumour cells by promoting cancer cell survival and proliferation. Therefore, the cell non-autonomous effects of PTEN deregulation must be considered and addressed in the development of successful therapies aiming at targeting cancers associated with PTEN depletion.

Regulation of PTEN expression

In addition to genetic loss or mutations, disruptions in the regulation of PTEN by various molecular mechanisms can generate a continuum of dysfunctional PTEN species and/or a spectrum of PTEN levels, which can then differently contribute to the pathogenesis of various inherited syndromes, cancers and other diseases. For instance, many different tumour types that harbour monoallelic mutations of *PTEN* do retain an undamaged remaining *PTEN* allele while displaying further or complete loss of PTEN protein immunoreactivity[93,99–101]. Many mechanisms are capable of transcriptional and post-transcriptional regulation of PTEN expression, including epigenetic silencing, transcriptional repression, regulation by miRNAs and disruption of competitive endogenous RNA (ceRNA) networks, which have all been shown to contribute to regulating PTEN levels. Furthermore, PTEN is subject to a range of other specific regulatory mechanisms, including PTMs, PTEN-interacting proteins, dimerization and secretion, that ultimately govern its protein levels, activity and function (see also following sections).

Genetic alterations of PTEN. *PTEN* was first identified as a tumour suppressor gene on human chromosome 10q23.3, a locus that is highly susceptible to aberrant genetic alterations in primary human cancers, xenografts and cancer cell lines[1,2]. Monoallelic or complete *PTEN* deletion is frequently observed in endometrial carcinoma, glioblastoma and prostate cancer[15]. The *PTEN* gene is also monoallelically mutated in the germ line of patients with PHTS, as well as in a range of sporadic cancers. A large study examined a diverse cohort of 3,042 individuals with Cowden disease and found that germline *PTEN* mutations are present in about 25% of individuals with the disease[102]. Additionally, 1,993 unique somatic *PTEN* mutations have been found in human cancers (see the Sanger Institute Catalogue of Somatic Mutations in Cancer (COSMIC) website). Both germline and somatic *PTEN* mutations have been identified in the

Senescence-associated secretory phenotype
(SASP). Effects mediated by the secretion of a range of proteins, including cytokines, chemokines and proteases by senescent cells. These effects are extremely diverse and include both autocrine and paracrine signalling, pro-tumorigenic and tumour-suppressive effects and pro-inflammatory and anti-inflammatory signalling.

Exosomes
Cell-derived vesicles that are present and secreted out of mammalian cells.

Competitive endogenous RNA
(ceRNA). A type of RNA that communicates with and regulates other RNA transcripts by competing for shared microRNAs.

Sporadic cancers
Cancers that occur in individuals who do not have a family history of that cancer or an inherited change in their DNA that would increase their risk of that cancer.

Cowden disease
An autosomal dominant multiple hamartoma syndrome that results most commonly from a mutation in the *PTEN* gene. The disease was named after the family in which it was first reported. Although the tumours are largely benign, individuals with Cowden syndrome have an increased risk of developing several types of cancer, including cancers of the breast, thyroid and uterus.

promoter and all nine exons of *PTEN*, with various types of mutation identified, including missense, nonsense, splice site variants, intragenic deletions and insertions and large deletions (FIG. 3a). Many missense mutations are functionally null and might serve as a dominant negative to inhibit wild-type PTEN catalytic activity[16], while many missense, nonsense and splice site mutations lead to unstable truncated proteins that are almost undetectable and thus are functionally comparable to the *PTEN* mono-allelic loss. In line with these multiple possible aberrations, *PTEN* haploinsufficiency importantly contributes to tumour initiation and progression[16]. Several tumour-derived PTEN mutations retain partial or complete catalytic function[103], suggesting that alternative mechanisms lead to inactivation of PTEN tumour-suppressive function. A compelling example is represented by mutation at Lys289, which alters PTEN subcellular localization[104].

Epigenetic and transcriptional regulation of PTEN. In addition to genetic alterations that partially or fully inactivate a given *PTEN* allele, emerging evidence demonstrates that *PTEN* expression is also suppressed through epigenetic mechanisms (FIG. 3b). Numerous studies have demonstrated hypermethylation of the *PTEN* promoter in cancer, especially in cancer types where *PTEN* is infrequently deleted or mutated, such as melanoma and lung cancer[105–108]. Additionally, *PTEN* transcription may be regulated by histone acetylation. In this respect, it has been shown that the transcription factor SAL-like protein 4 (SALL4) represses *PTEN* transcription by recruiting an epigenetic repressor, nucleosome remodelling and deacetylase (NuRD) complex, with histone deacetylase activity[109] (FIG. 3c).

Moreover, there are a number of putative transcription factor binding sites at the *PTEN* promoter, which may enable the regulation of PTEN expression at the transcriptional level. In this regard, through binding to the *PTEN* promoter, early growth-response protein 1 (EGR1), p53 and PPARγ activate *PTEN* transcription[110–112], whereas zinc-finger protein SNAI1 (SNAIL), the inhibitor of DNA-binding protein inhibitor ID1, and ecotropic virus integration site 1 protein homologue (EVI1; also known as MECOM) inhibit *PTEN* transcription[113–115]. Furthermore, Polycomb complex protein BMI1, transcription factor AP1 (JUN) and NF-κB repress *PTEN* transcription through mechanisms that are still poorly defined[116–118]. Finally, active NOTCH1 has also been reported to positively and negatively regulate *PTEN* transcription through C-repeat binding factor 1 (CBF1; also known as RBPJ) and transcription factor HES1, respectively[119,120]. Many of these PTEN transcriptional regulators have been implicated in cancer development and/or progression. Taken together, these findings suggest that the transcriptional control of PTEN lies at the intersection of pathways implicated in the regulation of tumour suppression and tumour promotion (FIG. 3c).

Post-transcriptional regulation of PTEN. PTEN is under extensive regulation by miRNAs. Specific miRNAs target mRNAs by binding to miRNA response elements usually located at the 3′ untranslated region (UTR) of target mRNAs[121]. A number of miRNAs have been found to participate in the regulation of metabolic disease and cancer by targeting PTEN[122,123] (FIG. 3d). A compelling example is the cluster of PTEN-targeting miRNAs of the miR-17~92 family, which is composed of three related, highly conserved, polycistronic mRNA genes that encode 15 miRNAs in total, overexpression of which contributes to lymphoproliferative disease and autoimmunity[124]. miR-21, one of the most frequently upregulated miRNAs in cancer, also directly targets and downregulates PTEN in a variety of human tumours, including hepatocarcinoma, ovarian and lung cancers[125,126]. Another example is miR-25, which negatively regulates PTEN levels in melanoma cells, downstream of ERK activation[127]. miRNA regulatory networks also enable oncogenes such as *MYC* to suppress PTEN protein levels through induction of miR106b~25 miRNA cluster, miR-19 and miR200c[128–130].

A further level of complexity in miRNA-mediated PTEN regulation is represented by the ceRNA network, which in this context is represented by the PTEN pseudogene 1 (*PTENP1*). *PTENP1* regulates PTEN protein levels through a novel non-coding function that confers new functions on pseudogenes[131]. Both *PTEN* and *PTENP1* share extensive sequence homology, and thus *PTENP1* mRNA can serve as a decoy to block the effect of specific miRNAs and boost PTEN expression[132] (FIG. 3d), suggesting that ceRNAs are important regulators of PTEN expression. Further combined bioinformatic and experimental approaches revealed the existence of an extensive network of ceRNAs that regulate PTEN levels. Functionally, these studies revealed that some protein-coding mRNAs served as ceRNAs to regulate PTEN expression through their new non-coding functions. Furthermore, PTEN and ceRNA levels were found to be co-regulated, and the identified ceRNAs could act as tumour suppressors through PTEN upregulation[133]. Further studies also demonstrated the in vivo relevance of the *PTEN* ceRNA network in tumour suppression[132,134]. Additionally, recent studies have revealed that PTEN is also regulated by long non-coding RNAs (lncRNAs), including lncRNA TUG1, CTB-89H12.4 and GAS5, which can function to sequester miRNAs that specifically target *PTEN* mRNA, thereby contributing to *PTEN* ceRNA networks[135,136] (FIG. 3d). Furthermore, new computational tools to identify novel *PTEN* ceRNAs have recently been developed[137,138]. Collectively, these findings lay the groundwork for additional studies to decipher the complexity, crosstalk and effect of tumour suppressors and their ceRNA networks under normal and pathophysiological conditions.

Fine-tuning by PTMs

PTEN is finely regulated by a number of PTMs (FIG. 3e and TABLE 1). While this layer of regulation can contribute to PTEN inactivation when aberrant, it also offers a therapeutic entry point towards PTEN functional enhancement and reactivation.

Ubiquitylation. Ubiquitylation is a PTM mostly known for its ability to tag protein substrates for proteasome-dependent degradation by covalent conjugation of poly-Lys11-linked, or poly-Lys48-linked, ubiquitin chains to the protein substrates[139,140]. Ubiquitylation also regulates

ERK
A serine/threonine-specific protein kinase that is involved in directing cellular responses to a diverse array of stimuli, such as mitogens, osmotic stress, heat shock and pro-inflammatory cytokines. It regulates cellular functions, including proliferation, gene expression, differentiation, mitosis and cell survival.

Pseudogene
A genetic unit transcribed into non-coding RNA that has a counterpart in another gene from which it is derived. The expressed pseudogenes have promoters, CpG islands and splice sites, although some do not contain introns because they were generated from mRNA retrotransposition. Pseudogenes have often lost their ability to encode proteins, for which they are often referred to as 'junk DNA'. They have now been functionally resurrected and attributed to a number of non-coding RNA functions, including their ability to act as competitive endogenous RNAs.

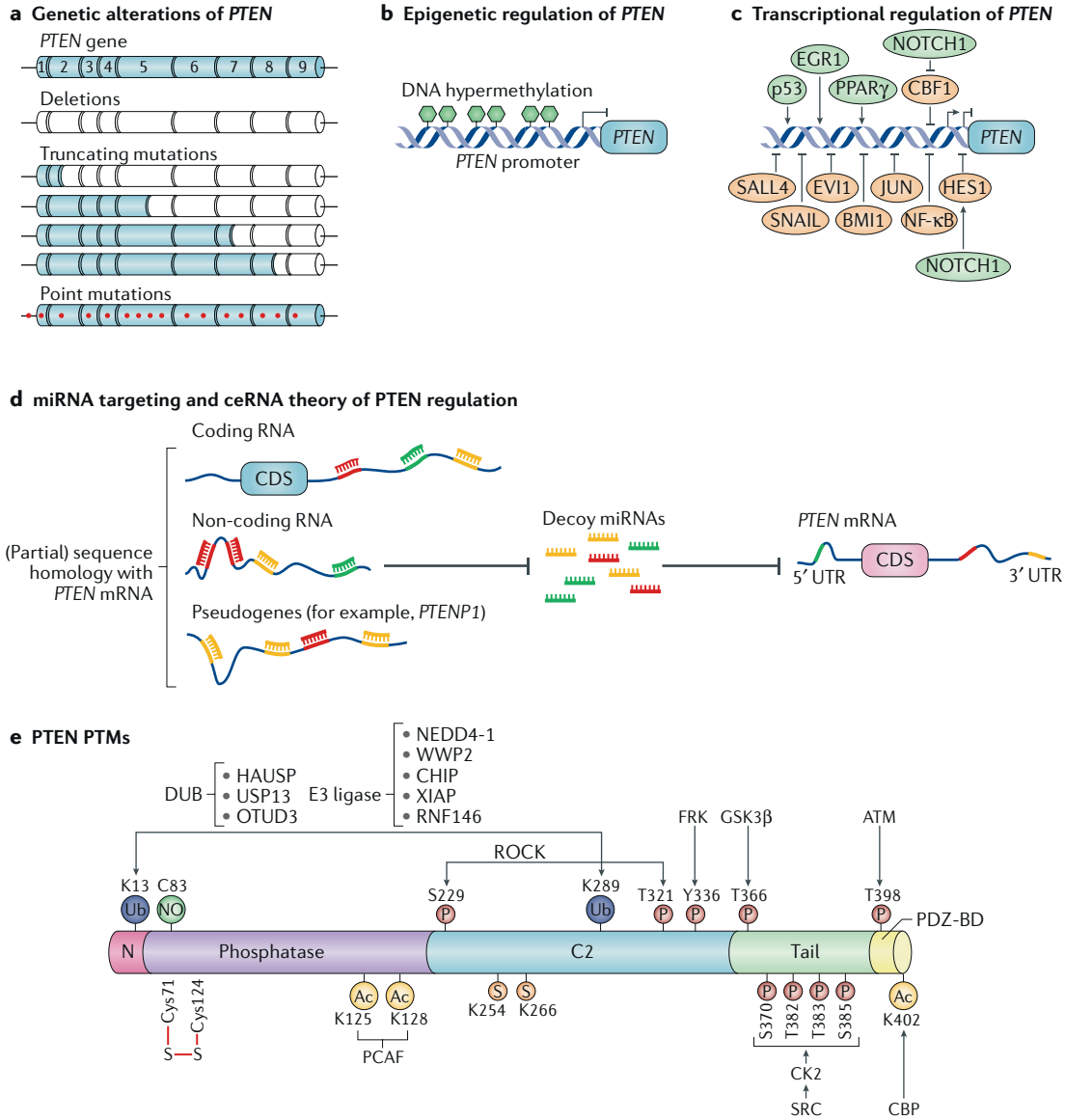

Fig. 3 | **Molecular mechanisms of PTEN regulation. a** | PTEN regulation by genetic alterations. Mutations have been found throughout *PTEN*, including large deletions, small deletions or insertions and point mutations. Allelic losses can result in complete deletion of the *PTEN* locus, whereas small deletions or insertion and point mutations can produce several PTEN truncated mutants that are functionally impaired. **b,c** | PTEN regulation occurs at the transcriptional level. *PTEN* silencing occurs through the methylation of its promoter with hypermethylation, reducing *PTEN* expression in various cancers (part **b**). *PTEN* transcription is also regulated by various transcriptional factors binding at its promoter (part **c**). Positive regulators are shown in green, whereas negative regulators are indicated in orange. **d** | The regulation of PTEN also occurs at the post-transcriptional level. Numerous microRNAs (miRNAs) negatively regulate PTEN expression. This negative regulation is counteracted by the networks of competitive endogenous RNAs (ceRNAs), which include various coding RNAs and non-coding RNAs with partial sequence homology to PTEN and PTEN pseudogene mRNAs. The ceRNAs act as a decoy for PTEN-targeting miRNAs, thereby boosting PTEN expression. **e** | PTEN is also tightly regulated by numerous post-translational modifications (PTMs) (see also TABLE 1). Ac, acetylation; ATM, ataxia telangiectasia mutated; CBF1, C-repeat binding factor 1; CBP, CREB-binding protein; CDS, coding sequence; CHIP, E3 ubiquitin-protein ligase CHIP; CK2, casein kinase 2; DUB, deubiquitylating enzyme; EGR1, early growth-response protein 1; EVI1, ecotropic virus integration site 1 protein homologue; HAUSP, herpesvirus-associated ubiquitin-specific protease; FRK, FYN-related kinase; GSK3β, glycogen synthase kinase 3β; Me, methylation; NO, nitrosylation; NF-κB, nuclear factor-κB; P, phosphorylation; NOTCH1, neurogenic locus Notch homologue protein 1; OTUD3, OTU domain-containing protein 3; PCAF, histone acetyltransferase PCAF; PDZ-BD, PDZ-binding domain; PPARγ, peroxisome proliferator-activated receptor-γ; rDNA, ribosomal DNA; RNF146, E3 ubiquitin-protein ligase RNF146; ROCK, RHOA-associated protein kinase; S, sumoylation; SALL4, Sal-like protein 4; SNAIL, zinc-finger protein SNAI1; Ub, ubiquitylation; USP13, ubiquitin C-terminal hydrolase 13; UTR, untranslated region; WWP2, NEDD4-like E3 ubiquitin-protein ligase WWP2; XIAP, X-linked inhibitor of apoptosis protein.

Table 1 | **Regulation of PTEN by post-translational modifications and interactions with other proteins**

Post-translational modifications	PTEN region involved	Effects on PTEN functions
PTEN post-translational modifications that regulate PTEN activity		
Ubiquitylation	N-terminal and C2 domain	• Polyubiquitylation leading to protein degradation • Monoubiquitylation to facilitate nuclear import
Phosphorylation	C-terminal phosphorylation (T366, S370, T382, T383 and S385)	• Promotes a closed and stable conformation • Reduces PTEN dimerization •Excludes PTEN from nucleus and is involved in DNA repair
Oxidation	Phosphatase domain (C124)	Inhibits lipid PPase activity
S-nitrosylation	N-terminal domain	• Inhibits lipid PPase activity • Decreases protein stability
Acetylation	• Phosphatase domain (K125 and K128) • C-terminal domain (K402)	• Inhibits lipid PPase activity • Increases interactions with PDZ-domain-containing interactors
Sumoylation	• C2 domain (K266) • C2 domain (K254)	• Increases association with plasma membrane • Induces nuclear import
PTEN-interacting proteins that regulate PTEN function		
PREX2	C-terminal tail	Inhibits lipid PPase activity
SIPL1	N-terminus and C-terminus	Inhibits lipid PPase activity
MAN2C1	Not known	Inhibits lipid PPase activity
MC1R	C2 domain	Increases PTEN stability
FRK	C2 domain	Increases PTEN stability
PICT1	C2 domain	Increases PTEN stability
DLG1	Not known	Increases PTEN stability
MAGI2	PDZ-binding domain	Increases PTEN membrane recruitment and activity
MAGI3	PDZ-binding domain	Increases PTEN membrane recruitment and activity
β-arrestins	C2 domain	Increases PTEN membrane recruitment and activity
Myosin V	C-terminal tail	Increases PTEN membrane recruitment and activity

DLG1, discs large 1; FRK, FYN-related kinase; MAGI2, membrane-associated guanylate kinase inverted 2; MAN2C1, α-mannosidase 2C1; MC1R, melanocortin receptor 1; PICT1, protein interacting with C terminus 1; PPase, phosphatase; PREX2, phosphatidylinositol-3,4,5-trisphosphate-dependent RAC exchanger 2 protein; SIPL1, shank-interacting protein like 1.

protein subcellular localization, vesicle trafficking and activation through different polyubiquitin chain types, such as Lys63-linked and Lys33-linked polyubiquitylation, and monoubiquitylation[104,141,142]. PTEN has been reported to be regulated by both polyubiquitylation and monoubiquitylation. Using a biochemical purification approach, NEDD4-1 was identified as an E3 ligase that triggers PTEN ubiquitylation[143]. Overexpression of NEDD4-1 not only triggers PTEN polyubiquitylation for proteasomal degradation but also increases PTEN monoubiquitylation at Lys13 and Lys289, which promote its nuclear import[104,143]. A number of independent studies have demonstrated that NEDD4-1 can trigger PTEN polyubiquitylation and degradation in different cancer settings[144–146]. Interestingly, several proteins have been described as able to modulate the association between PTEN and NEDD4-1. For example, the PY (Pro-Pro-x-Tyr)-motif containing the membrane proteins NEDD4-family-interacting proteins 1 and 2 (NDFIP1 and NDFIP2) and potent activators of NEDD4-1 have been shown to promote NEDD4-1-mediated PTEN polyubiquitylation and monoubiquitylation[147,148]. However, *Nedd4-1*-deficient mice and cells with RNAi-mediated *Nedd4-1* depletion showed no change in PTEN protein stability in vivo, suggesting the involvement of other E3 ligases in PTEN proteasomal degradation[149]. Four such candidates are NEDD4-like E3 ubiquitin-protein ligase (WWP2), X-linked inhibitor of apoptosis protein (XIAP), E3 ubiquitin-protein ligase CHIP (also known as STUB1) and E3 ubiquitin-protein ligase RNF146 (REFS[150–153]). However, these experiments are mostly carried out in cultured cells and/or in an overexpression setting. Therefore, additional data from in vivo mouse models or human samples are required to validate the role of these E3 ligases in PTEN down-regulation, as well as determine to what extent their oncogenic activity depends on PTEN degradation. In addition to E3 ligases, several deubiquitinases, including ubiquitin C-terminal hydrolase 13 (USP13) and OTU domain-containing protein 3 (OTUD3), are found to interact with and deubiquitylate PTEN, leading to its stabilization[154,155]. Additionally, monoubiquitylation of PTEN is reversed by the deubiquitinase herpesvirus-associated ubiquitin-specific protease (HAUSP; also

known as USP7), which leads to the nuclear exclusion of PTEN[156] (FIG. 2a).

Phosphorylation. One of the major PTMs regulating PTEN activity is phosphorylation. A search of phosphorylation sites in PhosphoSitePlus revealed that 24 phosphorylation sites on PTEN have been identified by global mass spectrometry analysis. The most prominent sites are Tyr46 in the PTEN phosphatase domain and phosphorylation sites located within the PTEN C-terminal tail on residues Thr366, Ser370, Thr382, Thr383 and Ser385. These sites are mainly targeted by SRC, casein kinase 2 (CK2) and glycogen synthase kinase 3 (GSK3β)[157–160] (FIG. 3e). PTEN phosphorylation is implicated in modulating its tumour-suppressive functions. For instance, human T cell acute lymphoblastic leukaemia (T-ALL) cells express high levels of PTEN protein but display constitutive PI3K–AKT activation. Two studies have shown that PTEN functional inactivation in T-ALL cells is associated with increased PTEN phosphorylation, resulting from either hyperactivation of CK2 or downregulation of NDRG2–protein phosphatase 2A (PP2A)-mediated PTEN dephosphorylation[161,162]. Functionally, phosphorylation of PTEN can affect its activity, cellular localization and protein stability. Several studies have shown that C-terminal tail phosphorylation promotes a closed and more stable PTEN conformation (FIG. 1b), which reduces its interaction with membrane phospholipids[163–165] or with other membrane-anchored PDZ domain-containing proteins, such as membrane-associated guanylate kinase inverted 2 (MAGI2) and MAGI3 (REFS[166–168]) (see next section), and thereby suppresses its activity. This closed phosphorylated PTEN is also less accessible to the ubiquitin ligase for polyubiquitylation than open non-phosphorylated PTEN, which leads to increased PTEN protein stability[158,168]. However, it should be noted that the effect of phosphorylation on PTEN protein stability seems to be cell-context dependent. For example, phosphorylation of PTEN on residue Thr366 leads to destabilization, rather than stabilization, of PTEN in glioma cell lines[169]. Additionally, phosphorylation of PTEN has been shown to affect PTEN subcellular localization. For example, phosphorylation of PTEN at the C-terminal tail region by ATM leads to its nuclear export[71] and sensitizes cells to DNA damage[68] (FIG. 2a). Recently, it has also been shown that phosphorylation of the PTEN C-terminal tail affects dimerization of the protein (see next section for details)[16].

Oxidation. Like other PTPs, PTEN is also sensitive to oxidation, as it possesses a catalytic Cys nucleophile. Numerous studies have shown that the catalytic activity of PTEN is fine-tuned by reactive oxygen species (ROS). ROS can oxidize the active Cys124 site, which in turn forms an intramolecular disulfide bond with Cys71 to suppress PTEN activity as a phosphatase[162,170]. Disulfide bond formation is inhibited by peroxiredoxin I (PRDX1), which protects PTEN from oxidation possibly through PTEN–PRDX1 direct physical interaction[171]. This oxidation-driven inactivation of PTEN can also be reversed by the chemical reduction of H_2O_2-oxidized Cys residues, which is predominantly mediated by thioredoxin[170]. This has been corroborated by analysis of thioredoxin-interacting protein (TXNIP) knockout mice. TXNIP is required to maintain sufficient levels of thioredoxin activity to reactivate oxidized PTEN and subsequently inhibit the PI3K–AKT signalling pathway[172]. Moreover, PTEN activity can also be indirectly inactivated by oxidation through regulation of PTEN interaction proteins. For instance, the oncogenic protein/nucleic acid deglycase DJ-1 (PARK7) binds PTEN and suppresses its catalytic activity. Oxidation of PARK7 results in an increase in its PTEN binding affinity, leading to a more profound decrease in PTEN lipid phosphatase activity[173]. In summary, an increase in intracellular oxidative stress levels in many cancer cells may trigger ROS-induced PTEN inactivation and the subsequent activation of the PI3K pathway.

S-nitrosylation. Several studies have shown that PTEN is also modified by *S*-nitrosylation, which, apart from oxidation, is another crucial redox mechanism that controls PTEN activity. The level of *S*-nitrosylation of PTEN is inversely correlated with PTEN protein levels in the early development of Alzheimer diseases from human clinical samples[174]. It is known that nitric oxide (NO) can trigger PTEN *S*-nitrosylation, leading to not only suppression of the lipid phosphatase activity of PTEN but also promotion of the downregulation of PTEN protein levels through NEDD4-1-mediated polyubiquitylation[174]. Consistent with these findings, another group has suggested that NO induces PTEN *S*-nitrosylation on Cys83 and subsequently inhibits its function and activity[175]. Recently, it was found that depletion of PARK2, which encodes the ubiquitin E3 ligase Parkin, contributes to increased 5′-AMP-activated protein kinase (AMPK)-mediated activation of endothelial nitric oxide synthase (eNOS; also known as NOS3), increased levels of ROS and a concomitant increase in oxidized NO levels, which drives inhibition of PTEN by *S*-nitrosylation and subsequent ubiquitylation[176]. With these findings, *S*-nitrosylation of PTEN has emerged as another PTM that tightly regulates PTEN activity and could serve as a therapeutic target.

Acetylation. Acetylation can also regulate PTEN activity and function. The histone acetyltransferase PCAF (also known as KAT2B) is known to interact with PTEN and can trigger its acetylation on Lys125 and Lys128 in response to growth factor stimulation[177]. These acetylation sites are located within the phosphatase domain. Hence, PTEN activity is inhibited when these sites are acetylated. In addition, PTEN has been shown to be acetylated by p300–CREB-binding protein (CBP) at Lys402, which is located at the C-terminal PDZ domain-binding motif. In turn, this acetylation affects the binding between PTEN and its various protein interactors (see next section)[178]. NAD-dependent protein deacetylase sirtuin 1 (SIRT1) can function as a PTEN deacetylase, and PTEN has been found to be hyperacetylated in *Sirt1*-deficient cells[178,179]. Upon *Sirt1* depletion, PTEN is also excluded from the nucleus[179], suggesting that acetylation modulates its subcellular localization.

Nucleophile
A chemical species that can donate an electron pair to an electrophile to form a chemical bond in relation to a reaction.

Sumoylation. PTEN was initially shown to be sumoylated at the Lys266 residue in its C2 domain, which subsequently increased its binding to the plasma membrane through electrostatic interactions[180]. This increased PTEN localization at the plasma membrane resulted in PI3K–AKT suppression and inhibition of anchorage-independent cell growth and tumour growth in vivo. In addition, PTEN is also sumoylated at Lys254, which improves PTEN nuclear retention, thereby supporting its nuclear function in DNA repair mechanisms[68] (FIG. 2a).

Role of protein–protein interactions

PTEN engages in a number of protein–protein interactions, which can largely affect its stability, activity and functions. These interactions involve various cellular proteins, including plasma membrane proteins, which regulate PTEN membrane localization and PIP_3 dephosphorylation, and proteins that modulate PTEN PTMs. PTEN molecules also bind to each other, forming dimers, which triggers full activation.

PTEN dimerization and activation. PTEN was recently found to homodimerize at the plasma membrane, a critical step towards its full activation[16]. Homodimerized PTEN is in an active conformation and thus exerts its full PIP_3 lipid phosphatase capability[16]. This study also demonstrated both in vitro, using biochemical and cellular assays, and in vivo, in genetically engineered mouse models, that catalytically inactive cancer-associated PTEN mutants do heterodimerize with wild-type PTEN and inhibit wild-type PTEN catalytic activity in a dominant-negative manner (FIG. 4a). A recent study provided a possible structural model for the PTEN dimer complex and confirmed that PTEN can indeed form homodimers in vitro[181] (FIG. 1c). This study also revealed that the C-terminal tail of PTEN stabilizes the formation of the dimer and that phosphorylation of the tail is associated with a closed conformation, which inhibits PTEN dimer formation[181] (FIG. 1b). It should be noted that PTEN protein purified from insect cells appears to be ubiquitously monomeric, suggesting that PTEN dimerization is cell-context dependent[182]. Nevertheless, the molecular mechanisms underlying the regulation of PTEN dimerization still remain largely elusive and demand further exploration. Importantly, strategies that favour PTEN dimerization may represent a promising therapeutic approach for cancer prevention and therapy.

Regulation by interactions with other protein partners. PTEN-interacting proteins can regulate PTEN phosphatase activity either directly or indirectly through altering its stability, subcellular localization and lipid membrane-binding affinity. Therefore, they have a major impact on PTEN tumour-suppressive functions (TABLE 1). Proteomic and functional overexpression library screens have revealed a number of PTEN-interacting proteins. PIP_3-dependent Rac exchanger 2 protein (PREX2)[183], shank-interacting protein like 1 (SIPL1; also known as SHARPIN)[184] and α-mannosidase 2C1 (MAN2C1)[185] function as novel negative PTEN regulators by directly suppressing PTEN lipid phosphatase activity. Notably, these negative regulators can increase the levels of phosphorylated AKT only in cells expressing PTEN, indicating that their cellular activity in the context of the PI3K–AKT pathway is based on PTEN inhibition. Furthermore, analyses of human clinical samples have shown that expression of these protein regulators correlates with loss of PTEN function only in PTEN-positive human cancer[183–185], suggesting they serve as possible new targets for future cancer therapies.

One common mechanism by which interacting proteins affect PTEN activity is regulation of PTEN protein stability. For example, melanocortin receptor 1 (MC1R) is mainly expressed in melanocytes and protects against ultraviolet damage by direct interaction with PTEN, shielding PTEN from WWP2-mediated degradation and consequently acting to suppress PI3K–AKT signalling. By contrast, melanoma-associated MC1R variants that do not interact with PTEN are associated with elevated levels of PI3K–AKT signalling following ultraviolet exposure and melanomagenesis[186]. Two other proteins, protein interacting with C terminus 1 (PICT1; also known as NOP53) and FYN-related kinase (FRK; also known as RAK), which are dysregulated in human neuroblastoma and breast cancer, respectively[187,188], have been reported to physically interact with PTEN and promote PTEN phosphorylation and stabilization[146,189], although the mechanisms underlying phosphorylation-mediated PTEN stabilization are unclear. Moreover, a mouse orthologue of *Drosophila melanogaster* discs large 1, *Dlg1*, interacts with and stabilizes PTEN protein in both Schwann cells and early-stage B-lineage progenitors. *Dlg1* is an essential tumour suppressor gene that controls epithelial cell growth and polarity of the imaginal discs of the fly in pupal development. Consequently, *Dlg1* loss disrupts PTEN functions and leads to abnormal myelination in the sciatic nerve and an expansion of leukaemogenic progenitors in murine models of B cell acute lymphoblastic leukaemia[190,191].

Apart from the regulation of PTEN protein stability, protein–protein interactions can also regulate PTEN subcellular localization (TABLE 1). Importantly, these interactions can assist targeting of PTEN to the plasma membrane, thereby activating its lipid phosphatase activity. For example, MAGI2 and MAGI3 bind to the PTEN C-terminal PDZ domain and facilitate its membrane recruitment and lipid phosphatase activity[166,167]. Additionally, the activation of RHOA-associated protein kinase (ROCK), a major downstream effector of small GTPase RHOA, triggers PTEN phosphorylation as well as its membrane recruitment and activation[192]. RHOA activation also leads to increased PTEN interaction with β-arrestins and their co-recruitment to the plasma membrane, which increases the lipid phosphatase activity of PTEN[193]. Finally, the actin motor protein myosin V directly interacts with PTEN and regulates PTEN movement to the cell membrane, which is necessary for its activity towards PIP_3 (REF.[194]). Further research is needed, however, to reveal the relevance of these interactions in human cancer.

Anchorage-independent cell growth
A type of growth where cells grow in the absence of a stable surface to which they can adhere. It is a key aspect of the neoplastic transformation, particularly with respect to metastatic potential.

Schwann cells
Cells in the peripheral nervous system that produce the myelin sheath around neuronal axons and function to support neurons. They are named after German physiologist Theodor Schwann, who discovered them in the 19th century.

Imaginal discs
Sac-like epithelial structures that are found inside the larvae of insects and become a portion of the outside of the adult insect after pupal transformation.

Sciatic nerve
The largest single nerve in humans and animals. It runs from each side of the lower spine through deep in the buttock into the back of the thigh and all the way down to the foot. It serves a vital role in connecting the spinal cord with the leg and foot muscles.

RHOA
A member of the RHO family of small GTPases, which function as molecular switches in signal transduction cascades. The RHOA protein promotes reorganization of the actin cytoskeleton and regulates cell shape, attachment and motility and is associated with cancer cell proliferation and metastasis.

β-arrestins
Multifunctional adaptor proteins that are best known for their ability to desensitize G protein-coupled receptors, thereby regulating a diverse array of cellular functions downstream of G protein-coupled receptors.

PTEN isoforms and PTEN secretion

As discussed above, regulation of the subcellular localization and activity of PTEN has been studied in much detail. Interestingly, recent studies have reported that PTEN can also be secreted extracellularly and taken up by neighbouring cells[17,18] (FIG. 4b). One group demonstrated that the canonical PTEN protein is packaged into exosomes and is transferred from one cell to another by means of these vesicles[18]. Interestingly, another group identified a translational variant of PTEN, which they termed PTEN-Long, and showed that it is evolutionarily conserved. PTEN-Long was found to be translated from an alternative start site 519 bp upstream of the ATG initiation sequence, adding 173 amino acids to the canonical PTEN[17] (FIG. 4b). PTEN-Long is a membrane-permeable variant of PTEN that can be secreted from cells and can be taken up by other cells directly. Indeed, PTEN-Long can be detected in human serum and plasma[17]. It contains a poly-Arg stretch, which shows sequence homology to permeable peptides, and thereby facilitates its cell penetration and subsequent inhibition of PI3K–AKT both in vitro and in vivo. PTEN-Long can therefore function in vivo as a therapeutic agent to suppress tumour growth, as demonstrated in various mouse models, with important therapeutic implications[17]. A subsequent study revealed that PTEN-Long can also localize in the mitochondria and interact with canonical PTEN to increase PTEN-induced putative kinase 1 (PINK1) protein levels, which in turn regulates mitochondrial function and energy production[195]. More recently, another N-terminal extended PTEN isoform, termed PTEN-β, has been identified (FIG. 4b). PTEN-β translation is initiated from an AUU codon upstream of and in-frame with the AUG initiation sequence for canonical PTEN. PTEN-β predominantly localizes in the nucleolus and physically interacts with and dephosphorylates nucleolin, which in turn negatively regulates ribosomal DNA transcription and ribosomal biogenesis[196]. As such, these studies demonstrate the complexity of this newly identified PTEN protein family and the diversity of its biological functions in cancer progression and normal development. Further quantitative studies, including analysis of human plasma and the serum proteome, are required to better understand how distinct PTEN isoforms are distributed between the intracellular and extracellular compartments. Finally, as various PTEN isoforms still share a high sequence homology with canonical PTEN, they may be modulated by the same or similar mechanisms. Importantly, the underlying mechanisms of PTEN-Long regulation and secretion remain unknown. Approaches aimed at boosting PTEN secretion and uptake by recipient cells would offer a novel therapeutic modality for cancer treatment.

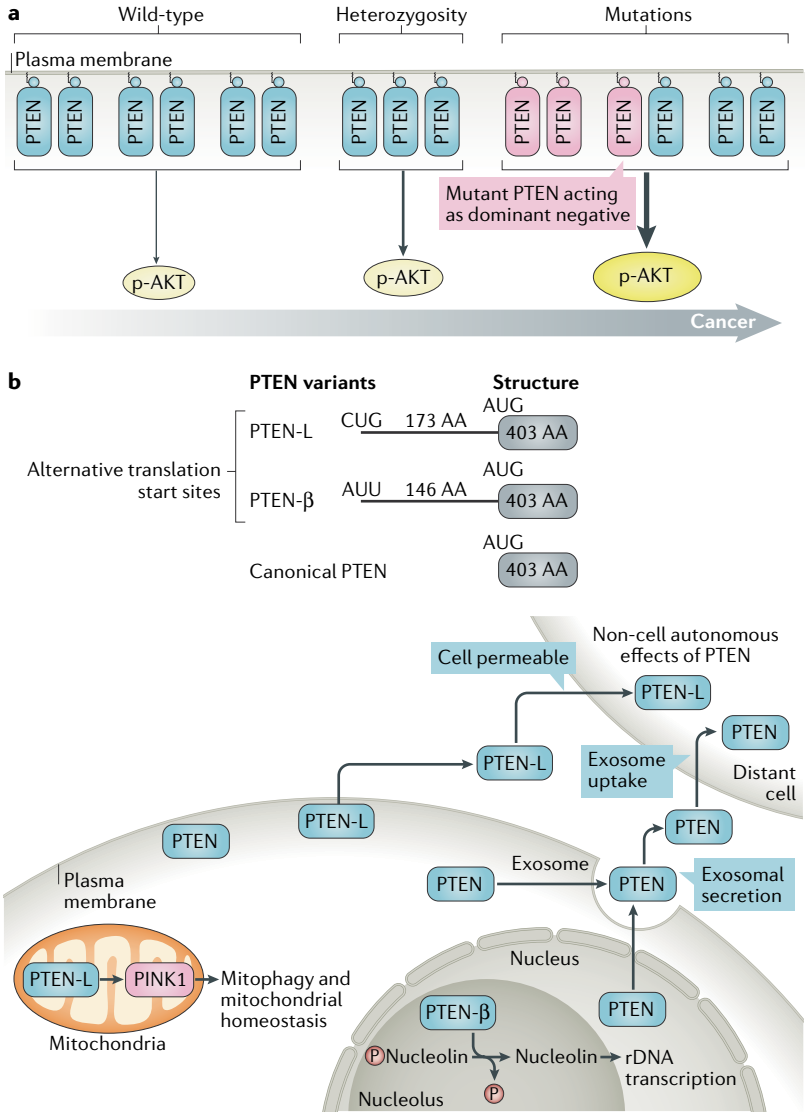

Fig. 4 | **Model of PTEN dimerization, activation and secretory regulation. a** | PTEN dimerization and activation at the plasma membrane. PTEN heterozygosity (*Pten*+/–) results in decreased levels of PTEN dimers and consequently increased AKT activation (p-AKT levels) and tumorigenesis. Heterozygous expression of PTEN harbouring cancer-associated mutations (pink) leads to the formation of catalytically inactive dimers, including with wild-type PTEN, which in turn promotes AKT hyperactivation and cancer progression. **b** | Isoforms of PTEN and PTEN secretion. Recent studies revealed the existence of translational isoforms of PTEN. The two identified isoforms, termed PTEN-Long (PTEN-L) and PTEN-β, are longer than canonical PTEN and translated from alternative start sites upstream from the canonical AUG start site. Like canonical PTEN, PTEN-L associates with membranes. PTEN-L is a membrane-permeable lipid phosphatase that is secreted from cells and can be taken up by neighbouring cells. Additionally, PTEN-L is found localized in the mitochondria where it can regulate mitochondrial function and energy production (by interacting with PTEN-induced putative kinase 1 (PINK1)). PTEN-β has been identified in the nucleolus, where it binds to and dephosphorylates nucleolin, thereby inhibiting transcription of ribosomal DNA, biogenesis of ribosomes and, in consequence, cell proliferation. Canonical PTEN can also be secreted in exosomes and can be taken up by distant cells, which together with PTEN-L secretion contributes to non-cell autonomous effects of PTEN.

Approaches for PTEN reactivation

Loss of PTEN function occurs in PHTS and in a wide spectrum of human cancers through a variety of mechanisms, as described above. The traditional pharmacological strategy to reverse the effect of PTEN inactivation is to antagonize the PI3K–AKT–mTOR pathway. However, the relief of feedback inhibition and subsequent activation of other oncogenic pathways caused by targeting PI3K–AKT–mTOR constitutes a major hurdle that limits the success of such therapies[197]. Additionally, PTEN exerts part of its tumour-suppressive function independently of the PI3K pathway. Therefore, increasing or restoring PTEN dosage itself may represent a more straightforward

Box 1 | **Opportunities for pharmacological PTEN reactivation after partial or complete loss of *PTEN* expression**

In cancers associated with PTEN dysfunction, tumour genotype determines which therapies should be considered for intervention (see Table). First, in tumours showing complete loss of *PTEN*, administration of therapeutic PTEN-Long protein or PTEN nanoparticles could be efficacious. Second, in tumours carrying monoallelic *PTEN* deletion or intact *PTEN* but harbouring defects in regulatory mechanisms, an approach geared at restoring or enhancing PTEN function and levels through potentiation of dimerization, transcription and post-transcriptional regulation could be employed. Lastly, in tumours harbouring monoallelic *PTEN* mutations, three therapeutic scenarios could be envisioned: reactivation of the remaining wild-type PTEN as aforementioned if the mutant PTEN is unstable; if the PTEN mutant acts in a dominant-negative manner to suppress wild-type PTEN protein function, combination of supplementation with PTEN-Long or PTEN nanoparticles together with reactivation of the wild-type protein; and gene editing to correct somatic *PTEN* mutations or even to engineer enhanced PTEN variants with improved activity and/or stability.

Applicable tumour type	Therapeutic intervention
Tumours with complete loss of *PTEN*	• Administer PTEN-Long protein • Administer PTEN nanoparticles
Tumours with monoallelic *PTEN* deletion or intact *PTEN*	• Increase PTEN dimerization • Use drugs to increase the activity of PTEN transactivators • Inhibit *PTEN*-targeting microRNA • Derepress epigenetic silencing or histone deacetylation • Target E3 ligase to stabilize PTEN protein
Tumours with monoallelic *PTEN* mutation	• Administer PTEN-Long protein • Administer PTEN nanoparticles • Block the dimerization between mutant and wild-type PTEN • Edit the *PTEN* gene to correct mutations or engineer into enhanced *PTEN* variants

and effective way to prevent and treat cancer and to benefit individuals with PHTS. Owing to a multilayered mode of PTEN regulation and the recently described presence of cell-permeable PTEN-Long isoform, restoration of PTEN appears to be feasible, although it would depend on the nature of the PTEN defect (BOX 1). Different strategies could be envisioned, including PTEN protein delivery, to enhance PTEN expression and activity.

PTEN protein delivery. Protein-based therapeutics remains challenging owing to degradation and low membrane permeability. However, the identification of PTEN-Long opens a new avenue to restoring PTEN function, particularly in tumours showing complete loss of *PTEN* (BOX 1). Additionally, one recent study has shown that intracellular delivery of PTEN protein using cationic lipidoids resulted in both a decrease in activated AKT and induction of apoptosis in *PTEN*-null prostate cancer cells, thus highlighting the potential of therapeutic PTEN protein delivery in cancer[198].

Enhancing PTEN expression. PTEN reactivation could also be achieved by attenuation of transcriptional or post-transcriptional repression through various methods, including enhancing the activity of PTEN transactivators (FIG. 3c), inhibition of PTEN-targeting miRNAs (FIG. 3d), derepression of epigenetic silencing (FIG. 3b) and targeting E3 ligases that promote PTEN degradation (FIG. 3e). These approaches are especially applicable to tumours not showing genetic *PTEN* alterations or still possessing a functional wild-type *PTEN* allele (BOX 1). However, such approaches would require genetic and quantitative analysis of the endogenous expression levels of PTEN modulators in individual patients to ensure the implementation of personalized therapies. There are also many more challenging hurdles to be overcome, including issues of delivery, specificity and toxicity, before these techniques could reach the clinic.

Enhancing PTEN activity. Another plausible PTEN reactivation strategy is to increase PTEN activity through various means, including potentiation of dimerization, promoting PTEN membrane association, gene editing by CRISPR–Cas9 to correct for somatic PTEN mutations or engineering PTEN variants with increased phosphatase activity. A recent study has identified PTEN mutations that exhibit substantially increased recruitment to the plasma membrane owing to increased association with PIP_2 (ePTEN). Upon expression in human cells, ePTEN decreases PIP_3 levels in the plasma membrane and subsequently reduces phosphorylation of AKT, cell proliferation and migration. Therefore, gene editing may provide a potentially powerful platform for restoring wild-type or engineering enhanced PTEN variants for cancer prevention and therapy[199]. However, considerable effort will be required to avoid off-target effects and to improve delivery and specificity of gene-editing techniques before such an approach can be applied towards PTEN restoration for cancer therapy.

Conclusions and perspectives

Since its discovery as a bona fide tumour suppressor, numerous in vitro and in vivo studies have highlighted the importance of PTEN functions within the cell, ranging from inhibition of cell growth, proliferation and migration to promotion of apoptosis, DNA damage repair and tumour-suppressive metabolic states. Consequently, the loss of PTEN function

PTEN-induced putative kinase 1
(PINK1). A mitochondrial serine/threonine-protein kinase encoded by the *PINK1* gene. PINK1 activity triggers the binding of the parkin protein to depolarized mitochondria, in turn inducing mitophagy. Mutations in this gene result in autosomal recessive Parkinson disease.

Nucleolin
The major nucleolar protein of growing eukaryotic cells. This protein is associated with chromatin and pre-ribosomal particles and functions to regulate ribosomal RNA transcription and ribosome assembly.

Lipidoids
Any materials having characteristics of a lipid.

leads to the disruption of many key processes, which is associated with disruption of organismal homeostasis, developmental defects and tumorigenesis. PTEN exerts much of its activity as a lipid phosphatase opposing the pro-survival and oncogenic PI3K–AKT–mTOR signalling pathway and integrates complex feedback loops within this pathway. PTEN also exerts PI3K–AKT-independent and protein phosphatase-dependent activities and functions within the nucleus. Additionally, the notion that subtle changes in PTEN levels result in cancer susceptibility and tumour progression highlights the pivotal role of various regulatory mechanisms of PTEN in tissue homeostasis and tumorigenesis. Accordingly, the elucidation of the mechanisms controlling PTEN protein levels and activity may offer novel targeted therapies for a wide variety of human cancers and inheritable syndromes associated with aberrations in PTEN-dependent networks.

Remarkably, even as our knowledge of the roles of PTEN as a tumour suppressor may seem extensive, groundbreaking discoveries in PTEN biology have still been made in recent years. Specifically, the findings that the full activity of PTEN is achieved by dimerization and that PTEN can be secreted to exert a cell non-autonomous effect on adjacent cells have provided new insights into PTEN function. Future studies elucidating the molecular mechanisms underlying the regulation of PTEN dimerization and PTEN secretion are key to fully defining the role of PTEN in tumorigenesis and identifying the best strategies to repair or remedy PTEN dysfunction for cancer prevention (BOX 1).

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