



● INVITED REVIEW

Selective neuronal *PTEN* deletion: can we take the brakes off of growth without losing control?

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Abstract

The limited ability for injured adult axons to regenerate is a major cause for limited functional recovery after injury to the nervous system, motivating numerous efforts to uncover mechanisms capable of enhancing regeneration potential. One promising strategy involves deletion or knockdown of the phosphatase and tensin (*PTEN*) gene. Conditional genetic deletion of *PTEN* before, immediately following, or several months after spinal cord injury enables neurons of the corticospinal tract (CST) to regenerate their axons across the lesion, which is accompanied by enhanced recovery of skilled voluntary motor functions mediated by the CST. Although conditional genetic deletion or knockdown of *PTEN* in neurons enables axon regeneration, *PTEN* is a well-known tumor suppressor and mutations of the *PTEN* gene disrupt brain development leading to neurological abnormalities including macrocephaly, seizures, and early mortality. The long-term consequences of manipulating *PTEN* in the adult nervous system, as would be done for therapeutic intervention after injury, are only now being explored. Here, we summarize evidence indicating that long-term deletion of *PTEN* in mature neurons does not cause evident pathology; indeed, cortical neurons that have lived without *PTEN* for over 1 year appear robust and healthy. Studies to date provide only a first look at potential negative consequences of *PTEN* deletion or knockdown, but the absence of any detectable neuropathology supports guarded optimism that interventions to enable axon regeneration after injury are achievable.

Key Words: *PTEN*; *mTOR*; spinal cord injury; corticospinal tract; motor system; axon regeneration; recovery of function

Introduction

Regeneration of injured or unhealthy axons holds great therapeutic promise for neurological disorders including acute trauma to the brain or spinal cord, stroke, and neurodegenerative diseases. The phosphatase and tensin homolog (*PTEN*) gene has emerged as an important regulator of axon regeneration, and recent findings by multiple groups support the potential of using *PTEN* as a therapeutic target. Our recent paper reports that long-term deletion of *PTEN*, a tumor suppressor gene, does not result in any major detectable pathology and may also enhance neuronal vitality (Gutilla et al., 2016). By selectively deleting *PTEN* in the motor cortex of young mice, we specifically assessed the effects of *PTEN* loss using the same approach that has been used to promote regeneration of the corticospinal tract after spinal cord injury.

PTEN's impact on axon regeneration was discovered in a seminal experiment in which conditional genetic deletion of *PTEN* promoted robust axonal regeneration of retinal ganglion cells (RGCs) following optic nerve crush (Park et al., 2008). The rationale for this experimental approach is rooted in the discovery of inhibitory intrinsic and extrinsic factors that prevent regeneration of injured axons (Schwab and Bartholdi, 1996; Fitch and Silver, 2008). One approach to overcoming the intrinsic inhibitory factors focuses on attempting to “recapitulate development”, in order to return adult neurons to a more

growth permissive state (Filbin, 2006). Growth cessation after the completion of development is thought to occur in part due to the onset of growth inhibiting genes being expressed.

In their landmark study, Park et al. (2008) tested whether axon regeneration could be enhanced if genes that normally repress cell growth were eliminated prior to axon injury. They specifically examined several known tumor suppressor genes including *PTEN*, *p53*, *retinoblastoma*, *Smad4*, *Dicer*, and *LKB*. The effect of each individual gene was studied using multiple strains of “floxed” mice, with each strain having only one of the aforementioned genes flanked by lox-P sites. The specific gene was deleted in the retina by injecting AAV-Cre into the vitreous humor of the eye prior to performing an optic nerve crush. Only deletion of the *PTEN* gene enabled axotomized RGCs to regenerate, though deletion of both *PTEN* and *p53* reduced retrograde degeneration of RGCs that otherwise occurred. The latter finding, not emphasized at the time, could mean that deleting *PTEN* enhanced RGC vitality such that the neurons could survive traumatic injuries that would normally cause cell death.

PTEN Deletion and Corticospinal Tract (CST) Axon Regeneration

Following the initial finding that linked *PTEN* deletion to

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enhanced neural regeneration, a follow up study tested whether neuronal *PTEN* deletion could also enhance regeneration after spinal cord injury. This study focused on regeneration of the CST, which mediates voluntary motor function. Damage to CST axons due to spinal cord injury is the cause of paralysis, and enabling regeneration of the CST is the best hope for restoring motor function after injury. Similar to Park et al. (2008), this study used floxed *PTEN* mice, and *PTEN* was deleted in the motor cortex of mice one day after birth by injecting AAV-Cre into the sensorimotor cortex. Then, as young adults, mice received spinal cord injuries. Tract tracing of CST axons revealed robust and unprecedented regeneration beyond the injury site (Liu et al., 2010).

Subsequent studies have confirmed and extended findings from these two papers. Genetic deletion of *PTEN* soon after spinal cord injury in adult mice, or knockdown of *PTEN* expression using short hairpin RNA (shRNA) against *PTEN* in adult rats a few days before injury was found to enhance the regenerative growth of the adult CST and recovery of skilled motor functions (Zukor et al., 2013; Lewandowski and Steward, 2014; Danilov and Steward, 2015). Remarkably, *PTEN* deletion also induced robust CST regeneration in the chronic injury setting one year following injury (Du et al., 2015). These discoveries further highlight the potential of using *PTEN* interference as a pro-regenerative strategy for treating adult spinal cord injury (Ramon-Cueto et al., 2000).

***PTEN*'s Role in Regulating Normal Neuronal Development and Function**

The *PTEN* gene is thought to exert its growth inhibiting effects through the PTEN protein's negative regulation of phosphoinositide 3-kinase (PI3K). As a phosphatase, *PTEN* converts active phosphatidylinositol (3,4,5)-triphosphate (PIP3) to inactive phosphatidylinositol (4,5)-bisphosphate (PIP2), resulting in diminished AKT and downstream mammalian target of rapamycin (mTOR) activation. Thus, deletion of *PTEN* leads to enhanced levels of PIP3, activation of AKT, and activation of mTOR. The mTOR pathway is well known for its ability to regulate cell growth and proliferation and *PTEN*'s upstream and non-redundant negative regulation of the mTOR pathway make it a promising pro-regenerative therapeutic target (Don et al., 2012).

It's here that we come to the theme of our review; is it possible to take the brakes off of such a powerful growth-promoting pathway without losing control? The logic behind testing *PTEN* was that it had been identified as a tumor suppressor gene. *PTEN* mutations are common in several cancers, and have been associated with developmental disorders including macrocephaly and autism spectrum disorders (Goffin et al., 2001; Hollander et al., 2011). Experimental studies in which *PTEN* was deleted during early development in particular cell types revealed neuronal overgrowth, brain enlargement, seizures, and premature death. These studies used mice with a lox-P flanked *PTEN* gene paired with Cre recombinase expression regulated under the control of promoters including neuron specific enolase (NSE, (Kwon et al., 2006)), glial fibrillary acid protein (GFAP, (Backman et al., 2001; Kwon et al., 2001; Fraser et al., 2004; Yue et al., 2005; Fraser et al., 2008; Wen et al., 2013)), Ca²⁺/calmodulin-dependent protein kinase II (CamKII, (Sperow et al., 2012)), and the dopamine active transporter (DAT, (Diaz-Ruiz et al., 2009)). In studies using NSE, GFAP, and CamKII promoter driven Cre expression, mice with *PTEN* deletion exhibit significantly higher postnatal mortality and premature death (~11 weeks of age for CamKII-Cre, (Sperow et al., 2012)). In the GFAP-Cre models, multiple groups have identified neurons with successful *PTEN* deletion in the cerebellum, hippocampus, and the

cerebral cortex (Backman et al., 2001; Kwon et al., 2001; Fraser et al., 2004, 2008).

The negative consequences following widespread neuronal *PTEN* loss during development necessitated an in-depth examination of the long-term consequences of deleting *PTEN* in the way that promotes axon regeneration. As a first step in assessing the potential risk, we employed the same experimental model as in our original report of CST regeneration following spinal cord injury (Liu et al., 2010). *PTEN* was deleted by injecting AAV-Cre into the sensorimotor cortex of floxed *PTEN* mice on postnatal day 1. Mice were then allowed to survive for at least one year, and motor function was tested in the final months prior to euthanasia (Gutilla et al., 2016).

Over several months of handling and testing, mice did not exhibit any obvious behavioral abnormalities or spontaneous seizures. General motor function was tested by open field activity and Rotorod, and brains were examined extensively for any evidence of tumors or other neuropathology. Mice with *PTEN* deletion exhibited normal exploratory activity in an open field and were slightly, though not significantly, impaired on the Rotorod. Most important, we found no evidence of tumors or other neuropathology in the area of *PTEN* deletion. Cortical motoneurons, the cells of origin of CST axons, appeared healthy and exhibited high levels of immunostaining for the phosphorylated form of ribosomal protein S6 (rpS6). rpS6 phosphorylation is considered a bioindicator of mTOR activation, so high levels of immunostaining for phosphorylated rpS6 indicates continued activation of mTOR more than a year after *PTEN* deletion.

The only histological abnormalities were: 1) cortical motoneurons lacking *PTEN* (identified by retrograde labeling following Fluorogold injections into the spinal cord) were substantially larger than control neurons; 2) there was visible disruption of the normal laminar organization of the cortex in the area of *PTEN* deletion, perhaps as a result of the increase in neuronal size; 3) the ratio of neuropil to cell bodies was higher in the region of *PTEN* deletion. Our speculation is that this is due to cellular hypertrophy including hypertrophy of dendrites, but we have not yet assessed this quantitatively.

Safety of *PTEN* Interference as a Therapeutic Strategy and Remaining Questions

While our study does not qualify as a safety study as would be required for preclinical development of a therapy, the mice survived without any ill effects for up to 18 months after *PTEN* deletion (considered early old age in mice). Other studies involving hundreds of mice and rats with *PTEN* deletion in the sensorimotor cortex report enhanced regeneration and improvements in motor function after spinal cord injury, and there have been no reports of negative effects. Taken together, our findings along with previous reports point to the possibility of targeting *PTEN* therapeutically without triggering untoward effects.

Despite providing an important first look at the long-term consequences of *PTEN* deletion, several questions remain unanswered. Other groups have reported seizures following deletion of *PTEN* early in development (Backman et al., 2001; Ogawa et al., 2007; Pun et al., 2012) as well as changes in the electrophysiological properties of neurons lacking *PTEN* (Fraser et al., 2008; Sperow et al., 2012; Williams et al., 2015). So far there have been no systematic studies of the physiological consequences of *PTEN* deletion in cortical neurons, but this warrants further investigation. Additionally, since most acute neurological traumas and neurodegenerative diseases occur in adults, it will also be important to assess the consequences of *PTEN* deletion in adults.

Articles with PTEN, regeneration, and axon in abstract

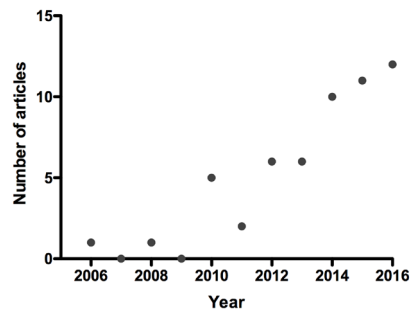


Figure 1 Increased number of articles related to *PTEN* and regeneration published per year since 2006.

A PubMed search done on May 21, 2016 using the keywords “PTEN, regeneration, and axon” in “Abstract” yielded a total of 56 papers. The graph plots numbers of publications per year. PTEN: Phosphatase and tensin homolog.

The ability to induce a robust growth capability in central nervous system neurons has broad implications even beyond the potential of enabling regeneration of axons after spinal cord injury. Park’s original study reported that in addition to promoting axon regeneration, *PTEN* deletion in retinal ganglion cells reduced retrograde cell death following optic nerve crush. It will be of considerable interest to assess whether *PTEN* deletion could prevent or reverse age-related deterioration of neurons such as neuronal atrophy or even prevent death that is observed in neurodegenerative diseases including Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis (ALS). Indeed, there have been reports that show that deleting *PTEN* in the substantia nigra reverses symptoms in an experimental Parkinson’s disease model and protects dopaminergic neurons from toxic insults (Diaz-Ruiz et al., 2009; Domanskyi et al., 2011).

The pace of research on *PTEN* related to neural regeneration is clearly accelerating. A PubMed search done on May 21, 2016 using the keywords “*PTEN*, regeneration, and axon” in “Abstract” yielded a total of 56 papers, with an increasing number being published each year (Figure 1). Indeed, 12 papers have been published in the first five months of 2016. This increased effort aimed at understanding *PTEN*’s role in neural regeneration will undoubtedly help to address the questions that still remain.

Author contributions: All authors participated in the organization of the article and the selection of topics to be covered. EAG wrote the majority of the text of the article, edited the figure and figure legend, and provided references. OS provided critical commentary, text, and figure design. All authors approved the final version of the article.

Conflicts of interest: OS is one of the co-founders of the company Axonis, which holds options on patents related to targeting *PTEN* to enhance regeneration after injury. The terms of this arrangement have been reviewed and approved by the University of California, Irvine in accordance with its conflict of interest policies.

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