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PTEN: A MOLECULAR TARGET FOR NEURODEGENERATIVE DISORDERS

Abstract

PTEN (phosphatase and tensin homologue deleted in chromosome 10) was first identified as a candidate tumour suppressor gene located on chromosome 10q23. It is considered as one of the most frequently mutated genes in human malignancies. Emerging evidence shows that the biological function of *PTEN* extends beyond its tumour suppressor activity. In the central nervous system *PTEN* is a crucial regulator of neuronal development, neuronal survival, axonal regeneration and synaptic plasticity. Furthermore, *PTEN* has been linked to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Recently increased attention has been focused on *PTEN* as a potential target for the treatment of brain injury and neurodegeneration. In this review we discuss the essential functions of *PTEN* in the central nervous system and its involvement in neurodegeneration.

Keywords

• AKT • Alzheimer's disease • Amyotrophic lateral sclerosis • Apoptosis • Axon • Development • Neuron • Parkinson's disease • PTEN • regeneration

Introduction

For many years the observation of loss of heterozygosity of chromosome 10 in human cancers, particularly glioblastoma, pointed to the existence of a tumour suppressor gene in chromosome 10. It was not until 1997, when three independent groups reported the first discovery of a candidate tumour suppressor gene, PTEN (*p*hosphatase and *t*ensin homologue deleted in chromosome 10) [1] /MMAC1 (*m*utated in *m*ultiple *a*dvanced *c*ancers-1) [2] or TEP1(TGF b regulated and epithelial cell enriched phosphatase 1) [3] which was mapped to chromosome 10q23. Somatic mutations of PTEN were detected in a variety of human cancers including glioblastoma, advanced prostate and endometrial cancers, thus it is now considered as one of the most frequently mutated genes in human malignancies. Germline mutations were also detected in almost 80% of patients suffering from the autosomal dominant diseases such as Cowden disease (CD) [4], Lhermitte Duclos disease (LDD) [5], and Bannayan-Zonana syndrome (BZS) [6]. These syndromes share characteristic developmental defects, benign tumours and increased susceptibility to thyroid and breast malignancies.

The *PTEN* gene consists of nine exons and encodes a 5.5kb mRNA [2]. It encodes a protein consisting of 403 amino acids with a relative molecular weight of 47 kDa [2]. Analysis of PTEN's crystal structure revealed that it consists of three main domains [7]. The amino terminal end (N –terminal), comprising 190 amino acids, carries the signature motif HCXXGXXR, which defines the family of protein tyrosine phosphatases (PTP) and dual specificity protein phosphatases (DSP). Within this motif, the Cys-124 and Arg-130 are essential for the catalytic function of the protein [7]. Most of the mutations are found to affect the N-terminal (Figure 1). Sequence analysis revealed that the catalytic amino terminal end bears homology to chicken tensin [2], an actin binding protein found in focal adhesions, and bovine auxillin [2], a protein which disassembles clathirin-coated vesicles. The evidence suggests that the N-terminal has additional roles to that of a phosphatase. The C-terminal domain (carboxyl terminal) consists of a C2 domain, which mainly functions in the binding of PTEN to the phospholipids in the plasma membrane [7]. The C-terminal domain also consists of a PDZ-binding motif which is responsible for protein-protein interactions [8] and two PEST sequences [9]. A known role for PEST sequences is to target proteins for proteolytic degradation. In contrast, studies have shown that PEST sequences play a role in the stability of the PTEN protein [9,10]. Finally, the tail of PTEN consists of 50 amino acids which is also essential for PTEN stability [7].

The discovery of PTEN in 1997 stimulated several research groups to identify its substrates. The finding by Mahaema and Dixon, that PTEN is a phosphatidylinositol phosphatase, was a major step towards the understanding of its function [11]. PTEN dephosphorylates specifically at position 3 of the inositol ring. PTEN opposes phosphatidylinositol 3 kinase (PI3K) and thus negatively regulates the pathway [11]. The PI3K/AKT signalling pathway has been widely reviewed (see review [12]). Briefly, binding of growth factors to receptor tyrosine kinases causes activation of PI3K which catalyses the phosphorylation of phosphatidyl-

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inositol 2 (PIP2) to generate the production of phosphatidyl-inositol 3 (PIP3) [13]. Generation of PIP3 results in the recruitment of the cytosolic protein, AKT, also known as PKB (protein kinase B), to the plasma membrane where it is activated and phosphorylated by phosphoinositide-dependent kinase-1 (PDK1) [14]. AKT is an essential mediator of several signalling pathways including cell survival, glucose metabolism and protein translation [15]. It exists in three isoforms: AKT1, AKT2 and AKT3 [15,16]. AKT3 is most abundant in the brain and testis [16]. AKT exerts its role as a cell survival and proliferation mediator by phosphorylating various downstream signals including: bcl2 antagonist of cell death (BAD) [1], glycogen synthase kinase 3-β (GSK3-β) [17], forkhead transcription factors (FHTR) [18], caspase-9 [19], nuclear factor- kappaB (NF- κB) [20] and mammalian-target-of rapamycin (mTOR) [21]. Of these targets, BAD is one of the most important molecules in regulating cell survival. PTEN antagonises AKT and inhibits cell survival and proliferation (Figure 2).

The mTOR protein is another essential downstream target of PTEN. It is a serine-threonine kinase which consists of two distinct complexes: mTOR complex 1(mTORC1) and mTOR complex 2 (mTORC2) [22]. mTOR regulates key cellular processes such as protein synthesis [22], autophagy [23], mitochondrial metabolism and biogenesis [22], which play an essential role in maintaining the integrity of postmitotic neurons (for a detailed description of mTOR signalling see review [22]). Defects in autophagy have been linked to neurodegeneration in conditions such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS)

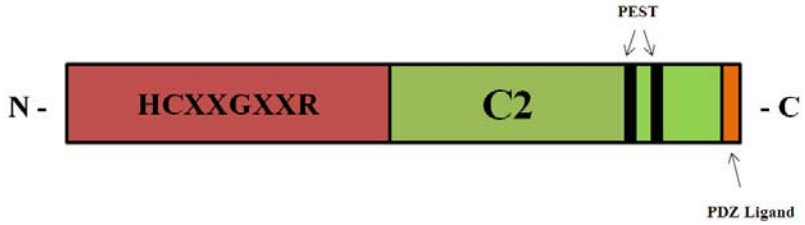

Figure 1. PTEN domains. Schematic diagram illustrating the structure of PTEN. The N-terminal domain contains the signature motif ***HCXXGXXR*** of protein tyrosine and dual-specificity phosphatases. The C2 domain facilitates the binding of PTEN to phospholipids in the plasma membrane. The PDZ binding motif is responsible for protein-protein interactions. The PEST sequences and PTEN tail are essential for PTEN's stability

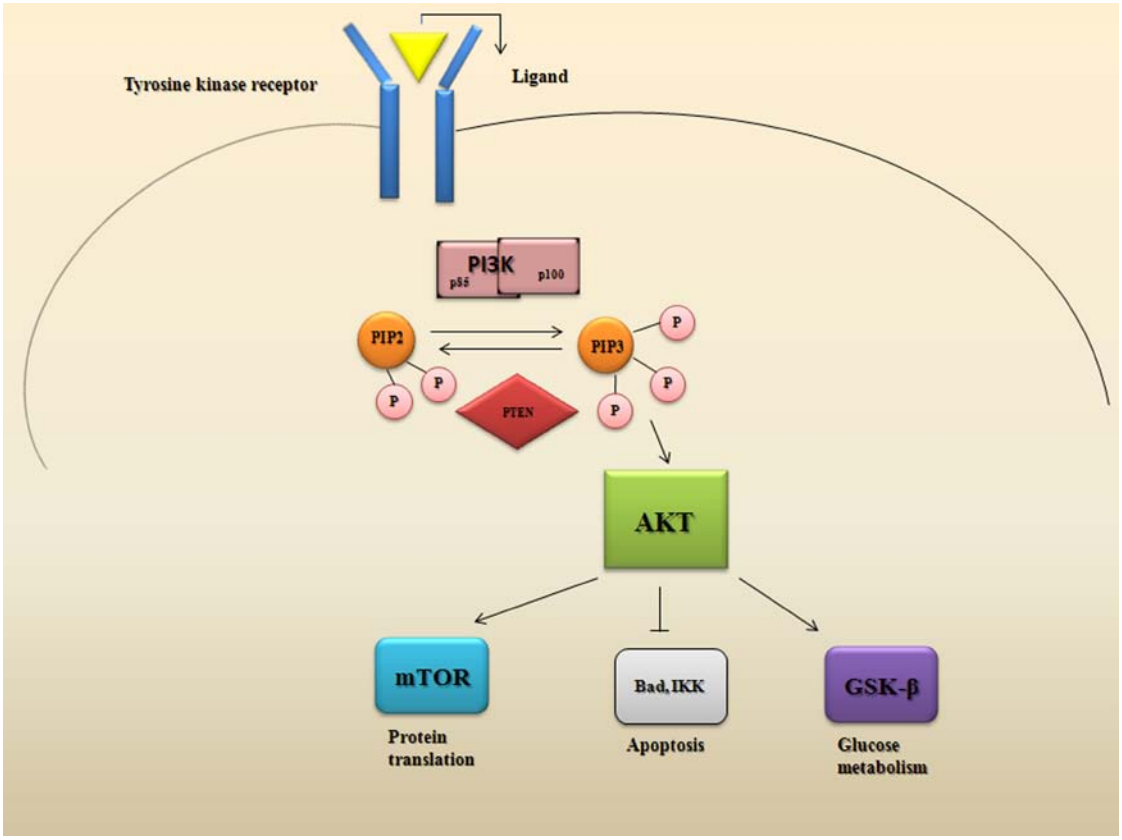

Figure 2. PTEN/PI3K/AKT pathway. The binding of ligands to tyrosine kinase receptors activates PI3K which catalyses the generation of phosphatidyl-inositol-3 (PIP3) from phosphatidyl-inositol-2 (PIP2). The production of PIP3 leads to the translocation of AKT from the cytoplasm to the cell membrane where it becomes phosphorylated and activated at Ser473 and THr308. Activated AKT phosphorylates several cytoplasmic substrates and regulates protein translation, glucose metabolism and inhibits apoptosis. PTEN dephosphorylates PIP3 and acts as a main negative regulator of the PI3K/AKT pathway.

[23]. Of interest, rapamycin, a selective inhibitor of mTORC1, was shown to be neuroprotective in experimental models of ALS [24], PD [25] and AD [26]. However in such chronic conditions the long term use of rapamycin may lead to inhibition of mTORC2 which can result in the inhibition of AKT and ultimately impair neuronal survival. In addition, the constitutive activation of autophagy could have detrimental effects on neuronal cells. Therefore further work is needed to investigate the long term safety of targeting mTOR in neurodegenerative diseases.

Although most of the evidence describes the role of PTEN in the PI3K pathway, recent progress has been made in understanding the association of PTEN with other signalling pathways. PTEN has been shown to inhibit cell migration by dephosphorylating focal adhesion kinase [27,28]. Ning and colleagues have previously demonstrated that PTEN is involved in the modulation of leptin and insulin signalling related to obesity [29]. They also showed that GSK-3β regulates PTEN by phosphorylation at position 366 priming site as a novel feedback mechanism [30]. In addition several studies have shown that PTEN negatively regulates the MAPK pathway, another important pathway in cell survival and proliferation [31,32].

Initially, much of the research focused on PTEN's role in the molecular pathogenesis of glioblastoma. This has unravelled a broader role for PTEN in the nervous system. In this review we will discuss the crucial functions of PTEN in the central nervous system (CNS) and the role of PTEN in neurodegenerative disorders.

PTEN in neuronal development

PTEN is widely expressed in the CNS where it localises to the cytoplasm and nucleus [33,34]. In *Drosophila melanogaster*, studies have demonstrated that PTEN regulates cell size, survival and proliferation during the development of the eye and wing [35,36]. To study the biological functions of PTEN during normal development in the mammalian system, PTEN knockout mice were generated by three independent groups [28,37,38]. Di Cristofano et al generated transgenic mice harbouring a loss of function mutation targeting exon 4 and 5 of the mouse PTEN gene [37] . The mice did not survive beyond 7.5 embryonic days. Suzuki's group targeted exon 3-5, and the mutant mice died by 9.5 embryonic days. The mice displayed enlarged caudal and cephalic regions [38]. In addition, Podyspanina and colleagues deleted exon 5 of the PTEN gene and their mutant mice died by 6.5 embryonic days [28]. Thus targeted deletion of the murine PTEN gene resulted in embryonic lethality, demonstrating that PTEN is essential for early embryogenesis. The variation seen in the life span of embryos could be due to the differences in genetic background and/or different techniques used to target PTEN. Of interest, none of the groups detected tumours in the embryos of the mutant mice. This could be due to the fact that the mice did not live long enough to develop tumours or may indicate that other genetic factors are required for tumorogeneis during early life.

To further study the tumour susceptibility associated with PTEN mutations, the three groups generated heterozygous mice of which the phenotype varied between the mouse models. The PTEN heterozygous mice (PTEN $^{+/-}$) were viable at birth but had a high rate of cancers of varying histological origin and autoimmune disorders due to lymphoid hyperplasia [28,37,38]. The differences in the techniques used to target PTEN could be associated with other molecular events that could account for the heterogeneity of the tumours observed in the mouse models. The regulation of apoptosis by the sex hormones (17 β-estradiol and testosterone) [39] may account for the phenotype of PTEN$^{+/-}$ mice observed by Podsypanina et al, which was strongly influenced by the sex of the mouse. These striking features should be taken into consideration where targeting PTEN is considered as a therapeutic target for neurodegenerative diseases. This may imply that females could be at a high risk of developing cancers. These mouse models emphasised the fact that PTEN is a crucial tumour suppressor gene with a key role in the regulation of proliferation.

Although PTEN mutations were frequently detected in advanced glioblastomas, none of the mutant mice developed neurological abnormalities or brain tumours. Hence the knock-out mice did not give an insight to the role of PTEN in the central nervous system or to its role in brain tumorogeneis.

To further explore the biological functions of PTEN during brain development, several research groups generated conditional PTEN knockout mice using the cre-lox technology. For example, PTEN $^{loxp-/-}$ mice were crossed with mice harbouring a nestin-promoter driven Cre transgene, which is mainly expressed in neuronal precursors by midgestation [40]. This strategy achieved almost complete deletion of PTEN in the CNS during early development. Mice were born with open eyes, enlarged brains and died shortly after birth. There was disorganised lamination of the brain structure and an apparent increase in cell size and proliferation of the neural progenitor cells. *In vitro* cultures of neurospheres from mutant mice confirmed the *in vivo* findings. Of note, deletion of PTEN in early gestation did not affect the differentiation of neural precursors in agreement with previous studies [33]. This model demonstrated that PTEN negatively regulates neuronal cell size and proliferation. Others studies have demonstrated that PTEN also regulates the migration and proliferation of stem cells in the adult mouse subventricular zone (SVZ) [41].

In Marino's et al mouse models, specific deletion of PTEN in the cerebellum during early gestation also led to a striking increase in cell size and disorganisation of the cerebellum lamination [42]. In line with previous studies, PTEN deficiency did not alter the capacity of the neuronal progenitors to differentiate. Surprisingly, the mutant mice showed a reduction in proliferation of cerebellar cells, unlike the finding of Groszer et al, where an increased proliferation of progenitor cells was confirmed by *in vitro* studies. This suggests that cerebellar precursors behave in a different manner to PTEN depletion when compared to other neuronal precursors. Another striking feature was the progressive loss and degeneration of Purkinje cells detected during early postnatal development. Specific deletion of PTEN in Purkinje cells showed that these features are likely to be cell autonomous. This suggests a possible role for PTEN and its downstream targets in

the neurodegeneration of specific postmitotic neurons. Although several studies have shown that AKT promotes neuronal survival, it is possible that postmitotic neurons such as Purkinje fibres, respond differently to the continuous activation of the PI3K pathway. Therefore further studies are required to identify the long term effects of PTEN down regulation on post-mitotic neurons.

Finally, Backman [43] and Kwon [44] groups generated transgenic mice where the expression of Cre was controlled by the glial fibrillary acidic protein (GFAP) promoter. Although targeted deletion of PTEN was expected to occur in the glial compartment, a selective deletion of PTEN was achieved in neuronal cells of the cerebellum and hippocampus during postnatal development, with no apparent PTEN deletion in stem cells. The mutant mice appeared healthy at birth, but eventually developed progressive macrocephaly, ataxia, tremors, seizures and sudden lethargy and died by 29-48 weeks [43,44]. The strikingly increased soma size of the neuronal cells accounted for the significantly enlarged brains of mutant mice. In agreement to Marino et al findings, there was no evidence of ongoing proliferation of neuronal cells or development of brain tumours. Both groups have shown activation of the PI3K pathway with increased AKT expression in PTEN null neurons, indicating that PTEN exerts its effects by negatively regulating this pathway [43,44]. The neurological features of these transgenic mice mimicked the characteristic features of LDD and thus provided an excellent model to further study the molecular pathogenesis of the disease.

We can conclude that PTEN plays an essential role in the regulation of neuronal cell size during early and late brain development. PTEN also regulates the proliferation and migration of neuronal precursors. These studies may have implications for the treatment of brain injuries. Deletion of PTEN following brain injury may decrease apoptosis and promote the migration of stem cells to facilitate brain repair.

PTEN and neuronal apoptosis

The lack of apoptosis in brain tumours associated with PTEN mutations raised an increasing interest in a possible role for PTEN in neuronal apoptosis. *In vitro* models of postmitotic neuronal cell death have shown that PTEN expression is altered in response to various apoptotic inducers [45]. For example, in primary cultured cerebellar granules serum withdrawal with potassium deprivation reduced the expression of PTEN mRNA [45]. While okadeic acid induces an initial increase in PTEN mRNA, this was followed by a reduction in PTEN expression [45]. Similar effects of serum withdrawal were also seen in cultured Neuroblastoma-2a cells [45]. Thus it seems that the reduction in PTEN expression is a compensatory mechanism by which postmitotic neuronal cells activate the PI3K/AKT pathway to enhance their own survival. In accordance with this, in the motor neuron cell line (VSC4.1), exposure to interferon-gamma was associated with increased PTEN expression and apoptotic cell death, reduced AKT, increased caspase 3 activity and BAX: Bcl ratio. Treatment of motor neuron cells with oestrogens inhibited PTEN and prolonged their survival [46]. Increased expression of PTEN and inhibition of the AKT pathway was observed in experimental models of chronic exposure to alcohol during gestation [47]. In this model the mice showed marked cerebellar hypoplasia and cell death indicating a correlation of PTEN with neuronal death [47]. In primary hippocampal neurons, over expression of wild type PTEN, decreased AKT expression and increased glutamate induced cell death [48]. On the other hand, over expression of mutant PTEN, increased AKT levels and significantly increased neuronal survival [48]. In addition $PTEN^{+/-}$ mice showed increased resistance to death induced by glutamate excitotoxicity [48]. *In vitro*, perioxynitrate mediates it neuroprotective effects by down regulating PTEN [49]. In line with these findings, it has been shown that hypothermia preserved the levels of phosphorylated PTEN, and attenuated the decrease in active p-AKT, leading to neuroprotection against ischemic injury [50]. Moreover there is accumulating evidence suggesting that downregulation of PTEN is neuroprotective in ischemic brain injury [51].

Although these studies clearly demonstrate the involvement of PTEN with neuronal apoptosis, the molecular mechanisms mediating these effects still remains under investigation. It was observed that in rat hippocampal neurons, PTEN physically associates with NR1 and NR2 subunits of N-methyl-D-aspartate (NMDAR), a glutamate receptor which is implicated in excitotoxicity induced neuronal death [52]. In models of brain ischemia, PTEN downregulation leads to a diminished extrasynaptic expression of NMDARs in addition to activating the AKT pathway [52]. This latest study elegantly demonstrated the dual protective role of the protein and lipid phosphatase activities of PTEN [52]. Recent studies revealed that PTEN deletion preserves the expression of GABA receptors, which are suppressed in brain ischemia [53]. Together these data provide evidence for the multiple functions of PTEN and emphasise its role in brain ischemia.

An intriguing question is how PTEN reaches its main targets, which are localised to the cell membrane. Studies in primary mouse cortical neurons, demonstrated that lactacytin induced neuronal apoptosis was associated with the cleavage of PTEN to a truncated form (50 kDa) which was found to accumulate in the insoluble fractions of the membranes [54,55]. These findings indicate that during neuronal apoptosis PTEN was translocated to the cell membrane where its targets are localised, and mediates its inhibitory effects on cell survival. Further studies revealed that lactacytin was associated with caspase 3 activation which induced the cleavage of PTEN to its truncated form [54]. Moreover, apoptosis induced in rat hippocampal cultures using staurosporine (STS) causes release of cytochrome c and activates caspase 3 and release of reactive oxygen species (ROS) [56]. In the latest study, PTEN was found to directly associate with the pro-apoptotic protein, BAX, in the cytoplasm and translocates with BAX to mitochondria during apoptosis. PTEN knockdown has reduced apoptosis induced by STS and release of ROS [56]. ROS are well known molecules implicated in the pathogenesis of neurodegenerative disorders such as ALS and PD. Further studies using different models of neurotoxicity, demonstrated that PTEN is a common regulator for ROS in pathological conditions [57]. PTEN knockdown reduced

the production of ROS independent of the antioxidant enzyme superoxide dismutase 2 (SOD2) [57].

It is clearly evident that PTEN is an important mediator of mitochondrial induced neuronal apoptosis. Since apoptosis and mitochondrial dysfunction are characteristic features in neurodegenerative disorders, down regulation of PTEN seems to be an attractive tool to prevent apoptotic death and delay the progression of neurodegenerative disorders.

PTEN and axonal regeneration

For many years numerous efforts to identify strategies to promote axonal regeneration has been a major challenge and with very limited success. The evidence suggests that inhibition of the mTOR pathway and hence decreased protein synthesis is a critical barrier for CNS regeneration. Activation of mTOR via PTEN deletion has been shown to promote axon regeneration of normal and diseased neurons. Park and colleagues utilised the optic nerve crush model to study axonal regeneration in mutant mice with conditional deletion of various growth control genes [58]. Among a variety of genes that were tested, mutant mice with PTEN deletion showed a significant increase in the survival of axotomised retinal ganglion cells (RGC) and a robust axonal regeneration of the injured optic nerves [58]. The mTOR pathway was inhibited in mature neurons and was further suppressed in axotomised adult neurons [58]. Further studies demonstrated that PTEN deletion promoted the compensatory sprouting of un-injured cortico-spinal tract (CST) and led to robust regeneration of injured CST axons which extended beyond the lesion site [59]. These findings suggest that PTEN deletion prevents dying-back axonopathy. Hence PTEN deletion attenuated the injury-induced mTOR down regulation promoting axonal regeneration and could be utilised as a novel strategy to treat neurological disorders where dying back axonapthy is considered as a major cause for neurodegeneration.

PTEN modulates other factors such as GSK3-β which also enhances axonal regeneration. An important observation by Jiang and colleagues was that inhibition of PTEN increased the number of growing axons in differentiating hippocampal neurons [60]. Activation of AKT and inhibition of GSK3-β, played a critical role in axonal growth [60]. In agreement with these findings, the increase in axonal length of hippocampal neurons induced by nerve growth factor (NGF) is mediated by the activation of casein kinase II (CK2), which phosphorylates and inhibits PTEN [61].

Recent studies revealed that the concomitant deletion of PTEN and suppressor of cytokine signalling 3 (SOC3) enhances a sustained axonal regeneration with an ability of axons to extend over long distances [62]. Furthermore PTEN deletion combined with induced intraocular inflammation also promotes axonal regeneration [63]. Hence PTEN acts synergistically with other factors to promote axonal regeneration.

Our team has recently reported that PTEN protein is enriched in motor neuron growth cones [64]. The same study revealed that PTEN depletion in purified spinal motor neurons of wild type mice enhances axonal growth and resulted in an increase in the size of growth cones [64]. In line with previous studies, this correlated with activation of the mTOR pathway. We have replicated these findings in purified motor neuron cultures from transgenic mouse models of spinal muscular atrophy (SMA). In addition we observed that PTEN deletion restored the levels of β-actin in growth cones [64].

Several studies have shown that PTEN is involved with synaptic development and plasticity. One study demonstrated that a 34 kDa fragment of PTEN was localised to the mouse neuronal dendrites [65]. In the weaver mouse model, PTEN was absent in Purkinje dendrites where there is defective synaptogenesis [65]. Moreover transgenic mice with conditional deletion of PTEN in the brain had enlarged dendrites and axons with defective synaptic transmission indicating a role for PTEN in synaptic plasticity [66]. Furthermore, recent studies have demonstrated that conditional deletion of PTEN during early postnatal development in the mouse auditory cortex enhanced the outgrowth of dendrites and spines that was associated with increased synaptic activity [67].Therefore evidence from these studies indicate that PTEN and its downstream effectors could be attractive therapeutic targets for the treatment of CNS injuries and neurodegenerative disorders.

Alzheimer's disease (AD) and PTEN

Alzheimer's disease (AD) is a neurodegenerative disorder characterised by the accumulation of intracellular neurofibrillary tangles and β-amyloid plaques. The neurofibrillary tangles consist of abnormally hyperphosphorylated tau, which has been implicated in the pathogenesis of AD [68]. AD Patients develop progressive deterioration in memory and cognition. A raised interest in the role of PTEN in AD came from previous studies suggesting that AKT is a crucial mediator of neuronal survival against β-amyloid neurotoxicity in experimental models of AD [69,70]. In addition, emerging evidence shows that GSK plays a crucial role in the regulation of tau phosphorylation [71-73]. Since PTEN is a main upstream regulator of GSK and AKT, this has been an incentive for researchers to explore the role of PTEN in AD.

Early genetic studies have linked late-onset AD (LOAD) to a locus on chromosome 10 where the PTEN gene is located [74]. However, further gene association studies showed that there was no association between PTEN polymorphisms and LOAD [75]. Griffin et al was the first to report dysfunction of PTEN signalling in AD [76]. They studied human brain autopsies of patients with AD and compared them with non-affected controls. They demonstrated that in AD, there is a loss of cytosolic AKT associated with increased recruitment of AKT to the particulate fractions [76]. This lead to a significant increase in AKT activation, which was detected in the early stages of AD and correlated positively with the disease stage suggesting a possible link with disease progression [76]. In the temporal cortex, they detected significant reductions in PTEN levels and loss of nuclear PTEN which also correlated positively with disease severity. Recent work has shown that PTEN may be inactivated by S-nitrosylation in AD and this

may account for the loss of PTEN observed [77]. Furthermore, in AD brains, PTEN was found to accumulate in intracellular fibrillary tangles, and co-localises with tau and senile plaques [78]. In accordance with these data, *in vitro* studies showed that over expression of wild-type PTEN reduces tau phosphorylation and aggregation indicating that PTEN protects against tau induced neurodegeneration [56,79]. Other studies failed to replicate these findings, and in contrast, reductions in the phosphorylated PTEN (inactive form) was found in some AD brains [80].

It is evident that deregulation of PTEN may contribute to neurodegeneration in AD. However, it remains unclear whether the altered distribution and loss of PTEN in AD, associated with constitutive activation of the PI3K/AKT pathway is a defence mechanism against neuronal injury or an association with disease pathogenesis and progression. Further studies using larger cohorts of AD brains are needed to determine the exact role of PTEN and downstream effectors of the PI3K pathway in AD.

PTEN and motor neuron disorders

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterised by selective degeneration of motor neurons and ultimately leading to death of patients within 3-5 years. The breakthrough discovery of superoxide dismutase 1 (SOD1) mutations in familial forms of ALS led to the generation of experimental mouse models that broadened our understanding of this complex neurodegenerative disorder [81]. Motor neuron cell death is a characteristic feature of ALS [82]. Although the role of apoptosis in ALS remains controversial, there is increasing evidence suggesting disequilibrium between pro- and anti-apoptotic factors leading to motor neuron death [82]. The selective vulnerability of motor neurons and the possible involvement of apoptosis suggest neuron specific regulations of survival signalling pathways. To date there is no definitive treatment for ALS. Thus understanding the signalling pathways leading to motor neuron degeneration is pivotal to identify novel therapeutic targets.

Early genetic studies have shown that molecules of the PI3K/AKT pathway are unregulated in response to motor neuron injury [83,84]. Further attempts to explore the signalling pathways in motor neuron injury proved that AKT plays an important role in mTOR neuronal survival and regeneration [85]. Cultured motor neurons deprived of trophic factors showed reduced PI3K activity and reduced levels of active AKT [86]. Moreover, transgenic mice over expressing bcl-2, an anti-apoptotic protein, were resistant to motor neuron injury. Furthermore, several studies have shown that increased motor neuron survival in ALS in response to trophic factors such as VEGF [87-89], IGF-1 [90,91], GDNF [92] and ghrelin [93] was mediated by the activation of the PI3K/AKT pathway.

Considering the importance of the PI3K signalling pathway in motor neuron survival, it is not surprising that increased attention has been focused to study the role of this pathway in ALS. Observations from several studies in human post-mortem tissue of ALS patients hint to a possible deregulation of the PI3K/AKT pathway. For example, genetic studies in our lab have shown decreased expression of the PTEN gene in cervical motor neurons from ALS patients [94]. This was associated with a concomitant increased expression of AKT and Protein kinase C genes. These findings are in agreement with previous studies where increased levels of the PI3K protein was detected in the particulate fraction of spinal cords of ALS patients, when compared to unaffected controls [95]. Although increased total AKT and S6K were also detected in the particulate fractions of the spinal cord, paradoxically this did not correlate with an increased activity [95]. Further studies on human ALS post-mortem tissue have shown a significant loss of the phosphorylated AKT (active form) protein in motor neurons [96]. It is unclear whether the activation of the PI3K pathway contributes to neurodegeneration or is a neuroprotective response in ALS. Since studies in human post-mortem tissue of ALS patients demonstrate changes at the end-stage of the disease, we speculate that although the surviving motor neurons are capable of activating the PI3K/AKT pathway, this adative response fails to reach significant protective levels in such a chronic and progressive disease. This hypothesis appears to be supported by our studies which demonstrated that targeted deletion of PTEN in vitro models of ALS [94] and SMA [64] significantly enhances the survival of transgenic $SOD1^{G93A}$ and SMA motor neurons [64]. It is reasonable that attempts to activate this pathway or over express its anti-apoptotic components should be explored to identify new treatment strategies.

Loss of active AKT was also observed in pre-symptomatic transgenic models of ALS, which remained to the end stages of the disease [96]. In addition, contradictory to the data from human studies, reduced PI3K protein levels were detected in pre-symptomatic mouse models which may account for the selective vulnerability of motor neurons [97,98]. The discrepancy in these findings with the human studies could be attributed to differences in models whereby the disease in mouse models is rapidly progressive. An elegant study by Liu et al, demonstrated that in the spinal cord injury model, PTEN deletion delayed the dying back-axonopathy seen in injured CST [59]. PTEN depletion has also been shown to promote axonal regeneration in the optic nerve crush model. It is therefore worth investigating whether PTEN depletion will delay degeneration of motor neurons *in vivo* in experimental models of ALS.

PTEN and Parkinson's disease (PD)

Parkinson's disease (PD) is the commonest cause of movement disorders in the elderly [99]. The pathological hallmarks of the disease are the degeneration and dysfunction of dopaminergic neurons [99]. The majority of cases are sporadic and recent discoveries of genetic mutations have been found to account for some of the familial forms [100]. Accumulating evidence suggest a relationship between PD and PTEN. PTEN regulates the function of PTEN-induced putative kinase 1 (PINK1), a protein kinase localised to the mitochondria where it suppresses the release of cytochrome c. PINK1 is well known to be mutated in familial forms of PD [101]. Studies have shown that downstream effectors of PTEN are involved in the pathogenesis of PD. Several studies have

demonstrated defective AKT signalling in PD [102]. Indeed, a marked reduction in total AKT and active AKT was detected in PD post-mortem brains [25,103]. In experimental models of PD, 6-hydroxydopamine (6-OHDA) a widely used PD mimic, inhibits the phosphorylation of GSK3 and AKT, which correlates with dopaminergic neuronal death [104]. This indicates that components of the PI3K/AKT pathway mediate the neurotoxic effects of 6-OHDA [104]. On the other hand, in neurotoxin models of PD, overexpression of AKT lead to neuroprotection from neuronal loss and axonal degeneration in dopaminergic neurons [105]. PD is also characterised by aggregation of the α-synuclein protein [106,107]. However the β-synuclein protein was found to directly interact and activate AKT, resulting in neuroprotection against neurotoxins that target the striatonigral system [108]. In addition, the neuroprotective effects of NGF in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD were also mediated by AKT activation [109].

Loss of function mutations in DJ-1 and Parkin genes have been detected in some familial cases of PD [110]. Although the functions of DJ-1 remains unclear, it is a known PTEN regulator whereby it inhibits its activity [111]. Targeted deletion of DJ-1 in dopaminergic neurons of *Drosophila*, leads to neuronal death and dopaminergic dysfunction [112]. Deletion of DJ-1 is associated with the release of ROS and an increased sensitivity to oxidative stress and was paralleled by inhibition of the PI3K pathway. Overexpression of PI3K attenuated the phenotype produced by DJ-1 deletion [112]. Similarly, inhibition of parkin is also associated with a suppression of the PI3K pathway [112]. In cultures of human dopaminergic SH-SY5Y cells, PTEN deletion rescued the neurotoxicity induced by MPP+ and reduces the release of ROS.

To further explore the role of PTEN in PD, two groups generated transgenic mice with conditional deletion of PTEN in the dopaminergic system, and both models shared similar phenotypes [113]. In the first model, PTEN*loxP* mice were mated with Slc6a3Cr transgenic mice carrying the *cre* - recombinase transgene, driven by the dopamine transporter (DAT) promoter for specific deletion in dopaminergic neurons by embryonic day 15 [113]. At this stage, the dopaminergic neurons have fully differentiated. PTEN-null neurons exhibited an increased soma size and dendrite thickness as well as increased cell number. Moreover PTEN depletion was found to protect against 6-OHDA mediated dopaminergic neurotoxicity [113]. The second research group crossed PTEN*loxP* mice with transgenic mice with *tifla* deletion, a crucial ribosomal RNA transcription factor, leading to suppression of mTOR and a phenotype which mimic PD. The mice exhibited a similar phenotype to the first described model [114], and showed up regulation of mTOR and neuroprotection of dopaminergic neurons with preservation of their physiological functions.

Together these data, provide emerging evidence for a relationship between PTEN and PD, in particular with reference to mitochondrial dysfunction suggesting PTEN acting as a common mediator for ROS release in pathological conditions such as PD. Previous studies have shown that PTEN directly interacts with serotonin receptors in dopaminergic neurons, which modulate their response to addiction drugs [52,115]. Hence PTEN mediated its effects on cellular functions independent of the AKT pathway. The fact that PTEN deletion promotes neuronal survival and modulates mitochondrial function implies that it has a dual neuroprotective effect on the dopaminergic system, making it an attractive novel target for the treatment of PD.

Conclusions

Since PTEN was identified, several lines of evidence confirmed its biological function in the CNS. The high frequency of mutations associated with glioblastoma multiforme and the neurological disorders detected in patients with germline mutations of PTEN [4,5,116], suggested a crucial role for PTEN in the CNS. PTEN has been demonstrated to play a critical role in the regulation of neuronal development, axonal growth and regeneration, synaptic plasticity, neuronal survival and differentiation [117]. The functions of PTEN are mediated by the PI3K/mTOR [58] and MAPK [31,32] pathways.

In recent years increasing attention has been focused on PTEN as a potential novel target for the treatment of neurodegenerative disorders. Despite the exciting data that has been reported, there are important questions that are yet to be answered before it can be translated to an effective therapy in humans. The first question is: *what is the long term safety impact of PTEN depletion in the CNS?* The progressive course of neurodegenerative disorders would require the long term inhibition of PTEN, if it is to be considered as a potential therapeutic target. There are a number of considerations that need to be taken into account before this approach is translated to the clinic. Inhibition of PTEN activates the mTOR pathway which has been linked to defective autophagy in AD and PD. In fact rapamycin is currently investigated as a potential treatment for AD and PD and hence it may antagonise the effects of PTEN inhibition. Furthermore, studies have shown that different neuronal cells behave differently when PTEN is inhibited, indicating specific neuronal functions of PTEN. The observation of degeneration of Purkinje fibres of the cerebellum which resulted from PTEN deletion suggests that constitutive activation of the PI3K pathway is possibly detrimental to specific neurons on the long term. In addition the risk of malignancy that may occur as a result of PTEN deletion still needs to be confirmed. Hence in vivo studies looking into the long term effects of PTEN inhibition in the adult CNS are of paramount importance. A wise approach is to aim at targeted PTEN deletion in specific neuronal population of interest. For example, we have demonstrated that inhibition of PTEN in normal and diseased motor neurons resulted in a significant increase in survival and axonal regeneration, particularly in the SMA model [64]. We have also shown that viral vectors used to deliver shRNA were an effective tool for a controlled inhibition of PTEN. This holds a promising future for the treatment of motor neuron disorders such as ALS and SMA. However it raises another important question: *how could you specifically inhibit PTEN in a specific target neuronal population such as motor neurons or substantia nigra?*

Viral vectors are now becoming an attractive tool for the efficient delivery of shRNA and *cre–lox* systems for targeted gene deletion. The inability of the viral vectors to cross the blood-brain barrier is a major barrier for their use in neurological diseases. The current approaches used in animal models could not be directly transferred to human. Thus refining the delivery techniques for viral vectors may enhance the targeted deletion of PTEN. Finally, *does PTEN regulate signalling pathways other than the PI3K/mTOR and MAPK pathways?* It is important to investigate whether PTEN regulates other signalling pathways as this may broaden our understanding of its functions in CNS, and may help to identify new therapeutic targets in the future.

We conclude that PTEN has a critical role in the CNS. Evidence so far has shown that PTEN is an attractive target for the treatment of neurological disorders. Further studies are needed to determine its long-term safety and to identify efficient tools for targeted deletion of the PTEN gene. Together these studies may ultimately lead to improved therapeutic strategies for neurodegenerative diseases.

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