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REVIEW

PTEN/PI3K and MAPK signaling in protection and pathology following CNS injuries

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Abstract **Brain and spinal cord injuries initiate widespread temporal and spatial neurodegeneration, through both necrotic and programmed cell death mechanisms. Inflammation, reactive oxidation, excitotoxicity and cell-specific dysregulation of metabolic processes are instigated by traumatic insult and are main contributors to this cumulative damage. Successful treatments rely on prevention or reduction of the magnitude of disruption, and interfering with injurious cellular responses through modulation of signaling cascades is an effective approach. Two intracellular signaling pathways, the phosphatase and tensin homolog (PTEN)/phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signaling cascades play various cellular roles under normal and pathological conditions. Activation of both pathways can influence anatomical and functional outcomes in multiple CNS disorders. However, some mechanisms involve inhibiting or enhancing one pathway or the other, or both, in propagating specific downstream effects. Though many intracellular mechanisms contribute to cell responses to insult, this review examines the evidence exploring PTEN/PI3K and MAPK signaling influence on pathology, neuroprotection, and repair and how these pathways may be targeted for advancing knowledge and improving neurological outcome after injury to the brain and spinal cord.**

Keywords spinal cord injury, traumatic brain injury, PTEN, MAPK, neuroprotection, axon regeneration

Introduction

Nearly 2 million Americans experience traumatic spinal cord (SCI) and brain injuries (TBI) each year (Loane and Faden, 2010; NSCISC, 2011), though unfortunately, no effective treatments are currently available. Investigating cell-specific responses, including signal pathways and associated proteins, is important for understanding spinal cord and brain pathology and neuroprotection, which could enhance therapeutic development. Two widely studied pathways involved in cellular responses in both normal and pathological conditions within the central nervous system (CNS) are the PI3K/Akt and MAPK pathways. These cascades are widely known for their roles in promoting survival, growth and proliferation (Chang and Karin, 2001; Cantley, 2002), however, their influence is not always beneficial following CNS injury. Much research has focused on protection of spared nervous tissue from progressive biochemical and inflammatory damage, and regeneration of damaged axons following primary mechanical trauma, both with limited success. Such trouble likely involves the complexities of these cellular responses following injury.

Unlike the peripheral nervous system (PNS), the CNS lacks inherent regenerative ability following injury (Schwab and Bartholdi, 1996). Contributing to this inhibition are myelin related proteins (Cadelli and Schwab, 1991), including myelin associated glycoprotein (MAG) (McKerracher et al., 1994), Nogo-A (GrandPré et al., 2000), and oligodendrocyte myelin glycoprotein (Omgp) (Wang et al., 2002). Astroglial-associated inhibitory molecules, including chondroitin sulfate proteoglycans (CSPGs), contribute to the

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extracellular matrix within the inhibitory glial scar (Dow et al., 1993). These examples highlight just a few of the obstacles in treating CNS injuries. Recent research, however, has shown remarkable advances in manipulating such barriers, even demonstrating the ability to promote robust CNS axonal regeneration (Park et al., 2008a; Liu et al., 2010b). Extensive literature currently exists on the variation and influence of intracellular signaling on neuroprotection, regeneration, and functional recovery following SCI and TBI, and tools are now available which hold the potential for promoting these benefits. The following sections highlight the PI3K and MAPK signaling pathways, their contribution to cell fate, and how these pathways may be modulated to improve neuroprotection and recovery following spinal cord and brain injuries.

PTEN and PI3K/Akt/mTOR signaling

The phosphatase and tensin homolog, PTEN, is highly expressed in adult CNS neurons (Cai et al., 2009; Liu et al., 2010b). Encoded by the *pten* gene mapped to chromosome 10q23, the 55 kDa PTEN protein is a dual-function protein tyrosine phosphatase that can dephosphorylate both proteins and lipids (Li et al., 1997). Its enzymatic active site, however, has more affinity for the latter, especially phosphatidylinositol-3,4,5-phosphate (PIP_3) (Lee et al., 1999). The physiological function of PTEN is highly important for processes including cellular proliferation and neuronal growth regulation (Dahia, 2000; Kwon et al., 2001). In addition, down-regulating PTEN's function or expression promotes axon regeneration and neuroprotection following CNS trauma (Park et al., 2008; Liu et al., 2010b; Zhang et al., 2007; Walker et al., 2012b). Beneficial effects of its inhibition are usually attributed to disinhibition of PI3K and downstream signaling through Akt (Zhang et al., 2007; Sury et al., 2011; Walker et al., 2012b) and the mammalian target of rapamycin (mTOR) (Shi et al., 2009, 2011; Zhong and Bowen, 2011) (Fig. 1).

PI3K signaling is often triggered by extracellular growth factor activation of a receptor tyrosine kinase (RTK) or G-protein coupled receptor (GPCR) (Engelman et al., 2006). Once active, PI3K can phosphorylate phosphatidylinositol-4,5-phosphate (PIP_2) to form PIP_3 (Engelman et al., 2006). PIP_3, a multipurpose secondary messenger, promotes activation of the survival kinase Akt (also known as PKB), and its membrane localization through activity of 3-phosphoinositide

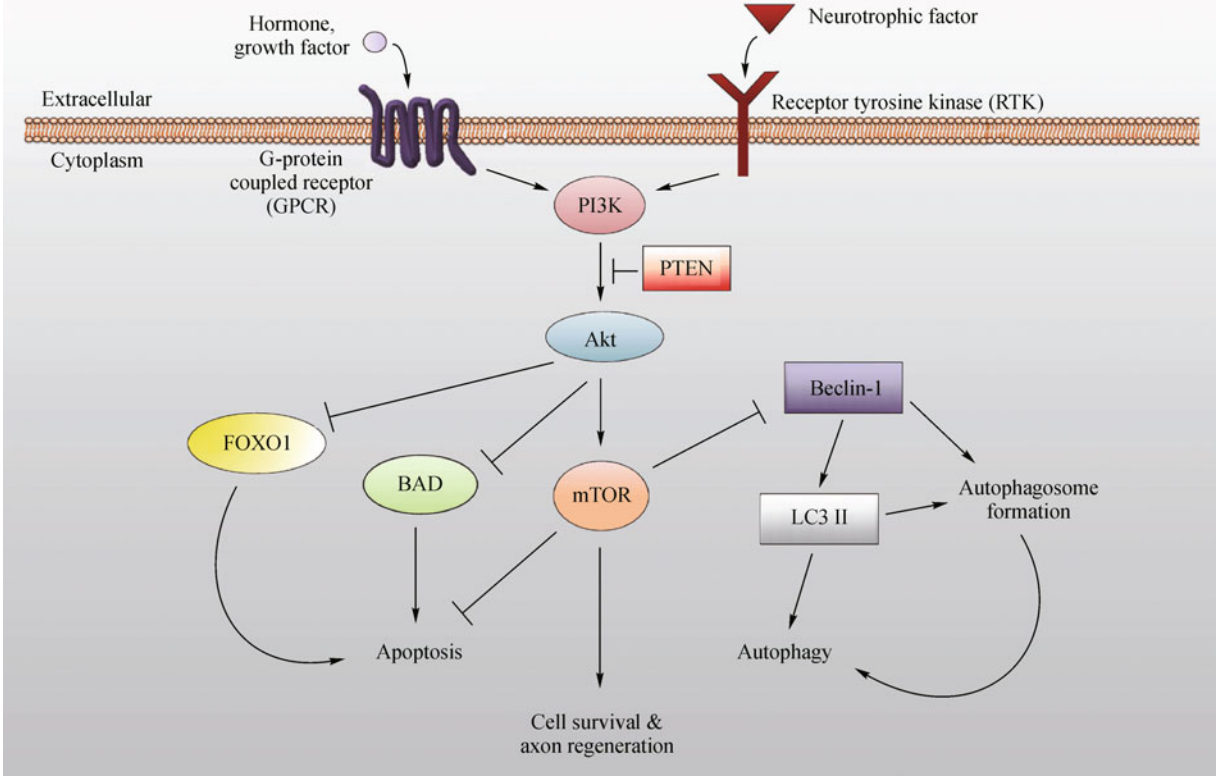

Figure 1 PTEN reduces PI3K/Akt signaling benefits on cell survival and regeneration. PI3K can be stimulated through RTK or GPCR-mediated signaling, promoting Akt inhibition of several apoptosis-associated proteins such as Bad and FOXO1, and promotion of pro-survival mediators such as mTOR. PTEN antagonizes PI3K, and the resulting reduction in downstream Akt and mTOR signaling promotes programmed cell death, i.e., apoptosis and autophagy. PI3K = Phosphatidylinositol 3-kinase; PTEN = Phosphatase and tensin homolog; mTOR = mammalian target of rapamycin; BAD = Bcl-2-associated death promoter; FOXO1 = Forkhead box protein O1; LC3 II = Microtubule-associated protein light chain 3 II.

dependent protein kinases (PDKs) (Alessi et al., 1997). Antagonizing PI3K in PIP_2 conversion, however, is PTEN. Thus, reduced PTEN activity and elevated PIP_3 production are essential for PI3K-mediated pro-survival signaling through Akt and its effectors (Fig. 1).

Akt phosphorylation decreases within the lesion area following SCI (Yu et al., 2005; Walker et al., 2012b), while increasing in neurons through a PI3K-dependent mechanism within the surrounding injury penumbra (Yu et al., 2005; Endo et al., 2006; Howitt et al., 2012). Akt phosphorylation at serine 473 peaks 8 h post-injury within this perilesional tissue (Yune et al., 2008), and diminishes through 24 and 48 h following trauma (Yune et al., 2008; Walker et al., 2012b). Similarly, phosphorylation at this site decreases rapidly within the injury epicenter following TBI, while transiently peaking at 4 h post-injury in the penumbra and co-localizing with its downstream effectors phosphorylated Bad and GSK-3β (Noshita et al., 2001). By 24 h post-TBI, apoptotic co-labeling with phospho-Akt is not observed (Noshita et al., 2001), further associating Akt activation with cell survival following CNS injury.

It is well known that mTOR inhibits the progression of apoptosis and autophagic cell death (Baehrecke, 2005; Levine and Yuan, 2005; Shang et al., 2010). mTOR activity is also linked to axonal regeneration following PTEN deletion (Park et al. 2008; Liu et al., 2010b; Sun et al., 2011). The understanding of the signaling steps between PTEN and mTOR involved in these events are not quite clear, though Akt activity is potentially involved based on its known effects and documented response to injury. Delayed phosphorylation of ribosomal protein S6 at serines 235/236, commonly used markers for mTOR activity, is observed 24 h post-SCI (Walker et al., 2012b). Further study could uncover a similar downstream mTOR activity pattern following TBI. mTOR, also known by the name FRAP, is a large serine/threonine kinase (289 kDa) responsible for detecting energy or nutrient variations within the cell, and is highly important in regulating key cellular functions in response to the energy or stress status of the cell (Proud, 2004). mTOR is activated upon phosphorylation at serine 2448, and functional interactions with other proteins forms two distinct enzymatic complexes, mTORC1 and mTORC2. mTORC1, the rapamycin-sensitive complex, can be activated indirectly through Akt via phosphorylation of the tuberous sclerosis complex protein 2 (TSC2) (Inoki et al., 2002), which prevents mTOR inhibition (Inoki et al., 2002; Jaeschke et al., 2002; Tee et al., 2002; Manning and Cantley, 2007).

Primary effectors of mTOR are ribosomal protein p70S6 kinase (p70S6K) and 4E binding protein-1 (4E-BP1) (Proud, 2002) (Fig. 1). Phosphorylation of p70S6K stimulates its phosphorylation of ribosomal protein S6, initiating a variety of translation-associated activities. Phosphorylation of 4E-BP1 by mTOR promotes translation, as well. Some of the most exciting aspects of mTOR's activation have been observed following PTEN inhibition or genetic deletion (Park et al., 2008; Liu et al., 2010b; Walker et al., 2012b; Zhong et al., 2012). A recent report suggested that exercise upregulates ribosomal protein S6 activity in spinal intermediate gray neurons at 10 and 31 days post-SCI (Liu et al., 2012b), posing an interesting question as to whether extensive behavioral testing or training activities in SCI and TBI research affect plasticity and neural tissue survival through mTOR-associated signaling.

Increased phospho-Akt in neurons of the injury penumbra (Yu et al., 2005; Endo et al., 2006; Howitt et al., 2012) suggests the natural upregulation of this pathway may represent an acute endogenous protective response to insult (Noshita et al., 2001), especially if followed by a progression of mTOR activation. Though this explanation is quite plausible, a better grasp of the temporal progression of intracellular prosurvival PI3K/Akt/mTOR signaling within penumbral neurons and glia is necessary to effectively identify specific signaling targets and therapeutic time windows for promoting neuroprotection and repair following CNS injury. Nevertheless, evidence exists suggesting that activation of Akt/mTOR, through PTEN inhibition or other means is likely neuroprotective and growth-promoting following injury to the CNS.

MAPK signaling

Mitogen-activated protein kinases (MAPKs) play many roles within the nervous system, including promotion of cell proliferation, neural plasticity and cell survival (Fig. 2). Cell damage and death may also be enhanced depending on the signal and specific MAPK involved. Three classes of MAPKs are known: extracellular signal-regulated kinases (Erks), p38 MAPKs, and c-jun N-terminal kinases (JNKs), also known as stress-activated protein kinases (SAPK), though referred to here as JNKs (Roux and Blenis, 2004). Each of these can be further categorized into different subclasses, including Erk 1/2 isoforms and the different JNK proteins, JNK 1, 2, and 3. Much has been published on general mechanisms of these pathways (Mielke and Herdegen, 2000; Chang and Karin, 2001), however, our understanding of how these pathways are involved in CNS injury and disease is incomplete. Like PI3K, MAPKs are activated via extracellular stimulation of a RTK or other receptor [e.g. a death receptor (Fig. 2)] by molecules including growth factors and inflammatory cytokines. These stimuli may incite cascades of molecules that can activate one or more of the MAPKs.

As an example of such signaling, a molecule known as Grb2 interacts with the intracellular domain of the RTK and activates a protein encoded by the gene, *son of sevenless* (*SOS*), which can exchange the bound guanidine diphosphate (GDP) for GTP on the coupled G protein, Ras. This mediates subsequent activation of the serine/threonine kinase, Raf (Geyer et al., 1997), which activates MEKs 1 and 2, kinases responsible for activating Erk 1/2 through

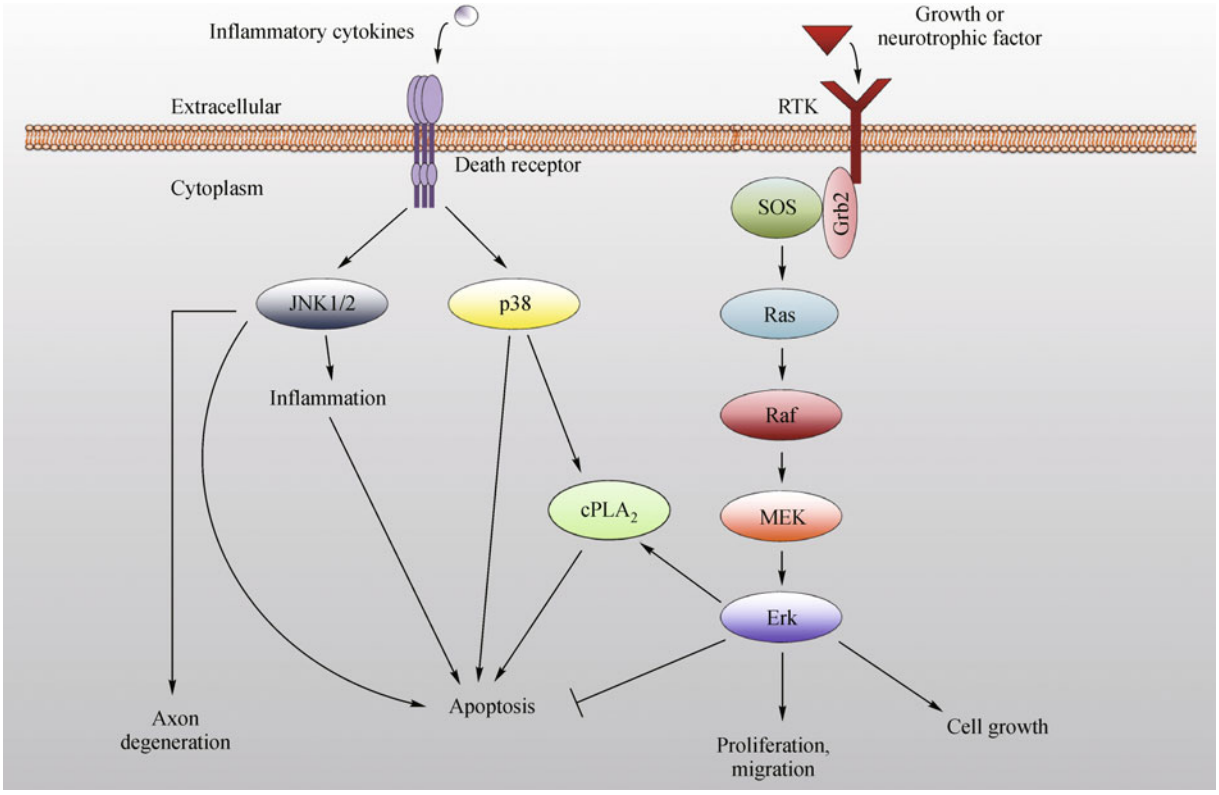

Figure 2 MAPK signaling influences cell survival and injury. p38 and JNK MAPKs are most commonly associated with pathology following CNS injury. Erk signaling is most often viewed as a positive influence on cell fate, though it can contribute to cell death as well. JNK = Jun N-terminal kinase; MAPK = Mitogen activated protein kinase; Erk = Extracellular signal-regulated kinases.

phosphorylation at Thr/Tyr residues of the Erk active domain (Pearson et al., 2001) (Fig. 2). A downstream cascade of phosphorylation and signaling of different effectors takes place, eventually ending in transcriptional or non-transcriptional regulation of various cellular activities, e.g. cell death and survival, proliferation, and even axon degeneration (Fig. 2) (Treisman, 1996; Johnson and Lapadat, 2002; White et al., 2007). Precise control over the timing, location, and duration of MAPK pathway stimulation is important for proper physiological function (Pouysségur et al., 2002).

Erk Signaling

Erk was the first of the MAPKs to be identified, and is the most studied and characterized (Rubinfield and Seger, 2005). Erk has several known isoforms, serving both cytoplasmic and nuclear functions, though Erks 1 and 2, with molecular weights of 44 and 42 kDa respectively, are the most commonly studied in neurological research. Erk signaling is often investigated due to its role in proliferation/replication and growth-related cellular activities (Sawe et al., 2008). These processes are important following CNS injury, however, the functional roles of Erk in the nervous system range from neural developmental regulation to cognition and memory. Various gene mutations can cause dysregulated Erk signaling, and are associated with multiple developmental nervous system abnormalities including X-linked mental retardation, Noonan syndrome, and neurofibromatosis type-1 (Samuels et al., 2009).

In neurotrauma research, the role(s) of Erk in neuroprotection and cell death is debated. Many cell types in the CNS express phosphorylated Erk after injury, including neurons, glia, and endothelial cells (Irving et al., 2000; Ferrer et al., 2003). Enhanced survival and neurite outgrowth has been reported in response to neurotrophin treatment and genetic overactivation of MEK and Erk (Segal and Greenberg, 1996). An elevation in Erk phosphorylation has also protected neurons against ischemic death in the brain (Sawe et al., 2008) and apoptosis following ghrelin administration post-SCI (Lee et al., 2010). Erk-mediated upregulation of brain-derived neurotrophic factor (BDNF), and downregulation of detrimental pro-nerve growth factor (pro-NGF), has also been shown to be neuroprotective following SCI (Lee et al., 2010; Wang et al., 2011). Erk can facilitate neural cell adhesion molecule (NCAM)-mediated neuroprotection, repair, and functional recovery, which may be a result of glial cell line-derived neurotrophic factor (GDNF)-mediated axonal regrowth (Zhang et al., 2009, 2010). Based on these results, therapeutically promoting upregulation of Erk signaling may enhance neuroprotection, repair and functional outcome of those with CNS injury.

These findings contrast with other reports suggesting a

pathological role of Erk activity upregulation following injury or stress (Alessandrini et al., 1999; Yu and Yezierski, 2005; Yu et al., 2010; Zhao et al., 2011). An inhibition of Erk 1/2 has been shown to prevent apoptosis following both SCI (Yu et al., 2010) and TBI (Zhao et al., 2012). Much of the disadvantageous effects of Erk activation following CNS injury have been associated with increased nociceptive signaling and behavior. Data to support such a role stem from studies using popular chemotoxic pain models that utilize paw, intrathecal, or intraspinal injections of formalin or quisqualic acid (AMPA) (Brewer and Yezierski, 1998, Yezierski et al., 1998; Brewer and Hardin, 2004; Yu and Yezierski, 2005; Wiley et al., 2009; Acosta-Rua et al., 2011; Chen et al., 2012a; Ohsawa et al., 2012). In SCI, Erk-related nociception may be associated with upregulation of NMDA receptor subunits NR1, NR-2A, and NK-1R (Yu and Yezierski, 2005).

In a recent study by Alter et al. (2010), Erk 1 deletion *in vivo* minimally affected spinal pain stimulation. However, an associated upregulation of Erk 2 was shown to play a much larger role in mediating the pain response. This provided interesting evidence that each Erk isoform may play variable roles dependent on the conditions of investigation or stimulation. Supporting the pathologic role of Erk 2 following SCI, a recent study specifically characterized its role following traumatic SCI (Yu et al., 2010). This study utilized both intrathecal and intraspinal RNAi technologies to knock-down Erk 2 protein and investigate the resulting effects on anatomical and functional recovery after contusive thoracic SCI. Erk 2 was found to promote white and gray matter tissue loss and functional deficits that were reversed by reduction of the protein.

This is the most direct evidence of Erk participation in specific pathology and functional disruption after SCI. We have observed significantly upregulated Erk 1/2 phosphorylation 1 day following contusive SCI (Walker et al. 2012a), lasting through at least 3 days post-injury, indicating a tissue-level activation and regulation of Erk signaling. Similar elevation in Erk activity occurs following TBI near the injury site, lasting for several days post-injury, and contributes to apoptotic cell death (Zhao et al., 2012). This suggests a coincidence of secondary injury progression and activated Erk increase following both SCI and TBI. In support of a link between Erk activity and cell death processes, we have recently demonstrated that *Gingko biloba* promotes cell survival and viability in spinal motor neurons by inhibiting Erk activity, leading to downregulation of cytosolic phospholipase A_2 ($cPLA_2$), a known detrimental effector following SCI (Liu et al., 2006; Titsworth et al., 2007; Zhao et al., 2011).

The means of Erk activation, such as RTK stimulation or interaction with PI3K signaling pathway proteins, may help define Erk's involvement in cell survival. For example, estrogen has been shown to mediate neuroprotection through Akt while simultaneously promoting Erk activity following SCI (Yune et al., 2008). Other suggestions to explain such dichotomy of function may involve cell- or time-specific reactions following injury, or differences in duration of activity that affect Erk's influence on cell death and survival. Another plausible rationalization is that Erk acts variably by cellular location, as Erk is known to translocate into the nucleus for involvement in transcriptional activities. Though there appears to be more negative impact on PI3K/Akt signaling than MAPK signaling following injury (Fig. 3), activation of both pathways likely fluctuates over time in different cell types. Many factors likely contribute to this pathway's diverse functions, and though the efficacy of Erk activation on neuroprotection is debated, it is undoubtedly important in multiple cellular processes following neural injury.

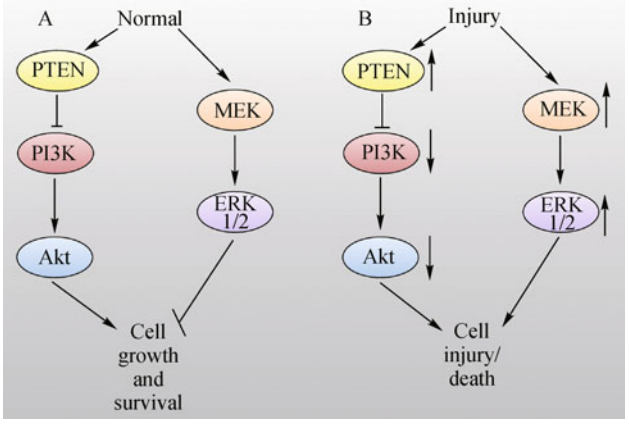

Figure 3 Potential injury-mediated alterations of PI3K-Akt vs. MEK-Erk signaling. (A) Under normal conditions, these two pathways exhibit a steady-state balance of activity and inhibition. (B) After CNS injury however, Erk activity may increase while Akt signaling decreases, reducing its downstream inhibition of cell death progression, with p-Erk playing a role in these events.

p38 and JNK signaling

p38 and JNK MAPK signaling pathways are commonly associated with cell responses to inflammation and stress. Specifically, p38 MAPK is activated by cell stress following injury, leading to downstream signaling through many effectors including $cPLA_2$, p53, and MAPK-associated protein kinase 2 (MK2), and is often associated with inflammatory responses (Saklatvala, 2004). Injured spinal neurons, astrocytes, and macrophages increase p38 MAPK expression (Ghasemlou et al., 2010). Active p38 MAPK expression significantly increases within minutes in cortical neurons after compressive brain injury, correlating with increased dendritic remodeling influenced by microtubule destabilization (Chen et al., 2010).

Activation of p38 MAPK can also be beneficial, promoting cell proliferation and survival (Krens et al., 2006), complicating the potential of therapeutically targeting its inhibition. Downstream MK2 phosphorylation by p38 MAPK instigates

cytokine production through transcriptional upregulation, perhaps providing a more appropriate target for inhibiting or modulating inflammatory events. It also enhances transcription leading to an increase in detrimental matrix metalloproteinases (Xu et al., 2006). A recent study by Ghasemlou et al. (2010) investigated the importance of MK2 in the progression of secondary inflammatory damage following SCI. Though many studies have examined cell signaling mechanisms associated with SCI, the identification of key functions of this particular pathway is novel and useful, providing insights into the tissue response and potential therapeutic targets for limiting secondary tissue damage following SCI and other CNS injuries.

An example concerns MK2's role in heat shock protein 27 (Hsp27) activity, and the formation of an oligomeric complex composed of Hsp27, MK2, p38, and Akt (Zheng et al., 2006). MK2 can mediate p38 incorporation into a complex with Akt and Hsp27 following peroxide stress, and upon activation of Akt, Hsp27 is released from the complex. Therefore it is reasonable that the loss of MK2 promotes neural protection from cellular stress such as nutrient deprivation (Mearow et al., 2002). From these findings, p38 mediation of neurodegenerative events is further supported.

Like p38 MAPK, JNK MAPKs are also commonly involved in instigating inflammation and cell death. Recent studies have shown that JNK signaling suppression is beneficial to cell survival following TBI (Tran et al., 2012) and ischemia/reperfusion injury (Chen et al., 2012b; Liu et al., 2012a). JNK activation also enhances axonal degeneration and reduces functional outcome following SCI (Yoshimura et al., 2011). JNK influence on cell death and degeneration is often mediated through pro-inflammatory or oxidative stress following injury (Kesherwani and Agrawal, 2012), and it has been suggested that JNK and p38 MAPK contribute to extended periods of injury-related pain (Kobayashi et al., 2008; Ji et al., 2009; Gao and Ji, 2010). Though p38 and JNK signaling play considerable roles in pathology following CNS injury, upstream and downstream signaling is quite complex, and deserves further analysis and discussion. The general signaling and influence of these two MAPK proteins is illustrated in Fig. 2 for comparative purposes to MEK-Erk signaling.

Tools for studying PI3K and MAPK-associated signaling in neural degeneration and repair

As a theme of this review, variation in cellular signal transduction enhances the complexity of unraveling mechanistic details of protection and pathology following CNS injury. The use of transgenic animals has allowed for more accurate and reliable assessments in such studies. Knocking-out specific signaling proteins affords discrete assessment of their role in cellular signaling effects. However, pharmacological approaches to experimental and clinical treatment are often more practical and accessible than genetic manipulation, even though such knockout investigations are critical to highlight potential targets for pharmacological therapeutics. Table 1 and Fig. 4 highlight some of the most commonly used chemical inhibitors, their targets, and functions for assessing the roles of particular steps of these pathways and for experimental assessment of their benefits through modulation in animal models of CNS injury and disease.

A large body of literature currently exists describing many processes and treatments that may act through stimulating the PI3K/Akt/mTOR axis or MAPK signaling in mediating neuroprotection. In general, many therapies may incite neuroprotective signaling through interaction and activation of extra- and intracellular domains of receptor tyrosine kinases (RTKs). We have shown that GDNF exerts beneficial effects through interaction with GFRα1 and its partner RTK, cRet, and potentially through neural cell adhesion molecule (NCAM) interaction on neurons (Zhang et al., 2009). GDNF

Table 1 PI3K and MAPK pathway inhibitors, their targets and actions

	Target	Action	Reference
PI3K pathway inhibitor			
Bisperoxovanadium (bpV)	PTEN	Inhibits PTEN phosphatase activity; Upregulates PI3K/Akt signaling	Schmid et al., 2004
LY294002	PI3 kinase	Blocks PI3K activity; Reduces phosphorylation of Akt at serine 473	Vlahos et al., 1994
Wortmannin	PI3 kinase	Blocks PI3K activity; Reduces phosphorylation of Akt at serine 473	Arcaro and Wymann, 1993
Akt inhibitor IV	Akt	ATP-competitive inhibitor of a kinase upstream of Akt but downstream of PI3K	Wang et al., 2006
Rapamycin	mTOR C1	Inhibits mTOR's ability to phosphorylate p70S6 kinase or 4E binding protein I	Kunz et al., 1993; Brown et al., 1994
MAPK pathway inhibitors			
U0126	MEK 1 and 2	Blocks MEK phosphorylation and activation of Erk 1/2	Favata et al., 1998
PD098059	MEK 1 and 2	Blocks MEK phosphorylation and activation of Erk 1/2	Dudley et al., 1995

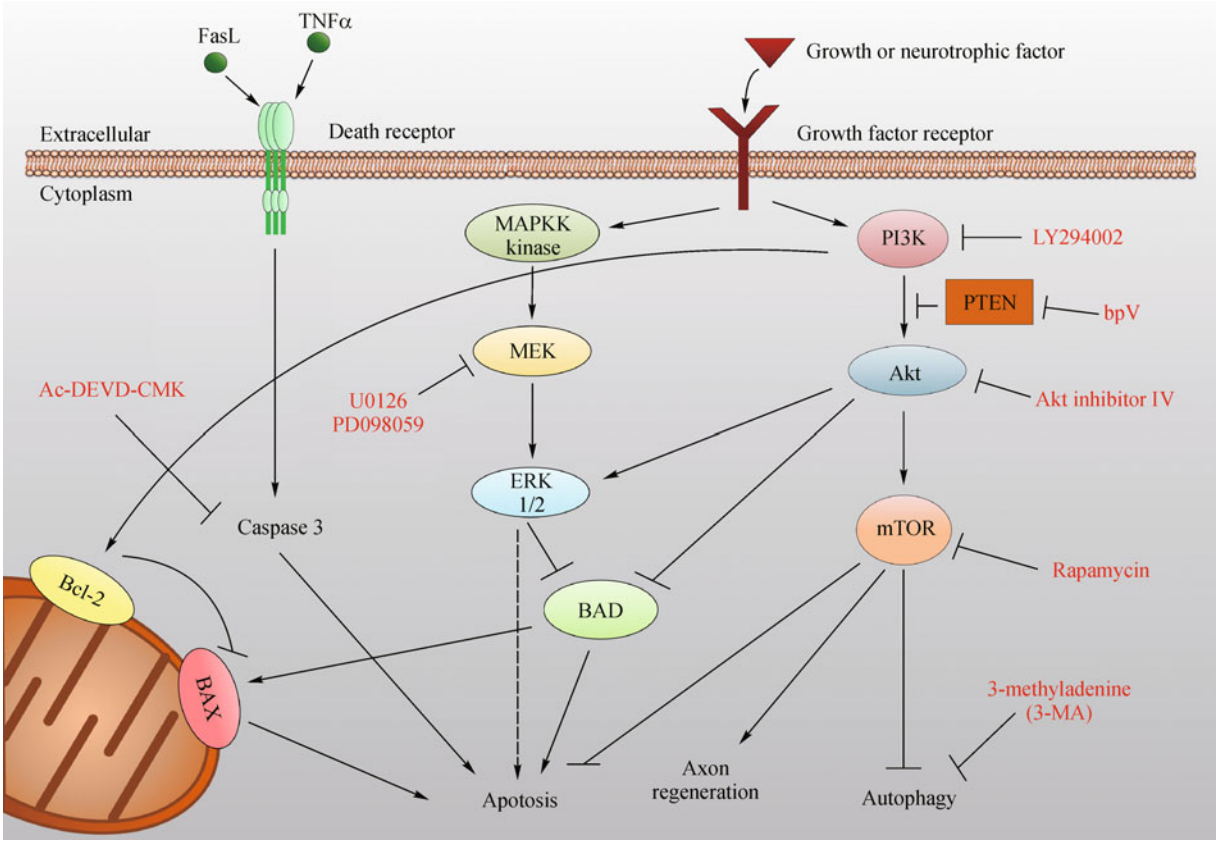

Figure 4 Overall PI3K and MAPK-Erk signaling is complex, but can highly influence the balance between cell survival and death. Multiple pathway inhibitors exist to further research cell signaling mechanisms. Though normal or pathological conditions can stimulate apoptotic cell death directly through death receptor and caspase activation, numerous signaling activities and interactions within a cell can indirectly influence cell fate. PI3K and MAPK pathways may individually, or through crosstalk, impact cell survival. For example, Akt may activate Erk to inhibit apoptosis. A variety of compounds targeting proteins within these cascades can help deepen our understanding of the complexities of PI3K and MAPK signaling in CNS pathology and protection. FasL = Fas ligand; TNFα = Tumor necrosis factor alpha; MAPKK kinase = Mitogen activated protein kinase kinase kinase; MEK = Mitogen activated protein kinase kinase; Erk = Extracellular signal-regulated kinases; PI3K = Phosphatidylinositol 3-kinase; PTEN = Phosphatase and tensin homolog; mTOR = mammalian target of rapamycin; BAD = Bcl-2-associated death promoter.

is known to promote neurite outgrowth *in vitro* via downstream Erk 1/2 signaling (Koelsch et al., 2010). *In vivo* however, Liu et al. (2010b) have shown that viral-mediated conditional deletion of PTEN in cortical neurons promotes enhancement in axon sprouting and regrowth in the spinal cord, with upregulated mTOR activity being a likely key intermediary in promoting such benefits. Previous work has also demonstrated this phenomenon following similar methods of PTEN deletion in an optic nerve injury animal model (Park et al., 2008). As such, both PI3K and Erk signaling can promote axonal regeneration depending on the stimulus and the conditions of the neurons under study.

Inhibition of PTEN by bisperoxovanadium

Knockout techniques provide new possibilities for intracellular upregulation of pro-survival signaling which can have beneficial effects that occur without extracellular stimulation by a trophic factor or ligand. However, deletion of an enzyme may have unintended effects on other aspects of cellular function. Pharmacological enzymatic disruption of signaling molecules like PTEN provides a much more convenient and less extreme method of assessing an enzymes' activity. For example, bisperoxovanadium compounds, also known as bpVs, specifically inhibit PTEN signaling, and have been used for promotion of neuroprotection in many CNS injury studies (Yang et al., 2007; Zhang et al., 2007; Nakashima et al., 2008; Sury et al., 2011; Walker et al., 2012b).

There are several members of the bpV family of compounds including bpV(pic), bpV(OHpic) and bpV (phen), all of which have high affinity and potency for inhibition of PTEN (Schmid et al., 2004). In neuroprotection studies using bpV compounds (Zhang et al., 2007; Nakashima et al., 2008; Yu et al., 2008; Liu et al., 2010a; Sury et al., 2011; Walker et al., 2012b), potentially detrimental systemic effects were not observed or reported, however,

more investigation is necessary to further verify if bpV has undesired off-target effects that may need consideration. Also, support for bpV as a CNS injury therapy requires further investigation for treatment of other injuries including TBI. Nonetheless, current evidence suggests that small molecule inhibition of PTEN lipid phosphatase function appears to be an effective, easily controlled, and relatively safe means of reducing the extent of tissue damage and enhancing resulting functional recovery. To investigate signaling protein effects, or to alter cell signaling in ways similar to bpV, a wide variety of chemical inhibitors are commercially available. Table 1 lists several commonly used PI3K/Akt/mTOR pathway signaling inhibitors, as well as MAPK inhibitors. Figure 5 illustrates bpV(pic)-mediated promotion of neuroprotection and PI3K/Akt/mTOR signaling following SCI.

PI3K/Akt/mTOR, autophagy, and apoptosis inhibitors

PI3K inhibitors include LY294002 and also wortmannin (Arcaro and Wymann, 1993; Vlahos et al., 1994). These are often used as potential therapeutics in cancer biology, due to the common upregulation of PI3K and Akt signaling observed in tumorigenic cells. However, they are also useful in determining PI3K or downstream pathway effects in different neurological conditions both *in vitro* and *in vivo*. Being that PI3K promotes Akt phosphorylation and activation, the range of applications for use of these compounds is large, spanning from acute effects of PI3K/Akt deactivation or activation following CNS trauma, to long-term anatomical and functional benefits or deficits observed when used therapeutically. Akt has many inhibitors, the most commonly

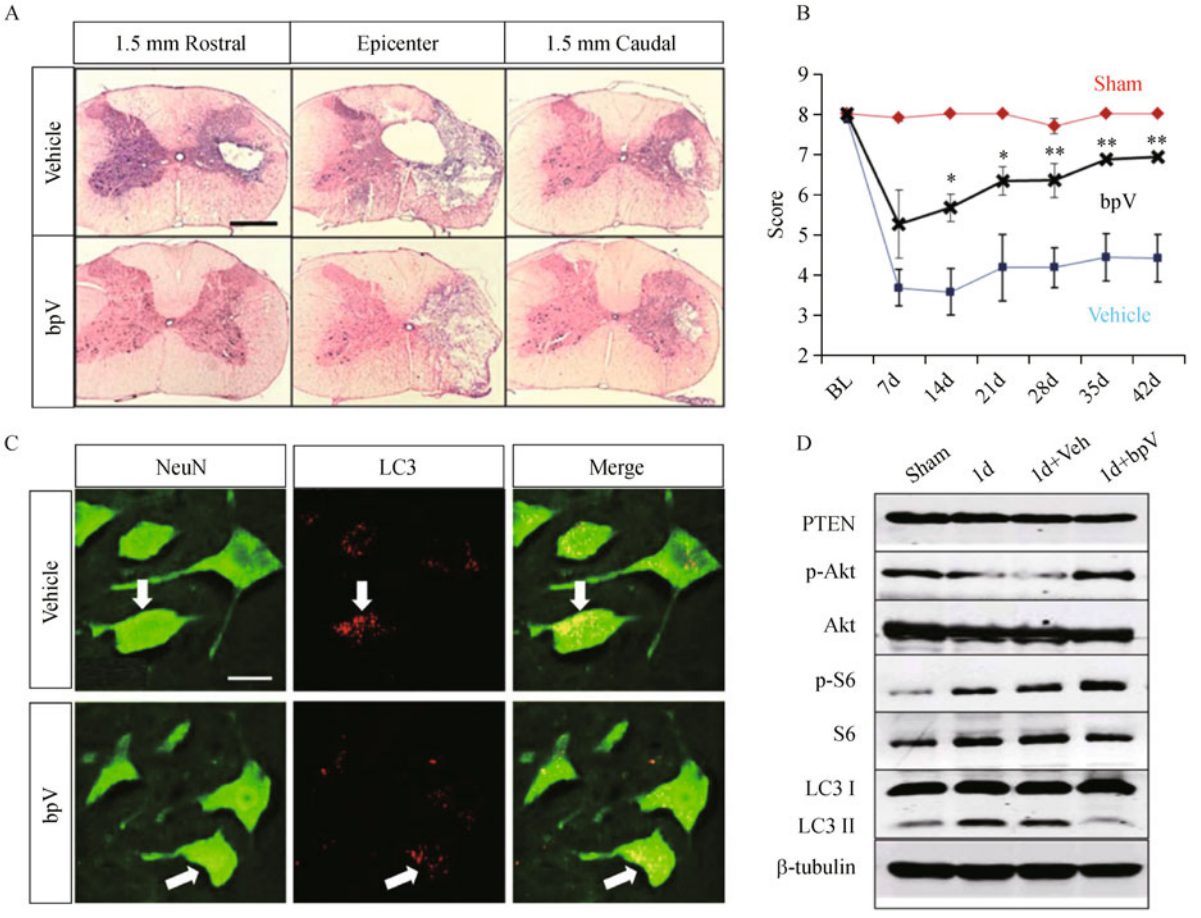

Figure 5 Neuroprotective and functional benefits of bpV(pic) treatment following SCI. (A) bpV(pic) reduced overall lesion volume following contusive cervical SCI. Cavitation common to both rat and human SCI is also reduced following bpV treatment in rats. Scale bar = 1 mm. (B) Sensorimotor functional outcome is also improved by bpV(pic) therapy, as determined by a treateating assessment. (C) Reduction of LC3-positive punctate autophagosomes are reduced in motor neurons following bpV(pic) treatment. Scale bar = 50 Ym. (D) Western blot analysis suggests bpV(pic) promotes Akt activity through inhibition of PTEN activity rather than expression. Also, reduction of autophagosome formation through diminished LC3 II/LC3I protein ratio after bpV treatment correlates with functional recovery and anatomical preservation, suggesting a potential mechanism of bpV-mediated neuroprotection. bpV(pic) = Dipotassium bisperoxo (picolinato) oxovanadate; LC3 II/I = microtubule associated protein light chain 3 II/I. LC3 I is the cytoplasmic form, while LC3 II is the lipidated form and classic autophagosome marker. * = $p < 0.05$; ** = $p < 0.01$ compared with Vehicle. [Modified from Walker et al. (2012a)].

reported being Akt inhibitor IV, which inhibits the ATP binding site of the enzyme (Kau et al., 2003), resulting in reduced Akt activity in tissue and cell samples. Again since Akt activation is tightly controlled by PI3K activity, PI3K inhibition also results in reduced Akt activity.

Rapamycin has long been known for its antibiotic function, and has been used as a therapeutic agent to elucidate mTOR influence on neuronal fate post-injury. Recent studies suggest rapamycin can promote autophagy and cell survival through mTOR inhibition after SCI (Sekiguchi et al., 2012) and stroke (Chauhan et al., 2011; Yan et al., 2011), while others suggest rapamycin-mediated autophagy promotes neurodegeneration following CNS injury (Grishchuk et al., 2011). These discrepancies are debatable, as described earlier, and may be injury type-dependent. Nonetheless, rapamycin has proven to be a useful tool in examining mTOR signaling both *in vitro* and *in vivo*. 3-Methyladenine (3-MA) (Seglen and Gordon, 1982) is now considered a commonly-associated autophagy inhibitor, and thus can be used in experiments to verify if progression of autophagy is pathologic or beneficial following injury, as well as used in conjunction with other pathway inhibitors, e.g. caspase inhibitor Ac-DEVD-CMK, (Fig. 4) to establish mechanisms of action within cells in response to injury, disease, or treatment.

MEK/Erk inhibitors

Two commonly used inhibitors used to aid in determining phospho-Erk's role in a particular reaction or other physiological outcome, are the chemicals PD098059 (Dudley et al., 1995) and U0126 (Favata et al., 1998) (Fig. 4). Both inhibitors target MEK's ability to phosphorylate and activate of Erk 1/2, thus serving also as Erk inhibitors as well. As non-competitive inhibitors with similar function, both PD098059 and U0126 inhibit Erk by binding in different conformations of MEK's active site. By preventing MEK's kinase active site from phosphorylating Erk, downstream activation is attenuated or inhibited. However, U0126 is suggested to be up to 100× more specific than PD098059 for MEK (Favata et al., 1998). Both compounds have been used *in vitro* and *in vivo* to assess Erk activity and its influence on cellular survival, proliferation and death. Due the complex effects MEK/Erk signaling may have in mediating cell responses in injury and disease, these two compounds can be used for analyzing and clarifying the mechanistic role Erk plays in different injuries and diseases of the CNS.

Summary

As research into the mechanisms of cell death and tissue degeneration following CNS injury continues, more information will be obtained which will aid in the development of effective, and specific therapies for preventing, or even reversing, the progression of pathology commonly observed following such injuries. Understanding the important roles and intricate internetwork communication and activity of intracellular pathways, is an important direction for making this progress. Nevertheless much more work is needed to effectively produce and validate therapies that target pathways such as PI3K/Akt and MAPK signaling. Current evidence, as described here, suggests that work toward understanding these complex signaling cascades is not only promising, but may be critical for achieving the goal of improved neurological outcome after brain and spinal cord injuries.

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Care and use of animals

All surgical and animal handling procedures mentioned were performed as approved under the Guide for the Care and Use of laboratory Animals (National Research Council) and the Guidelines of the Indiana University School of Medicine Institutional Animal Care and Use Committee (Approval #0000003163).

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