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The Role of PTEN in Neurodevelopment

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Abstract
PTEN is a lipid and protein phosphatase that regulates cell growth and survival. Mutations to PTEN are highly penetrant for autism spectrum disorder (ASD). Here, we briefly review the evidence linking PTEN mutations to ASD and the mouse models that have been used to study the role of PTEN in neurodevelopment. We then focus on the cellular phenotypes associated with PTEN loss in neurons, highlighting the role PTEN plays in neuronal proliferation, migration, survival, morphology, and plasticity.

PTEN Mutations and ASD

Autism spectrum disorder (ASD) is a neurodevelopmental disorder involving impaired social behavior, sensory hypersensitivity, repetitive behavior, and restricted interests. In the USA, 1 in 59 children receive an ASD diagnosis [1]. ASD is highly heritable, with genetics accounting for about half of ASD risk [2]. Approximately 50% of cases are expected to arise from the cumulative effects of common alleles each conferring a small degree of risk [3]. Copy number variations or single-gene mutations contribute to an estimated 10% of total ASD cases [4] and a larger proportion of simplex cases [5]. According to one estimate, as many as 30% of ASD cases may have a detectable genetic etiology [6]. Although they each account for only a small fraction of cases, single-gene mutations provide a useful starting point for dissecting the developmental etiology of ASD, since biological functions relevant to ASD etiology may be similarly disrupted in both idiopathic and syndromic cases. One gene associated with syndromic ASD risk is the phosphatase and tensin homolog gene *(PTEN)*. The PTEN protein is part of a signaling network that contains multiple ASD-associated gene products and represents a potentially common etiological mechanism for ASD and related neurodevelopmental disorders [7–9]. Here, we briefly review the PTEN protein and the phenotypes associated with PTEN loss of function in humans, before focusing on the structural and functional consequences of PTEN loss in vivo in animal models and what these tell us about PTEN function in neurons.

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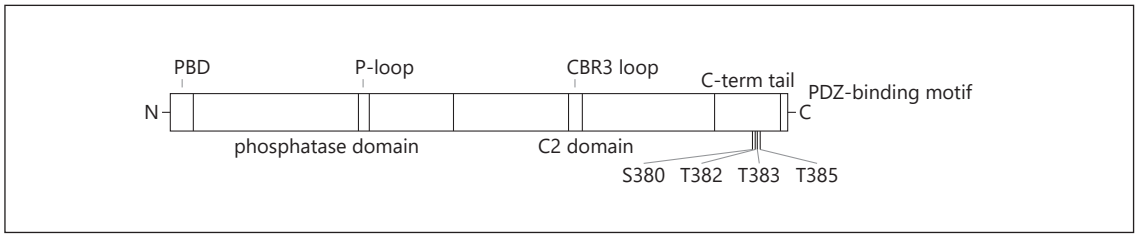

Fig. 1. Major structural features of PTEN. PTEN has an N-terminal PIP2-binding domain (PBD) and phosphatase domain (amino acids 7–185), containing the catalytic P-loop (123–130). A C2 domain (186–351) contains a CBR3 loop from 260–269, which mediates membrane binding. An unstructured C-terminal tail contains multiple phosphorylation sites which inhibit catalytic activity and membrane binding. The terminus of this tail contains a PDZ-binding motif (based on [33] and [58]).

PTEN was originally discovered due to its role as an oncogene [10, 11], and was quickly identified as the primary cause of the tumor syndromes Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and Lhermitte-Duclos disease, collectively referred to as PTEN hamartoma tumor syndromes (PHTS; OMIM #158350) [12–14]. Numerous cases were soon reported of individuals with both PHTS and ASD confirmed to have PTEN mutations [13, 15, 16], suggesting that PTEN is a risk factor for ASD. PTEN mutations have also been found in individuals with macrocephaly and ASD but lacking a PHTS diagnosis [17–23], indicating incomplete penetrance for both PHTS and ASD. Among individuals with both macrocephaly and ASD, PTEN mutations may be relatively common. The estimated frequency of mutations to protein-coding portions of PTEN in such patients ranges from <2 to 20%. Most of the variation is due to the sampling criteria; studies using a more restrictive definition of macrocephaly tend to find a higher mutation rate [17, 19–21, 23, 24]. Mutations to other members of the PI3K-AKT-mTOR pathway have also been found in cases of comorbid macrocephaly and ASD [25]. Multiple discoveries of de novo *PTEN* mutations confirm it is a risk gene for ASD [26–30]. Although the penetrance for ASD in humans with PTEN mutations is unknown, a retrospective chart review of patients with known PTEN mutations found macrocephaly in 66% and ASD diagnoses in 50%, with high frequencies of dermatological, gastrointestinal, and thyroid problems as well [31].

PTEN Biology

PTEN is a ubiquitously expressed and evolutionarily conserved dual-specificity protein and lipid phosphatase. In its best-characterized role as a lipid phosphatase, PTEN catalyzes the removal of the 3-phosphate from phosphatidylinositol (3,4,5)-phosphate (PIP_3), generating $PI(4,5)P_2$ and directly antagonizing the activity of the class I PI3 kinases (PI3K) [32–35]. PIP_3 recruits and activates a broad variety of effectors by their Pleckstrin homology domains, regulating a broad spectrum of biological functions including growth, survival, gene transcription, protein translation, cytoskeletal organization, and membrane trafficking [32]. The best characterized of these effectors is AKT, which in turn regulates signaling through mTOR and GSK3β. Meanwhile, PTEN's protein phosphatase activity targets a number of substrates, including MAPK signaling, which PTEN inhibits [36, 37]. PTEN consists of a phosphatase domain (residues 7–185), a C2 domain, which mediates membrane binding (186–351), and a 24-amino acid c-terminus tail (Fig. 1) [38]. An alternate transcript produced from a noncanonical translation initiation site produces small amounts of PTEN-L, which contains an additional 173 amino acids on its N-terminus [39]. PTEN-L is both secreted from the cell [39] and distributed differently within the cell, localizing preferentially away from the nucleus and toward the mitochondria [40].

PTEN is located throughout the cell, where regulation of its location is essential for its function. PTEN's lipid substrate PIP_3 is a membrane-bound phospholipid. Therefore, membrane-associated PTEN has the most catalytic activity [41]. PTEN localization to the nucleus contributes to its tumor suppressor activity [42] and its regulation of cell growth [43] while promoting cell survival [44]. Whether PTEN can access PIP3 in the nuclear matrix to regulate mTOR signaling is an open question as there has been evidence both for [43] and against this idea [45]. Monoubiquitination, SUMOylation, and protein-protein interactions increase the rate of PTEN translocation into the nucleus [42, 46, 47]. Impairing the ability of PTEN to localize

to the nucleus and associate with centromeres leads to an increased frequency of double-strand breaks in DNA [46, 48]. The tumor suppressor properties of nuclear PTEN do not depend on its phosphatase activity, but rather on its ability to associate with the anaphase-promoting complex [49]. However, nuclear PTEN does dephosphorylate PIP_3 in the nucleus to suppress neuron growth [43]. PTEN-L, the long form of PTEN, is preferentially distributed away from the nucleus [40]. The implications of nuclear PTEN for ASD remain an area of interest.

PTEN activity is dynamically regulated by phosphorylation. The kinase CK2 phosphorylates a cluster of residues on PTEN's C-tail; phosphorylated PTEN is degraded slower by proteasome [50]. In in vitro assays, the membrane affinity of PTEN depends on both the C2 domain and an N-terminal PIP_2-binding domain [41]. Phosphorylation of the C-tail prevents membrane binding [41]. The phosphorylated residues on the C-tail bind to the N-terminal PIP_2-binding domain, competitively blocking the sites of interaction with membrane-bound PIP_2 and the C2 domain [51–53]. The phosphorylation-dependent regulation of the subcellular localization of PTEN is dynamically regulated during cellular growth. For example, in axonal growth cones, most PTEN is phosphorylated and colocalizes with microtubules instead of the membrane [54]. Chemorepellent signals lead to the dephosphorylation of PTEN's C-tail, increasing its affinity for the membrane, where it opposes outgrowth and promotes collapse by dephosphorylating PIP_3 [55]. Meanwhile, downstream signaling of growth factor receptors activates CK2 to phosphorylate PTEN, thereby preventing interactions with the membrane [56].

Continued collection of exome and genome sequences from ASD cohorts, PHTS patients, and tumors has led to an expansive library of PTEN mutations associated with pathology. In ASD, tumor syndromes, and tumor tissue, mutations can impact PTEN function in a variety of ways. Mutations to an upstream regulatory element can dampen translation, causing PHTS [57], while point mutations associated with both ASD and cancer lose their function through depressed catalytic activity, instability, and altered subcellular localization [43]. There is a significant overlap between mutations associated with cancer and those associated with ASD [58]. However, it has been proposed that mutations severely affecting catalytic activity have a stronger association with PHTS and cancer, while mutations associated with PHTS and ASD are more likely to produce subtler alterations to stability or subcellular localization. For example, a comparison of the lipid phosphatase activity of several variants found in PHTS and ASD patients found that while a majority of PHTS-associated variants were completely inactive, most ASD-associated variants retained some lipid phosphatase activity [59]. In another study, a group of PTEN variants found only in ASD were stable and all, at least partially, rescued the morphology of PTEN-knockout neurons, while a group of variants associated with particularly severe PHTS completely lacked the ability to suppress AKT phosphorylation, suggesting a complete loss of function [60]. In a saturation mutagenesis approach in yeast, mutations implicated in PHTS and patient tumors had a greater impact on fitness than mutations implicated only in ASD, and they were more likely to target the catalytic pocket [61]. Collectively, these results suggest that mutations causing a complete loss of function are more likely to result in PHTS, while less severe mutations may contribute to ASD pathology despite retaining some function. However, it is notable that the severity and manifestations of PHTS vary broadly across the patient population, with cognitive function in individuals with PHTS ranging from normal to severely disrupted [62, 63]. Therefore, the likelihood of developing cancer or ASD in PHTS may depend on factors such as environmental insult of a permissive genetic background, and these factors may be distinct for the two conditions.

PTEN function is important in neurodevelopment. The most common clinical finding in humans with mutated PTEN is macrocephaly [31]. The increased head circumference of patients with PTEN mutations is driven by enlargement of the cerebellum, ventricles, and white matter, although cortex thickness remains normal [64]. Focal abnormalities in white matter are common [65], as are abnormalities of the vasculature [65–67]. It has been proposed that these white matter lesions could contribute to comorbid psychiatric diagnoses such as bipolar disorder, psychosis, obsessive compulsive disorder, general anxiety disorder, and developmental delay [68]. Hemimegalencephaly and focal cortical dysplasias have also been observed in patients with PTEN mutations [69, 70], and in patients with mutations elsewhere in the PI3K-AKT-mTOR pathway [69]. The cognitive outcomes of patients with PTEN mutations are highly variable. About half have an ASD diagnosis, while about one-third have diagnoses of developmental delay or intellectual disability, and about 1 in 5 have both ASD and a comorbid cognitive disorder [31]. A battery of cognitive function tests administered to patients with Cowden syndrome found that many individuals possessed normal intelligence, while executive and motor functions were the most likely categories to be impaired [63].

Mouse Models of PTEN Loss of Function

The high penetrance of PTEN mutations for ASD made PTEN a promising candidate to test in animal models. In the mouse, germline PTEN knockout is embryonic lethal [71]. Germline heterozygous and conditional knockout mice display altered neurodevelopment and ASD-associated behaviors, making them a promising model system for studying the biological basis of PTEN-associated ASD. Because PTEN mutations in humans are heterozygous, the most genetically similar animal model is mice that are germline heterozygotes. Like humans with PTEN mutations, PTEN heterozygous mice have an enlarged brain. In the mice, this is primarily due to increased proliferation of glia, although enlarged neuronal cell bodies also contribute [72]. These mice exhibit reduced social preference, social interaction time, and aggression, and an increased frequency of repetitive behaviors [72, 73]. The *m3m4* mutant strain, which carries 5 germline point mutations in PTEN's C2 domain, affecting subcellular localization, also has an enlarged brain with more glia. In this mouse, males are hypersocial, and both sexes have motor deficits [74]. Neuron-specific conditional PTEN knockout recapitulates many of the features observed in humans with PTEN mutations. In a GFAP-Cre × *Pten*$^{flx/flx}$ line, in which PTEN is knocked out early in the development of most cerebellar, hippocampal, and cortical neurons, the mice have larger brains with overgrown cerebella and enlarged ventricles, mirroring the characteristics of human Lhermitte-Duclos disease [75, 76]. In a different GFAP-Cre × *Pten*$^{flx/flx}$ line in which PTEN expression is also lost in glia, axons are hypermyelinated [77]. The NSE-Cre × *Pten*$^{flx/flx}$ mouse loses PTEN expression in a subset of forebrain neurons between birth and 2 weeks of age, leading to an enlarged brain, reduced social preference, impaired sensorimotor gating, learning deficits, and epilepsy [78, 79]. Therefore, PTEN mutant and neuron-specific conditional knockout mice recapitulate many of the symptoms associated with PTEN mutations in humans, including macrocephaly and behavior alterations that mimic the core symptoms of ASD.

Transgenic mouse lines in which PTEN loss is restricted to specific populations have provided insight to the contributions of specific circuits to altered behavior in PTEN knockout mice. Consistent with the established role of the hippocampus in the etiology of epilepsy, PTEN loss in the dentate gyrus of the hippocampus is sufficient to cause recurrent seizures [80, 81]. Social behaviors are complex and can be influenced by brain circuits that process reward, fear, motivation, and higher-order cognitive tasks. It is therefore perhaps unsurprising that PTEN deletions in a number of circuits can affect social behavior. In addition to the previously mentioned transgenic lines in which PTEN is lost in multiple brain regions, social behavior is impacted by restricted PTEN loss in midbrain dopaminergic neurons in a DAT-Cre driver line, in cerebellar Purkinje neurons in an L7-Cre driver line, and in cortical interneurons derived from the medial ganglionic eminence (MGE) in an Nkx2.1-Cre driver line [72, 82, 83]. It has been found that inhibiting hyperactive projections from the medial prefrontal cortex to the basolateral amygdala rescues the reduced social preference of PTEN haploinsufficient mice [84]. However, not all circuits implicated in social behavior mediate the altered behaviors associated with PTEN loss, since PTEN knockout in oxytocinergic neurons results in behavior similar to that of wild-type mice except for increased open-field activity [85]. A selection of PTEN conditional knockout lines and the associated behavioral alterations are summarized in Table 1. Together, these studies demonstrate that despite the effects of PTEN loss on the functions of other cell types in the brain, neuronal PTEN loss can drive ASD-associated behavior in a circuit-specific manner.

Proliferation, Differentiation, and Migration

PTEN is an important regulator of development from the earliest stages, regulating cell proliferation and cell fate. Mice with a homozygous PTEN deletion die before embryonic day 7.5 because of a failure for tissue to appropriately differentiate into endoderm, mesoderm, and ectoderm [71]. Mice lacking only 1 PTEN allele are viable and have increased cell proliferation during brain development. This extra cell proliferation primarily results in the excess production of glial cells as a result of increased signaling through GSK3β [86]. Brain overgrowth is evident starting at birth. The increased number of cortical neurons, first evident at E15.5, normalizes by adulthood, potentially due to increased postnatal apoptosis [86]. In adult mice heterozygous for PTEN, there are increases in the number of astrocytes, microglia, and oligodendrocytes [86]. Increases in progenitor proliferation and cell fate bias toward astrocytes and oligodendrocytes are also observed in a the *m3m4* homozygous mouse [74]. PTEN deletion can also alter the differentiation of cells at later stages of development; PTEN deletion in GABAergic neurons born in the MGE causes a decrease in the proportion that mature into somatostatin-positive interneu-

Table 1. Mouse models used to study PTEN loss of function in the context of neurodevelopment, and the behavioral, morphological, and physiological abnormalities observed in each

Strain	Targeted populations	Behaviors	Morphology and physiology	Reference
Pten$^{-/-}$	somatic	embryonic lethal	disrupted differentiation into endo-, meso-, and ectoderm	[71]
Pten$^{+/-}$	germline heterozygous	↓ social preference ↓ social novelty ↓ aggression ↑ marble burying ↑ tail suspension and forced swim ↓ dark-light emergence and open field	macrocephaly ↑ glia (astrocytes, oligodendrocytes, and microglia) neuronal hypertrophy ↑ axon growth	[72, 73, 84, 86]
m3m4	carries 5 germline point mutations to the noncanonical NLS	↑ social interaction (males) ↓ motor coordination	macrocephaly ↑ glia ↑ oligodendrocytes neuronal hypertrophy ↑ reactive gliosis	[74]
GFAP-Cre × *Pten*$^{flx/flx}$	DG, cerebellar granule neurons, some hippocampal and cortical neurons, by P0–P14	epilepsy	macrocephaly cerebellar hyperplasia neuronal hypertrophy	[75, 76]
GFAP-Cre × *Pten*$^{flx/flx}$	astrocytes, 80–90% of DG and cerebellar granule neurons, 50–80% of cortical neurons, by P14	epilepsy	macrocephaly cellular hypertrophy ↑ astrocyte proliferation hypermyelination ↑ dendritic spines ↑ spine size	[77, 117]
Emx1-Cre × *Pten*$^{flx/+}$; Emx1-Cre × *Pten*$^{fls/flx}$	cortical neurons, astrocytes, and oligodendrocytes, from corticogenesis	↓ social preference	macrocephaly somatic hypertrophy ↑ glia	[84, 86]
NSE-Cre × *Pten*$^{flx/flx}$	approximately 50% of excitatory forebrain neurons by the age of 4 weeks	epilepsy ↓ water maze ↓ social novelty ↓ social preference ↑ startle response ↓ time in open field	macrocephaly neuronal and dendritic hypertrophy mossy fiber sprouting ectopic dendrites ↑ dendritic spines	[78, 79, 92]
CamKIIα-Cre × *Pten*$^{flx/flx}$	excitatory forebrain neurons, at the age of 2–8 weeks	↓ water maze	normal cell size laminar-specific dendritic hypertrophy normal dendritic spines ↓ LTP ↓ LTD ↓ excitability	[104, 106, 107]
Nkx2.1-Cre × *Pten*$^{flx/flx}$	MGE-derived interneurons	↓ social preference ↓ novel object interaction	↑ PV/SST ratio cellular hypertrophy ↑ IPSCs (on pyramidal neurons)	[83]
DAT-Cre × *Pten*$^{flx/flx}$	midbrain dopaminergic neurons	↓ social preference ↓ social approaches (sex-dependent)	cellular hypertrophy ↑ axon growth ↑ DA release	[72, 100]
L7-Cre × *Pten*$^{flx/flx}$	Purkinje neurons	↓ social preference ↓ social approaches ↓ grooming normal memory	cellular hypertrophy ↑ excitatory drive ↓ tonic activity	[82]

NLS, nuclear localization sequence; MGE, medial ganglionic eminence; PV, parvalbumin; SST, somatostatin; DA, dopamine; IPSCs, inhibitory postsynaptic currents.

rons, and an increase in the proportion that mature to express parvalbumin [83].

The incidence of focal cortical dysplasia in humans with PTEN mutations [69, 70] implicates PTEN in neuronal migration. Postnatally generated PTEN-knockout granule neurons in the dentate gyrus migrate further from the subgranular zone, an effect prevented by treatment with the mTORC1 inhibitor rapamycin [87]. Ectopic cerebellar granule neurons have also been observed in conditional PTEN knockout lines [75]. In a model of mTOR hyperactivation driven by in utero electroporation with constitutively active Rheb, cortical neurons expressing have impaired migration, failing to reach the outer cortical layers and taking on the identity of deep-layer neurons instead [88]. Normalizing cap-dependent protein translation downstream of mTOR by overexpressing constitutively active 4E-BP rescues this cortical mislamination. It is currently unknown whether PTEN loss in the developing cortex results in similar mislamination, or whether currently existing examples of PTEN-associated migration defects are similarly dependent on cap-dependent protein translation.

Morphological Elaboration

Animal models of PTEN dysfunction demonstrate a role for PTEN in axon growth and guidance. Mice heterozygous for PTEN have an enlarged corpus callosum, and axons arising from cortical neurons are overgrown [84, 86]. The mossy fibers of PTEN knockout dentate gyrus granule neurons are overgrown and frequently have ectopic branches that project back to the dentate gyrus [79, 89]. Loss of tuberous sclerosis complex (TSC) results in a similar phenotype of axon overgrowth and ectopic projections [90, 91]. Treatment with rapamycin prevents the overgrowth of PTEN-null mossy fiber axons [92], implicating hyperactive mTOR signaling as a driver of axon outgrowth. However, by dynamically modulating PIP_3 at the growth cone, PTEN also has a direct mechanistic role in axon growth, branching, and guidance. Axon growth requires accumulation of PIP_3 at the leading edge of the growth cone. To maintain this pool of PIP_3, PTEN is sequestered away from the membrane [54]. Conversely, the presence of catalytically active PTEN at the growth cone prevents neurite outgrowth. When CK2, an inhibitory PTEN kinase, is prevented from phosphorylating PTEN, the axons of cultured hippocampal neurons fail to grow in response to nerve growth factor [56]. Likewise, the sites at which axonal filopodia sprout are predicted by PI3K-mediated transient increases in PIP_3 levels [93], allowing PTEN within the neurite to suppress the formation of new branches by controlling local PIP_3 levels. Finally, chemorepellent cues mediate growth cone collapse by recruiting active PTEN to the leading edge of the growth cone [54, 94]. In cultured chick dorsal root ganglion neurons, chemorepellent Sema3A activation causes PTEN to translocate from microtubules at the center of a neurite to the growth cone membrane, decreasing local PIP_3 levels and causing the growth cone to collapse [54]. Similarly, in hippocampal primary cultures, growth cone collapse mediated by Sema4D requires active PTEN. In this process, Sema4D binding to Plexin-B1 to signals through R-Ras GAP to dephosphorylate PTEN, allowing it to enter its active "open" conformation. Additionally, Plexin-B1 signaling also suppresses the activity of the inhibitory PTEN kinase CK2α to prevent PTEN inactivation [55]. Therefore, in addition to driving neurite outgrowth through mTOR activation, PTEN at axonal growth cones plays a direct mechanistic role in regulating outgrowth, branching, and collapse.

The somata and dendrites of PTEN mutant or knockout neurons are enlarged. Neuron-specific conditional knockout mice have enlarged neuronal cell bodies and dendrites in the cerebellum, cortex, and hippocampus, sufficient to cause macrocephaly without abnormally increased numbers of neurons [75, 76, 78]. In various transgenic and virally driven knockout models, neuronal somata are universally enlarged. This includes cerebellar Purkinje neurons [82], granule neurons of the dentate gyrus [95–97], pyramidal neurons in the auditory [98] and motor [99] cortices, and midbrain dopaminergic neurons [100]. Enlarged cell bodies and arbors are also seen in both GABAergic and glutamatergic neurons in vitro [101]. PTEN knockout in dentate gyrus granule neurons increases the number of primary dendrites, dendrites that arise directly from the soma [96], and it decreases the degree of self-avoidance within the dendritic arbor [97], affecting both the sampling of afferent input and the postsynaptic processing of that input.

Somatic overgrowth is gene dose-dependent. Viral shRNA-mediated PTEN knockdown in mature dentate gyrus granule neurons causes somatic hypertrophy [95, 102] to a lesser degree than complete knockout in newborn neurons [103]. In the cortices of PTEN haploinsufficient mice, somatic overgrowth is restricted to neuronal populations with higher endogenous levels of phosphorylated S6, such as layer 5 pyramidal neurons, indicating that populations with higher activity in downstream signaling cascades may be more susceptible to PTEN loss [84]. In later development, the effects of PTEN differ both by cell type and by cellular compartment. Dendritic overgrowth in the cortex of CamKIIα-$Cre^{+/-}$ × $Pten^{flx/flx}$ mice, in which PTEN is lost in forebrain excitatory neurons after several weeks of normal development, is restricted to the apical dendrites of layer 2/3 pyramidal neurons, with no effects on basal dendrites or to layer 5 pyramidal neurons [104].

Neuronal cell bodies in the *m3m4* mouse, which carries germline mutations reducing the ability of PTEN to associate with the nucleus and the cell membrane, are also hypertrophic [74], indicating that appropriate subcellular localization of PTEN is critical for its regulation of cell growth. Several ASD-associated point mutations in the C2 domain of PTEN decrease the amount of PTEN present in the nucleus; increasing the nuclear localization of 2 of these mutants (D252G and W274L), by adding a canonical nuclear localization sequence (NLS) to the protein, partially rescues the increase in soma size [43]. Overexpression of Y128L-NLS mutant PTEN, which has no protein phosphatase activity but retains some lipid phosphatase activity, partially rescues somatic hypertrophy in PTEN knockout neurons, while G129E-NLS, which is lipid-phosphatase dead but protein-phosphatase capable,

does not [43]. Thus, the nuclear lipid phosphatase activity by PTEN is important for regulating cell growth.

Mechanistically, both somatic and dendritic overgrowth of PTEN knockout neurons are likely to result from increased cap-dependent protein translation downstream of hyperactive mTORC1. A mechanistic evaluation of dendritic branching in cultured hippocampal neurons revealed that like PTEN knockdown, overexpressed or constitutively active PI3K, AKT, or Ras induce mTORC1-dependent dendritic overgrowth. Meanwhile, dendritic growth is reduced by knockdown of p70S6K or overexpression of 4EBP1 [105]. Somatic and dendritic hypertrophy of PTEN knockout neurons is rescued with rapamycin in vivo [87, 92, 104]. Overexpression of constitutively active Rheb in L2/3 pyramidal neurons results in somatic and dendritic hypertrophy similar to PTEN knockout; both of these phenotypes are rescued by coexpression of constitutively active 4EBP1 [88]. Collectively, these results suggest that PTEN dysfunction drives somatic and dendritic overgrowth by increasing cap-dependent protein translation downstream of mTORC1.

Synaptic Connectivity

PTEN regulates both the density and strength of glutamatergic synapses. A number of experimental models, including transgenic mice, and a variety of viral gene manipulation schemes have been used to examine the dendritic spine densities of neurons after PTEN loss. Remarkably, the various models show a wide variety of phenotypes, with some showing a robust increase, and others showing no change at all. Among transgenic mouse models, GFAP-Cre × *Pten*$^{flx/flx}$ and NSE-Cre × *Pten*$^{flx/flx}$ strains both have increased dendritic spine density in the cortex and hippocampus [77, 78]. In these models, PTEN is lost in about half the cortical and hippocampal neurons, and in nearly all granule neurons of the cerebellum and dentate gyrus. In the GFAP-driven line, this occurs prenatally, while the NSE-Cre driver line causes PTEN loss within the first 2 weeks after birth. A number of viral strategies to knock out or knock down PTEN have similarly resulted in increased dendritic spine density of dentate gyrus granule neurons, including lentiviral shRNA knockdown [95], retroviral Cre expression in a *Pten*$^{flx/flx}$ mouse [96], and retroviral CRISPR-Cas9-mediated knockout [103]. An AAV Cre-lox system likewise increases dendritic spine density on neurons in the auditory cortex [98]. In all of these models, increased dendritic spine density was associated with increased excitatory synaptic drive. Both strains of transgenic mice suffer from seizures [75, 79], and granule neurons and cortical neurons have both an increased frequency of spontaneous excitatory synaptic events and an increased amplitude of evoked events [96, 98]. These experiments convincingly demonstrate that, under the right conditions, PTEN loss can dramatically increase excitatory synapse formation on neurons, rendering them hyperexcitable.

Paradoxically, not all models demonstrate the same increase in excitatory synapse formation. CamKIIα-Cre × *Pten*$^{flx/flx}$ mice do not have increased numbers of dendritic spines in the cortex or in the CA regions of the hippocampus [104, 106]. Notably, the visual cortical pyramidal neurons in these mice are hypoexcitable with reduced responses to visual stimuli in vivo [107]. Like the GFAP- and NSE-Cre driver lines, these mice progressively lose PTEN expression in increasing numbers of cortical neurons with age, leading to mosaic knockout and affecting a majority of principal neurons in the cortex and hippocampus. The primary difference is that in the CamKIIα-Cre line, Cre expression comes on much later, starting at two weeks and affecting some neurons for the first time as late as 8 weeks of age. Therefore, the CamKIIα-Cre × *Pten*$^{flx/flx}$ mice first lose PTEN expression in circuits that are much more mature than in the other model systems. This suggests that with widespread PTEN loss in the brain, the effect on synapses depends on processes that occurred earlier in development.

A number of model systems rely on sparse viral infection to allow visualization using virally driven fluorophore expression. We recently showed that increased dendritic spine density after post-synaptic PTEN knockout in dentate granule neurons depends on the ability of those dendritic spines to find suitable presynaptic contacts, meaning that postsynaptic spine density cannot increase beyond the number of available boutons [108]. With sparse virally mediated knockout, we speculate the observed increase in dendritic spine density is primarily due to the ability of the PTEN knockout neurons to outcompete neighboring wild-type neurons for a limited pool of presynaptic terminals. One study that used cell-filling with a patch pipette to visualize knockout neurons in brain slices with dense AAV-mediated PTEN knockout found no change in the dendritic spine density of PTEN knockout neurons. Instead, the only change to the dendritic spines was in their morphology, which was enlarged and more mushroom-like [109]. This study was also performed in adult mice; later onset of PTEN loss could occur after critical periods for arborization have

closed, reducing the genesis or stabilization of new dendritic spines by preventing increases to the number of boutons. Since neurobiological deficits that emerge after developmental critical periods are more likely to be reversible after maturation, a close evaluation of differences between phenotypes in early- and late-onset conditional knockouts may inform which phenotypes might be more amenable to therapeutic interventions later in life.

The increased dendritic spine densities of PTEN knockout neurons are a source of increased synaptic connectivity and excitatory drive. PTEN knockout increases the frequency and the amplitude of excitatory synaptic events [95–98], even when no increase in spine density is observed [109]. Several aspects of the morphology and physiology of PTEN knockout neurons can influence their excitability, including the number of dendritic synapses, synaptic strength, the altered arrangement of branches in the dendritic arbor, and passive membrane properties [96]. The recruitment of presynaptic input appears to be unbiased, with granule neurons and cortical pyramidal neurons recruiting similar amounts of increased input from multiple distinct afferents [98, 108]. This increased recruitment of excitatory input is not counterbalanced by an increase in the formation of inhibitory synapses [95–97]. The principles by which PTEN governs the excitability of glutamatergic neurons likely apply to other cell types as well. For example, PTEN knockout in MGE-derived interneurons increased the frequency of inhibitory postsynaptic currents on cortical pyramidal neurons [83], suggesting that PTEN loss in GABAergic neurons makes them hyperactive as well. However, the seemingly linear relationship between PTEN function, recruitment of excitatory synaptic input, and hyperexcitability is complicated by the diversity of neurons within the brain. Like other neurons, PTEN knockout in cerebellar Purkinje neurons causes them to recruit increased excitatory input. Contrary to expectations, this increased excitation is insufficient to override other physiological changes (i.e., decreased input resistance) that render the neurons hypoactive. The end result of PTEN knockout in these neurons is a decrease in the tonic firing rate [82]. Therefore, a thorough analysis of ASD-relevant circuits in the PTEN knockout or heterozygous brain may be required to uncover cell-type- and circuit-specific effects of PTEN loss.

The density of dendritic spines is determined by balancing the rates of synapse formation and elimination. Similar to axon growth cones, the formation and motility of dendritic filopodia are driven by PIP_3 accumulation at the tip [110], suggesting that PTEN could inhibit inappropriate spine formation in the wild-type brain by translocating to spine tips to prevent extension or promote collapse, as in axonal filopodia. A large portion of the excess dendritic protrusions on PTEN knockout neurons have a filopodial morphology, suggesting an increase in de novo spine formation [96]. However, in an analysis of the mechanisms of spine density regulation by PTEN, dendritic spine density was regulated by the protein phosphatase function of PTEN but not its lipid phosphatase function, since the expression of the G129E mutant protein, which has protein phosphatase activity but lacks lipid phosphatase activity, was able to rescue dendritic spine density in PTEN-null neurons, while the Y138L point mutant protein, which is selectively protein-phosphatase dead, was not [111]. Results from models of TSC also support an mTOR-independent role for PTEN in the regulation of dendritic spine density, since the loss of TSC proteins does not increase excitatory synaptogenesis [101, 112, 113]. In contrast, hyperactive mTOR could be the driving force behind any deficit in dendritic spine elimination, since dysfunctional autophagy due to mTOR hyperactivation has been implicated in increased spine density due to a lack of autophagy-dependent synapse pruning [114]. A more nuanced analysis is required to determine both the relative contributions of spine formation and spine elimination to the increased connectivity that occurs with PTEN loss, and also which catalytic activities of PTEN are most relevant to each process. This will in turn inform which mechanisms make the most promising therapeutic targets.

Compared to its postsynaptic effects, the effects of PTEN on the presynapse have received less attention. Axonal arbors of PTEN knockout neurons are larger, and in some circuits the frequency of en passant boutons along the axon is increased [84, 89], meaning the total number of synapses in the PTEN deficient brain is likely to be increased. The effects of PTEN loss on synaptic function (similar to that on dendritic spine density, may be age-dependent. In the hippocampi of GFAP-Cre×*Pten*$^{flx/flx}$ mice, basal transmission is decreased [77], while in the NSE-Cre×*Pten*$^{flx/flx}$ mouse it is temporarily increased during a window in early adulthood [115]. In autaptic cultures, PTEN loss causes an increase in the size of the readily releasable pool in both GABAergic and glutamatergic neurons. However, the spontaneous release rate is decreased in this system, while the paired-pulse ratio is increased, indicating a decrease in the efficiency of vesicle fusion [116]. Although treatment with rapamycin rescued the increase in the number of presynaptic vesicles, TSC1 knockout neurons in the same model system do not have

increased vesicular pool size or altered release rates [101]. Therefore, the changes to the vesicular pool and synaptic release may require the coactivation of mTOR and one or more other signaling pathways downstream of PTEN.

Conclusion

PTEN is an upstream regulator of a signaling pathway in which many proteins are implicated in ASD, making it an especially interesting target of study in ASD etiology. The functions of PTEN in brain development are diverse. PTEN and the signal cascades it regulates influence brain development during cell proliferation and differentiation, migration, neurite outgrowth, synaptogenesis, and myelination. It is not an understatement to say that PTEN influences every stage of neurodevelopment, including plasticity in the mature brain. At this point, it is unclear which, or what combination, of this constellation of cellular changes forms the neurobiological basis for the symptoms of ASD. As our understanding of the cellular roles and mechanisms of PTEN improves, so too will our understanding of the etiology of its associated neurodevelopmental disorders, including ASD, epilepsy, and cognitive impairment, potentially leading to the development of new diagnostic and therapeutic tools.

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Author Contributions

P.D.S. wrote the manuscript, R.V.S. edited the manuscript, and B.W.L. conceived and edited the manuscript.

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