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REVIEW

Distinct roles for ERK1 and ERK2 in pathophysiology of CNS

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Abstract **Mitogen-activated protein kinases ERK1 and ERK2 have been implicated in various pathophysiological events of the CNS, but their specific roles in cell processes under physiologic and pathological conditions remain to be determined. ERK1/2 was originally identified as a kinase activity that mediates neuronal survival and neuroprotection, but it was subsequently found that ERK1/2 also plays a critical role in neurodegeneration. This dichotomy makes it difficult to target ERK1/2 for neuroprotection. Accumulating evidence suggests that ERK1 and ERK2 may play distinct functions in a variety of cell fate decisions. In this review, I summarize recent evidence for distinct roles for individual ERK isoforms in pathophysiology of the CNS.**

Keywords ERK isoform, cell death, cell proliferation, neurodegeneration, neuroprotection

Introduction

ERK1 (a 44 kDa protein) and ERK2 (a 42 kDa protein) are extracellular signal-regulated protein kinase members of mitogen-activated protein kinase (MAPK) family (Chen et al., 2001). They are encoded by distinct genes, but share 83% homology at the amino acid level and are expressed in all tissues (Cargnello and Roux, 2011), with high levels in the brain. In resting cells, ERK1 and ERK2 have a cytoplasmic localization and are associated with the cytoskeleton, but upon activation, ERK1/2 can translocate to the nucleus or to membrane specializations. Activation of ERK1/2 by phosphorylation of threonine and tyrosine residues mediates an effect of the growth factor signaling cascades that involve activation of Ras, Raf, MEK and ERK1/2 and are associated with cell survival and proliferation (Hetman and Gozdz, 2004).

Activation of ERK1/2 occurs in response to growth factors, ligands for G-protein coupled receptors, glutamatergic signaling, oxidative stress, proinflammatory mediators, cytokines, apoptotic signaling, ischemia, neurotrauma, osmotic stress, and microtubule disorganization (Roux and Blenis, 2004; Cargnello and Roux, 2011). The ERK1/2 signaling cascade regulates phosphorylation of target proteins leading to short-term functional changes, and transcriptional regulation of gene expression resulting in long-term adaptive changes (Seger and Krebs, 1995; Adams et al., 2000; Kolch, 2000; Cargnello and Roux, 2011).

ERK1/2 was originally identified as a kinase activity that is associated with neuronal survival and neuroprotection (Hetman and Gozdz, 2004), but it was subsequently found that ERK1/2 also plays a critical role in secondary damage mechanisms resulting from a number of neurodegenerative diseases, stroke, CNS injury, and autoimmune diseases of the CNS (Colucci-D'Amato et al., 2003; Cheung and Slack, 2004; Chu et al., 2004; Lu and Xu, 2006; Subramaniam and Unsicker, 2006; Zhuang and Schnellmann, 2006; Otani et al., 2007; Mebratu and Tesfaigzi, 2009; Subramaniam and Unsicker, 2010). This dichotomy makes it difficult to exploit ERK1/2 inhibition for therapeutic strategies in CNS diseases.

Elucidation of how ERK1/2 controls cell death and cell proliferation will help us understand completely ERK1/2 function in neurodegeneration and neuroprotection. Most current evidence for a biologic role of ERK1/2 signaling in cell proliferation or death and neuroprotection or neurodegeneration has relied on the use of MEK inhibitors (Hetman and Gozdz, 2004; Lu and Xu, 2006; Subramaniam and Unsicker, 2010; Cargnello and Roux, 2011). However, it is controversial whether the individual ERK isoforms share their pathophysiological functions or play distinctive roles due to the difficulty for MEK inhibitors to determine the specific contribution of each isoform to pathophysiological functions. Recently, this issue has been addressed by

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generating ERK1- and ERK2-deficient mice or utilizing RNAi approaches to overcome the non-specific inhibition of ERK isoforms by MEK inhibitors. Accumulating evidence suggests that ERK1 and ERK2 may play distinct functions (Matsumoto et al., 2005; Agrawal et al., 2006; Lloyd, 2006; Vantaggiato et al., 2006; Krens et al., 2008a; Krens et al., 2008b; Nakazawa et al., 2008), emphasizing the importance of identifying the influence of individual ERK isoforms in the neurodegeneration. Unfortunately, isoform specific inhibitors of ERK1 and ERK2 are not available. Therefore, understanding the specific functions of individual ERK isoforms is paramount for designing strategies that can spare the neuroprotective effects of ERK1/2 activity while attenuating the resultant pathology of ERK1/2 activation after neurodegeneration.

This review summarize recent evidence for distinct roles for individual ERK isoforms in pathophysiology of the CNS including cell death and proliferation of different cell types, excitotoxic damage, inflammatory responses, cognitive dysfunction, locomotor dysfunction, pain and downstream targets under physiologic and pathological conditions. Furthermore, this review also discuss the neuroprotective strategies involved in targeting individual ERK isoforms.

Distinct functions for ERK1 and ERK2 in physiology of the CNS

Distinct functions of ERK2 and ERK1 in cell processing during development

Despite their strong sequence homology, several studies have shown that ERK1 and ERK2 play specific roles in cell processes and brain function during development. Knockout of ERK2 is embryonically lethal (Yao et al., 2003), while ERK1 null mice are viable and do not exhibit any gross abnormalities compared to wild-type littermates (Selcher et al., 2001), which suggest that ERK2 is essential for the physiologic roles during development. In addition, ERK2 has been shown to be necessary for cell proliferation, while ERK1 functions as a negative regulator of cell proliferation by antagonizing ERK2 (Vantaggiato et al., 2006).

Important roles of ERK2 in neurogenesis have recently been investigated by conditional inactivation of murine ERK2 gene in neural progenitor cells of the developing cortex (Satoh et al., 2007; Samuels et al., 2008; Satoh et al., 2011b). ERK2 conditional knockout mice showed impaired proliferation of neural progenitors in cortex and ventricular zone, reduced generation of neurons, decreased cortical and ventricular thickness, and profound learning and memory deficits in water maze and eight-arm radial maze (Satoh et al., 2007; Samuels et al., 2008; Satoh et al., 2011b). ERK2 deficiency in humans is associated with microcephaly, impaired cognition, and developmental delay (Samuels et al., 2008). These results indicate that ERK2 promotes neurogenesis and neural differentiation, and is required for normal cognition and memory function during neural development. ERK1 seems not associated with learning as ERK2 knockdown mice showed unaltered ERK1 expression and were not compensated for in learning and memory. However, ERK1 deletion in ERK2 knockdown mice showed increased impairment of neurogenesis (Satoh et al., 2011b).

A requirement of ERK2 in glial progenitor proliferation was also reported by Imamura et al. (2010). They generated CNS-specific ERK2 knockout and ERK1$^{-/-}$ mice to investigate the individual and combined roles of ERK1 and ERK2 in the proliferation and differentiation of radial glial progenitors during cortical development. They demonstrated that ERK2 deficiency caused proliferation defects in radial glial progenitors within the ventricular zone, and a severe disruption of lamination in the cerebral cortex, while loss of ERK1 did not impair radial glial progenitor proliferation. However, in both CNS-specific ERK2 knockout and ERK1 1$^{-/-}$ mice, both ERK1 and ERK2 are required for radial glial maintenance and cortical lamination (Imamura et al., 2010).

Little is known about the specific function of ERK1 and ERK2 in cell processes of oligodendrocyte progenitors into mature oligodendrocytes, although the importance of ERK1/2 for oligodendrocyte progenitor proliferation and differentiation was previously recognized (Stariha and Kim, 2001). A recent study demonstrated that ERK1 knockout did not affect oligodendrocyte differentiation (Fyffe-Maricich et al., 2011). In contrast, loss of ERK2 resulted in fewer galactocerebroside-expressing mature oligodendrocytes in cortical cultures *in vitro* and a delay in the appearance of differentiated oligodendrocytes and in the expression of myelin basic protein, but not changes in the proliferation of oligodendrocyte progenitor cells *in vivo* (Fyffe-Maricich et al., 2011). These findings suggest that ERK2 contributes to a distinct function in the timing of oligodendrocyte differentiation and forebrain myelination but is not critical for the proliferation or survival of oligodendrocyte progenitor cells.

Under normal conditions, mature, myelinating oligodendrocytes are post-mitotic and do not proliferate. However, NGF treatment or complement complex C5b-9 can activate ERK1 in oligodendrocytes and induce proliferation of a subset of mature oligodendrocytes which was blocked with the use of an MEK inhibitor, thus suggesting the involvement of ERK1 in oligodendrocyte proliferation (Stariha and Kim, 2001).

Distinct roles of ERK2 and ERK1 in brain function during development

ERK2 conditional knockout (CKO) mice with specific deficiency of ERK2 in CNS showed viable, fertile, and a normal appearance, but marked deficits in multiple aspects of brain function (Satoh et al., 2007; Samuels et al., 2008; Satoh et al., 2011a; Satoh et al., 2011b). It was demonstrated that

ERK2 CKO mice with 20%–40% reduction of ERK2 showed deficits in learning and long-term memory, while ERK1 deficient mice did not show a significant impairment in learning ability (Satoh et al., 2007; Samuels et al., 2008; Satoh et al., 2011b). ERK1 knockout mice exhibit enhanced synaptic plasticity and learning, thought to be due to increased association of ERK2 with MEK (Mazzucchelli et al., 2002; Lloyd, 2006). ERK2 conditional knockout (CKO) mice in CNS also showed marked anomalies in several social behaviors including elevated aggressive behaviors, deficits in maternal nurturing, poor nest-building, low levels of social familiarity and social interaction, and decreased anxiety-related behaviors (Satoh et al., 2011a). ERK1 inhibition with MEK inhibitor SL327 in ERK2 CKO mice did not affect these brain functions. These results indicate that ERK2, but not ERK1, serves an important physiologic role in these social behaviors (Satoh et al., 2011a). On the other hand, ERK1 knockout mice showed enhanced fear extinction and reduced depression (Tronson et al., 2008). Moreover, recent studies provide evidence that ERK2 signaling in the hippocampal dentate gyrus contributes to the antidepressant effects of testosterone (Carrier and Kabbaj, 2012), while ERK1 participates in cocaine-sensitive mGluR5-dependent long-term depression in the Bed Nucleus of the Stria Terminalis (Grueter et al., 2006).

Distinct functions of ERK1 vs. ERK2 in pathology of the CNS

Distinct functions of ERK1 and ERK2 in excitotoxic damage and apoptosis of neurons and oligodendrocytes under pathological conditions

Glutamate toxicity has been strongly implicated a number of neurological disorders, including brain ischemia, neurodegenerative diseases, and neurotrauma (Tzschentke, 2002; Hallett and Standaert, 2004; Hynd et al., 2004; Wang and Qin, 2010). ERK1 and ERK2 are activated in excitotxicity-associated events (Yu and Yezierski, 2005; Lu and Xu, 2006; Zhuang and Schnellmann, 2006; Subramaniam and Unsicker, 2010; Wang and Qin, 2010; Yu et al., 2010). NMDA receptor-induced signaling can cause persistent action of ERK1/2 and also induces apoptosis of neurons *in vitro* and *in vivo*. Inhibition of ERK1/2 protects neurons from glutamatergic toxicity (Stanciu et al., 2000; Subramaniam and Unsicker, 2010). ERK1/2-mediated neuronal apoptosis has also been confirmed in various models of glutamate-induced neurodegeneration and CNS diseases (Yu and Yezierski, 2005; Lu and Xu, 2006; Zhuang and Schnellmann, 2006; Subramaniam and Unsicker, 2010; Wang and Qin, 2010; Yu et al., 2010).

ERK1 activation has been recently showed to protect against glutamate-induced cortical neuron death (Liu et al., 2009) and NMDA-induced retinal cell death, while ERK1 knockout is reported to exacerbate NMDA injury (Nakazawa et al., 2008). These results suggest that ERK1 plays a critical protective role against NMDA-induced neuronal death.

Excessive glutamate is also toxic to oligodendrocytes and to axons in white matter through AMPA receptors under excitotoxic conditions (Park et al., 2004; Matute et al., 2007; Matute, 2011). Although oligodendrocytes and astrocytes express glutamate receptors (Park et al., 2004), oligodendrocytes are particularly susceptible to the AMPA receptor-induced damage after neural injury, which undergo both necrosis and apoptosis at early time points, with apoptosis continuing at chronic time points. A recent study showed direct evidence that ERK1/2 activation mediates AMPA-induced oligodendroglial death *in vitro* and in a multiple sclerosis (MS) model (Domercq et al., 2011). Inhibition of ERK1/2 with U0126 significantly reduced AMPA-induced oligodendroglial death in the MS model. Therefore, ERK1/2-mediated glutamate toxicity plays major role in oligodendroglial death in the early phase postinjury resulting in axonal degeneration. Although oligodendroglial progenitor cell proliferative response occurs through activation of growth factor-ERK1/2 signaling starting at 2 weeks post-injury, this endogenous oligogenic response is insufficient against oligodendroglial loss and demyelination.

We recently showed that AMPA receptor-induced excitotoxic spinal injury leads to the injury-induced activation of the ERK→ELK-1 signaling cascades and transcriptional regulation of NMDA receptors and substance P receptors (Yu and Yezierski, 2005). Moreover, ERK2 siRNA reduces AMPA receptor-mediated cell death after excitotoxic injury (Yu et al., 2010). This result suggests the involvement of ERK2 in AMPA receptor-mediated cell death.

Distinct functions for ERK1 and ERK2 in activation and proliferation of microglia and astrogliosis after neuroinflammatory disease

Neuroinflammatory responses are the common pathophysiological hallmarks of neurodegenerative disorders, cerebral ischemia, and neurotrauma which include activation and proliferation of microglia and astrogliosis, infiltration of immune cells, activation of the adaptive immune system, and production and release of pro-inflammatory mediators (Fleming et al., 2006; Pajoohesh-Ganji and Byrnes, 2011; Pizza et al., 2011). ERK1/2 has been shown to undergo activation, resulting in pro-cell proliferation in microglia and astrocytes in response to a wide range of stimuli including inflammatory and immune stimuli, cytokines, nitric oxide, Fas ligand, growth factor withdraw, and various animal models of ischemia, neurotrauma and neurodegenerative diseases (Kaminska, 2005; Ji et al., 2009; Kaminska et al., 2009).

In these glial cells, ERK1/2 either directly promotes cell proliferation and indirectly activates mitogenic and inflammatory transcription factors (such as NF-KB) at both delayed

and chronic phases postinjury, leading to inflammatory responses, astrogliosis, and astroglial scar formation during neuroinflammation (Kaminska, 2005; Kaminska et al., 2009). Once inflammatory responses are activated, microglia and astrocytes release complex neurotoxic molecules toxic to neurons and oligodendrocytes, leading to progressive death of neurons and oligodendrocytes after ischemia, CNS injury, and neurodegenerative diseases (Park et al., 2004; Kaminska, 2005; Keane et al., 2006; Suter et al., 2007; Zhao et al., 2007; Kawasaki et al., 2008; Hulsebosch et al., 2009; Kaminska et al., 2009; Bardoni et al., 2010). Moreover, activation of astrocytes also releases scar-forming molecules and axonal regeneration inhibitors, such as chondroitin sulfate proteoglycans (CSPGs) which, in turns, cause glial scar formation and inhibition of axonal regeneration (Brambilla et al., 2009).

Although direct evidence on specific roles for ERK1 vs. ERK2 in proliferation of microglia and astrocytes is not available, indirect evidence indicates that ERK2 enhance cell proliferation, while ERK1 appears to have an antagonistic function, resulting in a reduction of cell proliferation (Lloyd, 2006; Vantaggiato et al., 2006; Indrigo et al., 2010). Moreover, ERK2 has been shown to mediate oxidative stress-induced activation of NF-KB that is a key transcription factor for upregulation of pro-inflammatory mediators (Zhou et al., 2011).

ERK1 deletion exacerbates neuroinflammation, demyelination, and paralysis after experimental autoimmune encephalomyelitis

Experimental autoimmune encephalomyelitis (EAE) is a Th1-mediated autoimmune disease of the CNS and has been widely used as an animal model for multiple sclerosis, a human demyelinating disease (Agrawal et al., 2006). Recent studies suggest that ERK1 plays an important role in controlling the Th1/Th2 balance in T cells and in antigen-presenting cells (APCs). ERK1 does not affect the development of T cells, B cells, or dendritic cells (DCs) *in vivo* (Nekrasova et al., 2005; Agrawal et al., 2006), but suppresses autoimmune Th1 immune responses and plays an important role in Th2 differentiation and survival (Agrawal et al., 2006). Considering the roles of ERK1 in the T cell-mediated autoimmune responses, Agrawal et al. examined the effect of ERK1 deletion on the development and progression of paralytic symptoms following experimental autoimmune encephalomyelitis (EAE). The results demonstrated that $ERK^{-/-}$ mice exhibit increased white matter damage and paralytic symptoms, develop severe neuroinflammation and demyelination in the spinal cord, and exacerbated EAE, compared with their wild-type counterparts (Nekrasova et al., 2005; Agrawal et al., 2006).

ERK2, but not ERK1, is required for CD8 T cell activation, proliferation and survival following stimulation of the TCR alone (D'Souza et al., 2008). ERK2 also plays a key role in efficient generation of antigen-specific IgG-bearing B cells because B cell-specific ERK2-deficient mice showed impaired T cell-dependent immune responses and reduced proportion of antigen-specific IgG1-bearing cells and the subsequent number of IgG1 antibody-secreting cells (Sanjo et al., 2007).

ERK2 knockdown reduces AMPA receptor-mediated cell death, white matter damage, and locomotor deficits after traumatic spinal cord injury

Recent studies demonstrated that ERK1/2 is sequentially activated in neurons, oligodendrocytes, microglia, and astrocytes after neural injury, and in turn mediates multiple seconday mechanisms implicated in the development and progression of locomotor deficits (Yu and Yezierski, 2005; Zhuang et al., 2005; Zhao et al., 2007; Genovese et al., 2008; Yu et al., 2010). Excitotoxic damage of neurons, oligodendrocytes (OLs), and axons (white matter), glial cell proliferation and glial scar formation, and pathological autoimmune responses are the underlying cause of locomotor deficits following thoracic spinal cord injury (SCI) (Park et al., 2004; Byrnes et al., 2007; Ankeny et al., 2009; Brambilla et al., 2009). Given the roles of ERK2 in excitotoxic neuronal death (Yu et al., 2010), NF-KB activation (Zhou et al., 2011), and cell proliferation, while ERK1 protects against excitotoxicity and neuroinflammation and regulates autoimmune responses, selective inhibition of ERK2 is of great importance in attenuating secondary damage and locomotor deficits while sparing the neuroprotective effects of ERK1 activity after SCI.

To examine whether ERK2 is involved in the injury-induced secondary injury and motor deficits after SCI, we used intrathecal administration of ERK2 siRNA or intraspinal microinjection of lentiviral-ERK2 shRNA to knock down spinal ERK2 following SCI in rats. Intrathecal treatment of ERK2 siRNA significantly reduced AMPA receptor-induced apoptosis after excitotoxic spinal cord injury. Intraspinal administration of the lentiviral ERK2 shRNA significantly improved locomotor function and white matter tissue sparing 6 weeks following severe SCI. These data suggest that ERK2 signaling is a deleterious contributor to excitotoxic cell death, the white matter damage, and associated motor deficits of spinal injury (Yu et al., 2010).

Distinct functions of ERK 1 and ERK2 in pain hypersensitivity

The important roles of ERK1 and ERK2 signal transduction pathways in the regulation of pain-related gene expression and the development and maintenance of pain hypersensitivity after inflammation, peripheral nerve injury (PNS) and CNS injury have been well documented (Ji and Woolf, 2001; Song et al., 2005; Yu and Yezierski, 2005; Zhuang et al.,

2005; Zhao et al., 2007; Ji et al., 2009). In particular, ERK1/2 is activated in spinal cord dorsal neurons, microglia, and astrocytes, and implicated in the enhanced excitability of spinal cord dorsal neurons in models of inflammatory pain and neuropathic pain. The activated ERK1/2 targets multiple downstream genes implicated in the development and maintenance of pain hypersensitivity, including glutamate and substance P receptors in nociceptive neurons and pro-inflammatory mediators (such as NF-KB, TNF-α, IL-1β, iNOS, COX2, PGE2, etc.) in microglia and astrocytes. ERK1/2 inhibition with MEK inhibitors (PD98059 and U0126) has proven to be an effective approach to suppress multiple pain-related genes and alleviate pain in several animal models of neuropathic pain (Ji and Woolf, 2001; Song et al., 2005; Yu and Yezierski, 2005; Zhuang et al., 2005; Zhao et al., 2007; Ji et al., 2009). Thus, ERK1/2 inhibition represents a rational therapeutic target for the treatment of pain hypersensitivity. However, ERK1/2 also contributes to anti-nociception (Siuciak et al., 1994; Pezet et al., 2002). For instance, BDNF, NT-3, and opioid receptor-mediated antinociception involves ERK1/2 pathways (Siuciak et al., 1994; Narita et al., 2002; Pezet et al., 2002). Although much is known about the roles of ERK1/2 in pain hypersensitivity, little is known about the specific functions of individual ERK isoforms. Recently, two studies on the distinct functions of ERK1 vs. ERK2 have been reported. Xu et al. utilized a ERK2 siRNA AAV vector to selectively inhibit the expression of ERK2 in neurons of the adult mouse spinal cord dorsal horn and demonstrated that ERK2, but not ERK1, knockdown attenuates complete Freund's adjuvant-induced pain hypersensitivity. These results indicate that ERK2 in the spinal cord dorsal horn is required for the development of inflammatory pain hypersensitivity (Xu et al., 2008). Using ERK1 knockout mice, Alter et al. revealed that ERK1 was not required for formalin-induced spontaneous behaviors, complete Freund's adjuvant-induced heat and mechanical hypersensitivity, and spared nerve injury-induced mechanical hypersensitivity (Alter et al., 2010). These results support a predominant role for ERK2 in the pain hypersensitivity.

Specific downstream substrates/targets and mechanisms of ERK1 and ERK2 in biologic function

More than 150 putative ERK1/2 substrates/targets have been identified to date (Yoon and Seger, 2006). However, only a few of specific substrates/targets for individual ERK isoforms are identified and summarized herein. In response to different stimulation, such as under physiologic or pathological conditions, individual ERK isoforms have been shown to regulate specific downstream targets positively or negatively. Under physiologic conditions, ERK1/2 plays an important role in cell proliferation. Individual ERK isoforms control cell proliferation through several mechanisms.

The N-Terminal domain of ERK1 responsible for the functional differences with ERK2

ERK1 and ERK2 are present in the cytoplasm of quiescent cells. Upon extracellular stimulation, active ERK1 and ERK2 accumulate in the nuclei of cells. Although the mechanisms responsible for nuclear accumulation of ERK1/2 remain unclear, a novel nuclear translocation sequence (NTS) located within the kinase insert domain was recently identified as a new nuclear translocation mechanism for ERK1/2 (Cargnello and Roux, 2011). More recently, Marchi et al. demonstrated that ERK1 shuttles between the nucleus and cytoplasm at a much slower rate than ERK2 due to the N-terminal domain of ERK1 and that reduced nuclear shuttling of ERK1 causes a strong reduction of ERK1 ability to carry proliferative signals to the nucleus. This mechanism significantly contributes to their differential functions in cell proliferation (Marchi et al., 2008).

Requirement of ERK2 for the cell cycle progression from G1 to S-phase

Analysis of gene disruption experiments demonstrated that proliferation rate, mitogenic factor requirement, magnitude of DNA synthesis stimulation, timing of re-entry into the cell cycle from G0, and the expression of ERK2 were unchanged in ERK1 deficient embryonic fibroblasts as compared to wide-type cells (Meloche and Pouyssegur, 2007). These results support that ERK1 is not required for the cell cycle progression, embryonic development and normal growth or fertility. The conditional ERK2-deficient mice were viable and appeared relatively normal. But the loss of ERK2 reduced cyclin D1 expression and pRb phosphorylation, impaired proliferation and self-renewal ability of neural stem cells in the ventricular zone, upregulated the JAK-STAT signaling pathway, reduced G1/S cell cycle in neural progenitor cells (NPC), downregulated neural stem cell markers, including Tenascin C NR2E1 and Lgals1, and upregulated several glial lineage markers, including GFAP, Ptprz1, Aldoc, S100β, and GLAST, during development. These results suggest that ERK2 is important both for the cell cycle progression and proliferation of neural stem cells in the VZ and in the maintenance of NPC multipotency by suppressing the commitment of these cells to a glial lineage during embryonic development (Imamura et al., 2008).

ERK1 antagonizing ERK2 signaling

Recent studies demonstrated that ERK1 genetic deletion or RNAi silencing promotes the proliferation of fibroblasts, whereas ERK2 RNAi reduces cell proliferation (Indrigo et al., 2010). Vantaggiato et al. also demonstrated similar results in cell proliferation with individual ERK knockdown and revealed a significant interplay between ERK1 and ERK2 in cell proliferation. For instance, loss of ERK1 caused a

marked mitogen-stimulated phosphorylation of ERK2 in ERK1 deficient embryonic fibroblasts and overexpression of ERK1, but not ERK2, attenuates NIH 3T3 cell proliferation and transformation. These results suggest that ERK1 acts as a negative regulator of cell proliferation in fibroblasts by restraining ERK2-dependent signaling. Based on these studies, it has been proposed that ERK1 and ERK2 have opposite roles in cell proliferation, with ERK1 antagonizing ERK2 signaling (Lloyd, 2006; Vantaggiato et al., 2006).

Distinct gene targets of ERK1 and ERK2 knockdown embryos

Analysis of microarray based on gene expression profiling of ERK1 and ERK2 knockdown in zebrafish embryos confirmed the specific function of ERK2 in the initiation, maintenance and patterning of mesoderm and endoderm formation and suggested that ERK1 and ERK2 have both common and distinct gene targets. ERK1 targets specific gene involved in dorsal-ventral patterning and subsequent embryonic cell migration, while ERK2 controls genes involved in cell-migration, mesendoderm differentiation and patterning (Krens et al., 2008a).

Distinct targets of ERK1 and ERK2 in innate and adaptive immune responses

The ERK1 and ERK2 have also been shown to play distinct roles in downstream substrates and targets on innate and adaptive immune responses. A major target of ERK 1 is the production of cytokines and antibodies. $ERK1^{-/-}$ mice showed enhanced IL-12p70 and reduced IL-10 production in response to TLR stimulation in DCs, reduced production of antigen-specific IgE and expression of Th2 cytokines IL-4 and IL-5 through its effect on JunB and BIM, higher IFN-γ production and lower IL-5 production in MOG35-55-primed T cells, and enhanced total antibody (IgG1, IgG2a, and IgG2b) titers in serum, compared with wide-type mice (Nekrasova et al., 2005; Agrawal et al., 2006; Goplen et al., 2012).

Two potential reasons for the preferential dependence of CD8 cells on ERK2 are the consequence of differences in the expression levels of ERK1 and ERK2, with ERK2 being expressed at much higher levels, and in the expression of downstream target Bcl-2 family members Bcl-XL and Bim. $ERK2^{T-/-}$ CD8 T cells showed reduced expression of the prosurvival members (Bcl-2 and Bcl-x) and increased levels of the proapoptotic member (Bim) compared with the WT controls. Importantly, $ERK1^{-/-}$ CD8 T cells did not show these differences with similar stimulation. ERK2 supports survival and generation of antigen-specific IgG1-expressing B cells through Bcl-2 survival signal (Sanjo et al., 2007; D'Souza et al., 2008).

Distinct targets of ERK1 and ERK2 under pathological conditions

ERK1 and ERK2 also play distinct roles in downstream targets including transcription factor NF-KB, glutamaterigic signaling, oxidative stress, and caspase 3 activation under pathological conditions. In a Barrett's EA cell line FLO cells transfected with pNF-KB-Luc, ERK2 knockdown with siRNA significantly reduced pNF-KB luciferase activity following acute oxidative stress (Zhou et al., 2011), suggesting that ERK2 activation may mediate H_2O_2-induced activation of NF-KB that is a major contributor to proliferation of microglia and astrocytes, inflammatory responses, and astroglial scar formation after neuronal injury (Brambilla et al., 2009). Deletion of ERK1 gene has been shown to reduce expression of c-Fos and enhance NMDA-induced neuronal death (Nakazawa et al., 2008), while ERK2 knockdown reduces AMPA receptor-mediated activation of caspase 3 (Yu et al., 2010). These data suggest that ERK1 activation may have protective effects during retinal excitotoxicity, while ERK2 activation contributes to apoptosis.

Conclusions: targeting individual ERK isoforms for neuroprotection

Evidence is accumulating that ERK1 and ERK2 have distinct functions in physiology and pathology of the CNS (Fig. 1). ERK2 activation is implicated in the secondary injury mechanisms and neurological dysfunction under pathological conditions, while ERK2 also plays essential physiologic roles in brain development and function. ERK1 antagonizes ERK2 signaling and protects against excitotoxicity, inflammation, and autoimmune disease of CNS. ERK2 reduction using viral vector shRNAs has been shown to protect against excitotoxicity, inflammation, motor disability and chronic pain after neuronal injury, representing a promising therapeutic strategy for spinal cord injury and pain hypersensitivity. Considering the evidence that ERK1 deletion exacerbates excitotoxicity, inflammation, demyelination, paralysis, and EAE, enhancement of ERK1 activity with gene therapy may provide neuroprotective strategy for autoimmune disease of the CNS and neurotrauma. Therefore, targeting individual ERK isoforms with reduced ERK2 and/or enhanced ERK1 activation may provide novel therapeutic strategies for inhibiting the pathological consequences of ERK2 over-activation, while sparing physiologic aspects of ERK function.

However, ERK2 knockdown as a treatment for neurodegenerative diseases, stroke, and traumatic brain injury, particularly in children, is not without potential consequence as ERK2 deficient human and mice show developmental delay or mental retardation.

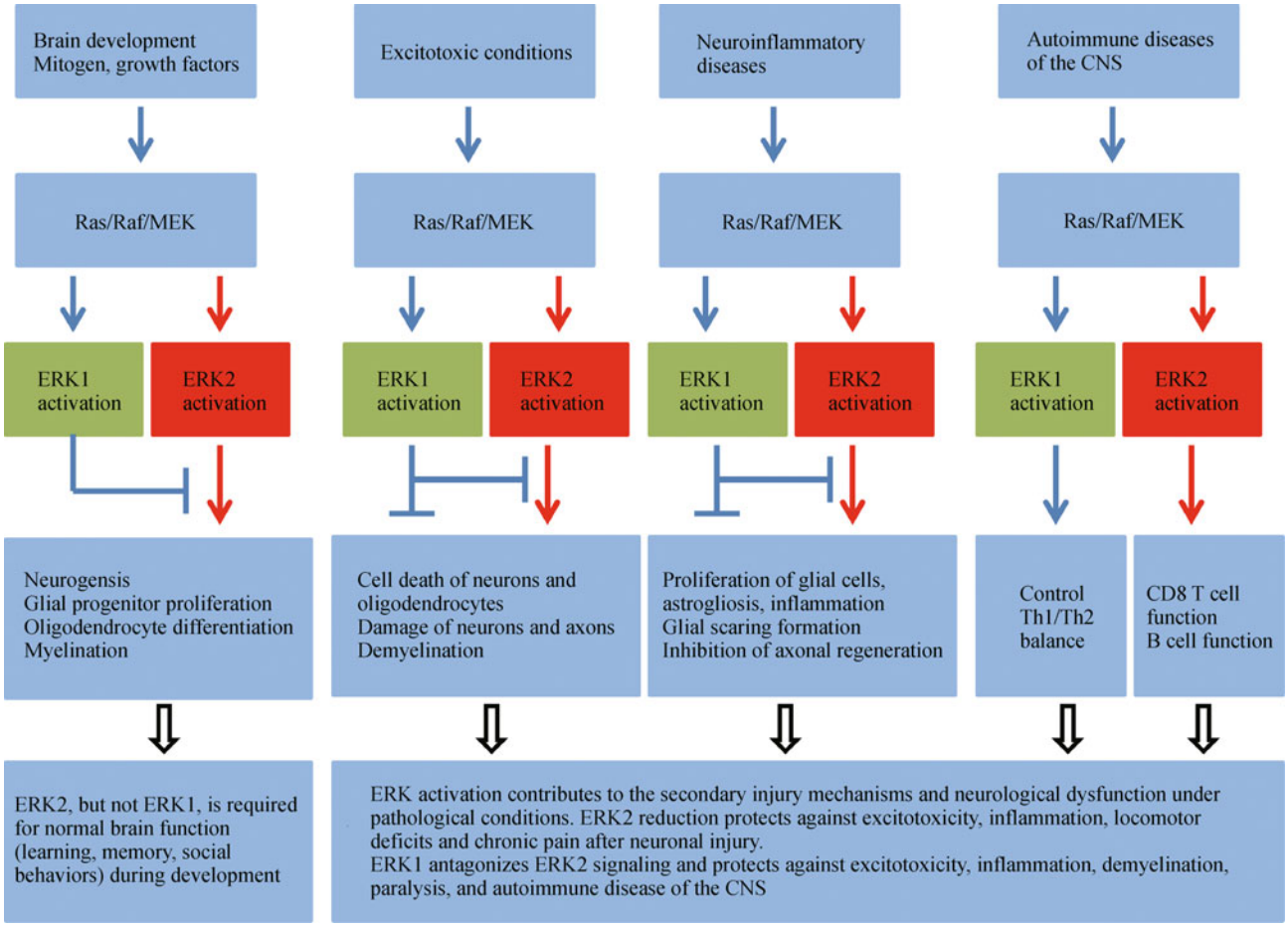

Figure 1 A diagram summarizing recent evidence for distinct functions for individual ERK isoforms in pathophysiology of the CNS.

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