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Zinc and the ERK Kinases in the Developing Brain

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Abstract

This article reviews evidence in support of the hypothesis that impaired activation of the extracellular signal-regulated kinases (ERK1/2) contributes to the disruptions in neurodevelopment associated with zinc deficiency. These kinases are implicated in major events of brain development, including proliferation of progenitor cells, neuronal migration, differentiation, and apoptotic cell death. In humans, mutations in ERK1/2 genes have been associated with neuro-cardio-facial-cutaneous syndromes. ERK1/2 deficits in mice have revealed impaired neurogenesis, altered cellularity, and behavioral abnormalities. Zinc is an important modulator of ERK1/2 signaling. Conditions of both zinc deficiency and excess affect ERK1/2 phosphorylation in fetal and adult brains. Hypophosphorylation of ERK1/2, associated with decreased zinc availability in cell cultures, is accompanied by decreased proliferation and an arrest of the cell cycle at the G0/G1 phase. Zinc and ERK1/2 have both been shown to modulate neural progenitor cell proliferation and cell death in the brain. Furthermore, behavioral deficits resulting from developmental zinc deficiency are similar to those observed in mice with decreased ERK1/2 signaling. For example, impaired performance on behavioral tests of learning and memory; such as the Morris water maze, fear conditioning, and the radial arm maze; has been reported in both animals exposed to developmental zinc deficiency and transgenic mice with decreased ERK signaling. Future study should clarify the mechanisms through which a dysregulation of ERK1/2 may contribute to altered brain development associated with dietary zinc deficiency and with conditions that limit zinc availability.

Keywords

Zinc; Brain; ERK; Brain development; Zinc deficiency; MAPK; Neuron

Introduction

Decreased zinc availability during gestation can have major deleterious effects on brain development. Several factors that are not yet fully understood can contribute to the teratogenicity of developmental severe zinc deficiency and to the potential deleterious effects of marginal zinc availability during gestation. Among them, oxidative stress,

imbalance of growth factors, impaired cell proliferation, and deregulated apoptotic cell death, could be involved (Uriu-Adams and Keen 2010). In fetal rat brain, extracellular signal-regulated kinase (ERK1/2) activation is markedly affected as a consequence of marginal dietary zinc deficiency. This review will discuss the relevance of both zinc and ERK1/2 for normal brain development, the role of zinc in the modulation of ERK1/2 in the brain, the putative role of ERK1/2 in zinc toxicity, and the potential involvement of ERK1/2 deficits in the altered brain development associated with zinc deficiency.

Zinc deficiency is also associated with altered behavioral patterns in experimental animals and in humans. Although the deleterious effects of zinc deficiency on behavior and cognition are accepted and reported to occur in human populations, there is limited knowledge on the mechanisms underlying those effects (Black 2003). This review will also discuss the adverse effects of zinc deficiency on behavior and the similarities found with those observed in ERK1/2 deficits. Finally, the role of zinc and ERK1/2 in the development and treatment of depression will be used as an example that highlights the relevance of this field to mental health.

Zinc and Brain Development

A significant risk of marginal zinc nutrition during pregnancy and early development has been shown to occur not only in the developing world, but worldwide (Briefel et al. 2000). Severe zinc deficiency can be deleterious for the conceptus, but also marginal dietary zinc levels during gestation can lead to major changes in signaling cascades that are central to brain development (Aimo et al. 2010b). Besides a low dietary zinc intake or poor food zinc availability (Briefel et al. 2000; Walsh et al. 1994), other conditions during pregnancy, including diabetes, maternal infections, chemotherapy, stress, and toxin exposure (e.g., arsenic, ethanol, or herbicides) can reduce fetal zinc availability (Coyle et al. 2009; Taubeneck et al. 1995; Taubeneck et al. 1994). In several of these conditions either brain teratogenicity or altered behavior has been reported in the offspring.

Zinc deficiency has a major adverse impact on prenatal and early postnatal development (reviewed in Uriu-Adams and Keen 2010). Seminal work from Hurley and Swenerton demonstrated for the first time that gestational severe zinc deficiency causes structural malformations in mammals (1966). In humans, pregnancy complications occur in a genetic disorder called acrodermatitis enteropathica, characterized by a defect in the zinc transporter Zip4 that causes decreased zinc absorption (Brenton et al. 1981). While Zip4 knockout mice die in utero, heterozygous embryos present malformations of the brain; and postnatally, the brain, heart, and skeleton (Dufner-Beattie et al. 2003). A deficit of Zip4 is associated with embryonic exencephalia, hydrocephalus, anophthalmia, and anopia (Dufner-Beattie et al. 2003). Similar teratogenicity is observed as a consequence of severe dietary zinc deficiency during gestation (Hurley and Swenerton 1966). Brain teratogenicity in Zip4 knockout mouse embryos was proposed to result from impaired neurogenesis (Dufner-Beattie et al. 2003). While gestational zinc supplementation increases neural progenitor proliferation in mice (Azman et al. 2009), zinc deficiency has also been shown to affect neurogenesis in the adult brain. Severe zinc deficiency imposed to adult rats or mice decreases neural progenitor cell proliferation in the dentate gyrus of the hippocampus (Corniola et al. 2008; Suh et al. 2009).

Severe zinc deficiency both during lactation and during adulthood has been shown to increase hippocampal apoptosis (Gao et al. 2009; Xu et al. 2011). Culture in zinc deficient media decreases the proliferation of IMR-32 neuroblastoma cells, causing an arrest of the cell cycle at the G0/G1 phase and the induction of apoptosis (Adamo et al. 2010). Although other mechanisms may also contribute to the teratogenicity of zinc deficiency, it is now clear that zinc availability can affect the balance of neurogenesis and apoptosis.

While zinc deficiency imposed late in development does not cause obvious malformations, postnatal zinc deficiency can affect brain development. Seminal work from Dvergsten et al. (1983) demonstrated the adverse impact of zinc deficiency on cerebellum development. They showed that severe zinc deficiency imposed during a critical period of rat cerebellum development (first postnatal 3 weeks) causes the persistence of the external granule cell layer, a thinner molecular layer and a decreased internal granule cell layer (Dvergsten et al. 1983). Suggesting an impaired neurogenesis, a lower number of cerebellum granule cells are observed in zinc deficient rats (Dvergsten et al. 1983). Zinc deficiency also affects the differentiation of basket, stellate (Dvergsten et al. 1984b), and Purkinje (Dvergsten et al. 1984a) neurons, which present a significantly impaired dendritic arborization. Thus, zinc deficiency during critical periods can have adverse impacts on brain development even when gross malformations are not detected.

Marginal zinc availability during early development does not cause overt signs of impaired brain development but may have subtle effects with significant consequences for brain function. In rats that were fed a marginal zinc diet during gestation, no obvious alterations in maternal and fetal outcome were observed (Aimo et al. 2010b). However, altered protein thiol homeostasis, tubulin oxidation, and impaired tubulin polymerization were observed in brains from marginally zinc deficient fetuses at embryonic day (E) 19 (Mackenzie et al. 2011). Furthermore, several signaling cascades, including ERK1/2, are affected by marginal zinc deficiency at E19 (Aimo et al. 2010b). Marginal zinc nutrition during gestation also affects the expression of Zn transporters, N-methyl-D-aspartate sensitive glutamate receptor (NMDAR) subunits (Chowanadisai et al. 2005), and myelin protein profiles in the postnatal rat brain (Liu et al. 1992). In mice, marginal zinc deficiency during gestation decreased the expression of nestin, a neurofilament often used as a marker for neural/glia progenitors (Wang et al. 2001). Although zinc may directly effect nestin transcription or translation, it is likely that these results reflect impaired proliferation, differentiation, or survival of progenitor cells. Alterations in hippocampal morphology (fewer cells and increased density) accompanied by impaired performance on a learning and memory test was observed in adult rats exposed to marginal zinc deficiency during gestation and lactation (Halas et al. 1986; Kawamoto and Halas 1984). These experiments demonstrate that decreased zinc availability, even mild or only at select windows of time during brain development, can have deleterious effects on brain physiology that persist into adulthood. Although marginal zinc availability during gestation does not cause major structural abnormalities, alterations in key signaling cascades could irreversibly affect brain cellularity and connectivity.

The Role of ERK1/2 in Neurodevelopment

The ERK1/2 kinases are members of the mitogen activated protein kinase (MAPK) family that is highly conserved among eukaryotes. ERK1 and ERK2 have greater than 95% amino acid identity among humans, mice, and rats (Charest et al. 1993). The amino acid sequences of the 44 kDa MAPK ERK1 (also known as MAPK3 and p44 MAPK) and of the 42 kDa MAPK ERK2 (also known as MAPK1 and p42 MAPK) are 84% identical (Boulton et al. 1991). ERK1 and ERK2 are activated by the same kinases, MEK (mitogen activated ERK kinase) 1 and 2, and appear to have highly overlapping substrate specificity (Gonzalez et al. 1991; Zheng and Guan 1993). A wide variety of extracellular stimuli are capable of activating the ERK1/2 cascade. Brain derived neurotrophic factor (BDNF) provides an example relevant to neural development (Fig. 1). BDNF binds to the extracellular domain of tropomyosin-receptor-kinase B (TrkB) leading to its activation, and autophosphorylation. The adaptor protein Shc binds to phosphotyrosine residues in the cytosolic domain of TrkB. Grb2 binds to Shc and recruits the guanine nucleotide exchange factor Son of Sevenless (Sos) to release GDP from the small GTPase Ras. Then, Ras binds GTP and activates Raf to phosphorylate MEK1/2 which subsequently phosphorylates ERK1/2. Once activated, ERK1/2 phosphorylates target proteins in the cytosol or nucleus to regulate important cellular functions such as proliferation, differentiation, apoptosis, and synaptic plasticity (reviewed in Minichiello 2009).

Cell culture experiments have implicated ERK1/2 signaling in nearly every aspect of neural development, but the physiological relevance of this data remains unclear. A group of genetic syndromes, collectively known as neuro-cardio-facial-cutaneous syndromes (NCFC), are associated with alterations in ERK signaling (reviewed in Samuels et al. 2009). While detailed analysis of brain development has not been conducted in these patients, it is clear that structural and functional abnormalities of the central nervous system are a common feature. Mental retardation, schizophrenia, bipolar disorder, and autism are among the neuropsychological disorders associated with NCFCs. Microdeletions of a region that contains the gene for ERK1 are associated with 1% of all cases of autism (Kumar et al. 2008; Weiss et al. 2008). These autistic individuals also exhibit facial and cardiac defects typical of NCFCs. Samuels et al. analyzed lymphoblast ERK2 levels from two NCFC patients with microcephaly and cognitive deficits associated with deletions encompassing the ERK2 gene (2008). These patients had approximately 61% of the ERK2 concentration found in normal individuals, suggesting that mutations in one of the four alleles for ERK1/2 may be sufficient to produce severe structural and behavioral abnormalities. In general, mutations that increase ERK1/2 activity can result in macrocephaly, while mutations that decrease ERK1/2 activity can result in microcephaly (Samuels et al. 2009) suggesting that ERK1/2 activity can control the expansion of human neural progenitor cells.

The recent use of transgenic mice has enabled significant advancements in understanding the role of ERK1/2 in brain development and function. While ERK1 knockout mice have no gross morphological abnormalities and only a subtle behavioral phenotype (Mazzucchelli et al. 2002), ERK2 knockout mice die in utero (Satoh et al. 2007). The role of ERK2 in neural development was studied by conditional deletion of ERK2 in neural precursor cells using the nestin promoter to drive Cre recombinase expression. Surprisingly, these mice developed

grossly normal brain structures, and no behavioral abnormalities were initially detected (Heffron et al. 2009). However, more detailed analysis revealed a decreased proliferation of neural progenitor cells at E15.5 and E17.5 resulting in a reduced number of astroglial cells, and thinning of cortical layers II/III. These results suggest a defect in late born cortical neurons and glia. At postnatal days (P) 35–40 these mice develop astrogliosis, collagen deposition, and increased microvasculature of the frontal cortex (Imamura et al. 2008; Imamura et al. 2010). A recent report also showed these animals have impaired social behavior and long term memory (Sato et al. 2011). Another mouse model was developed to delete ERK2 in radial glia, using the glial fibrillary acidic protein (GFAP) promoter to drive Cre recombinase expression. These mice present decreased proliferation of intermediate progenitor cells, reduced cortical thickness, decreased number of neurons, increased number of astrocytes, and delayed oligodendrocyte differentiation, with no change in oligodendrocyte progenitor survival or proliferation (Fyffe-Maricich et al. 2011; Samuels et al. 2008). This structural phenotype was accompanied by impaired long-term memory.

ERK1 knockout mice have recently been used in combination with conditional deletion of ERK2 in neural progenitor cells expressing Cre recombinase under the nestin promoter (Imamura et al. 2010). These mice present decreased proliferation of progenitors in the ganglionic eminence and disruption of their tangential migration resulting in a reduced number of cortical oligodendrocytes and inhibitory interneurons (Imamura et al. 2010). At birth, ERK1/2 double knockout mice have increased numbers of apoptotic cells and abnormal neurons with a stem cell-like cytology. A further decreased neural progenitor cell proliferation was found in the ERK1/2 double knockout compared to the ERK2 conditional knockout mice. These findings suggest that total ERK1/2 activity controls the proliferation of certain late born progenitor cells and the differentiation of subpopulations of neurons and glia during fetal brain development (Fyffe-Maricich et al. 2011; Imamura et al. 2010).

In summary, mice with ERK1 and ERK2 deficits have provided strong evidence on the critical role of these kinases for brain development. In models of ERK2 deficiency an increased ERK1 phosphorylation occurs, while ERK1 knockout mice present increased ERK2 phosphorylation (Mazzucchelli et al. 2002). Because these kinases are activated by the same kinases and have overlapping substrate specificity; the fact that ERK1 deficits exacerbate the defects of conditional ERK2 knockout mice suggests that both kinases might compensate for each other during brain development having, at least in part, overlapping functions.

ERK Modulation by Zinc

In the central nervous system endogenous zinc released from synaptic vesicles stimulates ERK1/2 activity. In neurons zinc is not only a structural/functional component of proteins, membranes, and chromatin; but is also sequestered in synaptic vesicles of a subset of neurons by the zinc transporter ZnT3 (Cole et al. 1999). The most thoroughly investigated example of vesicular zinc function is at the terminals of mossy fibers projecting from the dentate gyrus to CA3 in the hippocampus where zinc is released from glutamatergic vesicles in response to depolarization-induced calcium influx (Ketterman and Li 2008). In the synapse, zinc may affect neurotransmission directly by inhibiting receptors such as the

NMDAR (Molnar and Nadler 2001) or indirectly by modulating cell signals such as ERK1/2. Several methods have implicated synaptic zinc in the activation of ERK1/2 signaling. As discussed below, ERK phosphorylation increases with zinc supplementation and decreases when synaptic zinc is eliminated (Besser et al. 2009; Sindreu et al. 2011).

Regulation of ERK1/2 by zinc may be involved in mechanisms of synaptic plasticity contributing to learning and memory. It was initially reported that a genetic deficit of ZnT3 did not affect behavior in mice (Cole et al. 2001). However, an impairment of hippocampus-dependent spatial memory and contextual discrimination accompanied by decreased hippocampal ERK1/2 phosphorylation was recently observed in these mice (Adlard et al. 2010; Sindreu et al. 2011). Transfection of the dentate gyrus with a dominant negative form of MEK1 resulted in memory impairments similar to those observed in ZnT3 knockout mice (Sindreu et al. 2011). These findings support previous observations that zinc and ERK1/2 are involved in mechanisms of synaptic plasticity such as long-term potentiation in the hippocampus (Takeda et al. 2009; Kanterewicz et al. 2000). However, the literature is inconsistent with regard to the role of zinc in potentiation of the mossy fiber-CA3 synapse (Reviewed in Nakashima and Dyck 2009). In addition, pharmacological inhibitors of ERK1/2 signaling disrupt long-term potentiation at CA1 but not CA3 (Kanterewicz et al. 2000). Although zinc may stimulate ERK1/2 to modulate synaptic plasticity in the hippocampus, the mechanistic details remain obscure.

In the hippocampus endogenous zinc can modulate cell proliferation by regulating ERK1/2 signaling. The subgranular zone of the dentate gyrus is one of the few sites where neurogenesis occurs in the adult brain. In this area, markers of neurogenesis (Ki67 and doublecortin) are decreased in ZnT3 knockout mice and in rats exposed to dietary zinc deficiency or injected intraperitoneally with the zinc chelator clioquinol (Suh et al. 2009). Zinc released from mossy fibers has been proposed to activate ERK1/2 in postsynaptic CA3 neurons as well as presynaptic mossy fibers (Besser et al. 2009; Sindreu et al. 2011). Induction of status epilepticus in mice by intraperitoneal injection of the muscarinic acetylcholine receptor agonist pilocarpine stimulates neurogenesis while injection of U0126 (a MEK1/2 inhibitor) into the dentate gyrus attenuates this effect (Choi et al. 2008). Interestingly, this pilocarpine-induced model of status epilepticus stimulates the release of zinc from mossy fiber terminals (Suh et al. 2001). Along with the findings from zinc deficiency and depression discussed below, the above findings support a significant role for zinc in the activation of the ERK1/2 pathway leading to cell proliferation.

While it is well established that zinc can stimulate ERK1/2 signaling in a wide range of cell types, various mechanisms can underlie this effect including: (i) the capacity of zinc to modulate select protein phosphatases and kinases, (ii) the direct binding of zinc to cell surface receptors, and (iii) the activation of matrix metalloproteinases (MMPs) by zinc (Fig. 2).

Indirect evidence suggests that zinc can affect ERK1/2 activation through the modulation of select phosphatases. Decreased ERK1/2 phosphorylation was observed in mossy fibers of ZnT3 knockout mice (Sindreu et al. 2011). While there were no differences in MEK1/2 phosphorylation, ERK1/2-directed tyrosine phosphatase activity was increased in

hippocampal lysates from Znt3 knockout mice. Furthermore, addition of zinc to these lysates reduced ERK1/2-directed tyrosine phosphatase activity. This suggests that zinc released from mossy fibers enters presynaptic axons and stimulates ERK1/2 signaling by directly inhibiting tyrosine phosphatase activity (Sindreu et al. 2011).

Zinc could indirectly activate cell surface receptors by inhibiting the activity of specific kinases. Zinc has been proposed to inhibit C-terminal Src kinase (Csk) leading to the activation of TrkB and the downstream ERK1/2 cascade at the mossy fiber-CA3 synapse in the absence of neurotrophic factors (Huang et al. 2008). In vitro, zinc directly inhibits Csk kinase activity with an IC_{50} of 0.5 μ M. Csk inhibits the Src family kinases (SFK) by phosphorylating tyrosine 530. Treatment of BDNF-deficient neurons with zinc decreases SFK phosphorylation at tyrosine 530 which increases its activity to phosphorylate TrkB (Zambuzzi et al. 2008). Once activated TrkB can stimulate the ERK1/2 cascade as described above. Accordingly, in neuronal cultures from BDNF null mice, zinc supplementation increases the levels of TrkB and ERK1/2 phosphorylation (Huang et al. 2008).

Direct activation of cell surface receptors by zinc can also stimulate the ERK1/2 cascade. Zinc can bind to the recently identified zinc receptor, also known by the systematic name G-protein coupled receptor 39 (GPR39). GPR39 is a member of the ghrelin receptor family initially proposed to be the receptor for obestatin, a peptide derived from proghrelin. However, cells expressing GPR39 respond to zinc but not to obestatin (Holst et al. 2007). CA3 neurons in the mouse hippocampus express a GPR39 receptor that is responsive to zinc. Recent evidence shows that zinc binds to GPR39 and activates G_{α_q} to stimulate phospholipase C leading to calcium influx and activation of the ERK1/2 cascade (Besser et al. 2009). However, further experiments using GPR39 knockout mice or RNA interference approaches are needed to definitively demonstrate that zinc can act through GPR39 to induce ERK1/2 phosphorylation.

With regard to MMPs, in rat cortical neurons (RCN) zinc causes the activation of extracellular MMP-2 and MMP-9. Activated MMPs stimulate the cleavage of pro-BDNF to the active form (Hwang et al. 2005). Zinc supplementation increases the activity of MMP-2 and MMP-9 in the brains of triple transgenic mice that model Alzheimer's disease (Corona et al. 2010). Accordingly, the BDNF concentration is increased in the brains of zinc supplemented mice and rats (Corona et al. 2010; Cieslik et al. 2011). By increasing MMP activity zinc can increase the concentration of active BDNF leading to increased phosphorylation of ERK1/2.

Zinc can also stimulate transcription of the BDNF gene. Zinc supplementation leads to increased BDNF mRNA expression in the adult rat brain (Cieslik et al. 2011). The zinc finger transcription factor cyclic-AMP responsive element binding protein (CREB) can stimulate transcription of BDNF (reviewed in Minichiello, 2009). Although the mechanism of zinc induced BDNF expression has not been elucidated, zinc deficiency decreased CREB phosphorylation in mice (Gao et al. 2011) suggesting that zinc could modulate CREB activity. ERK1/2 phosphorylates the kinases RSK and MSK which can directly phosphorylate CREB (Impey et al. 1998). By activating ERK1/2, zinc might stimulate

CREB to increase the expression of BDNF which; after translation, secretion, and cleavage; could function as an autocrine or paracrine factor to further activate local ERK1/2 signaling.

The above evidence (summarized in Fig. 2) indicates that zinc can have a major role in ERK1/2 activation, exerting modulatory actions at various levels. However, further study is needed to demonstrate which mechanisms are responsible for the decreased ERK1/2 phosphorylation resulting from dietary zinc deficiency.

The Role of ERK1/2 in the Neurotoxicity of Zinc

At toxic levels zinc may stimulate ERK1/2 dependent cell death. In the brain, toxic levels of zinc can occur in certain pathological conditions such as epileptic seizures and transient ischemia. During transient ischemia zinc accumulates in affected neurons before cell death. Supporting toxicity, injection with a zinc chelator decreased cell death in a rat model of transient ischemia (Koh et al. 1996). While toxic levels of zinc cause oxidative stress by inhibiting the electron transport chain in mitochondria (Ye et al. 2001), the toxicity of zinc in the brain may also require ERK1/2. In fact, the MEK inhibitor U0126 prevented mitochondrial dysfunction and cell death in neuronal- glial cell cultures exposed to 100 μ M zinc (He and Aizenman 2010). Furthermore, injection of MEK inhibitors PD98059 or U0126 before cerebral ischemia results in a decreased infarct size (Alessandrini et al. 1999); (Namura et al. 2001). However, interpretation of these results depends upon the specificity of these compounds, and U0126 can inhibit other kinases, such as p38 and Akt (Davies et al. 2000). These inhibitors have also been used to support a neuroprotective role for ERK1/2 signaling during transient ischemia. For example, treatment with leptin before transient ischemia reduced cell death, but PD98059 abrogated this effect (Zhang et al. 2007). Although signaling through the ERK1/2 cascade may be necessary for both toxic and protective mechanisms during transient ischemia, further research using specific techniques for gain and loss of function experiments is required to elucidate whether or not the ERK1/2 cascade is necessary and sufficient to cause cell death when zinc accumulates at toxic levels in the brain.

Zinc Deficiency, ERK1/2, and Brain Development

Zinc deficiency differentially affects the MAPKs both in cell cultures and E19 rat brain. Incubation of IMR-32 human neuroblastoma cells in zinc deficient media for 24 h caused a lower phosphorylation of ERK1/2, and a higher phosphorylation (45 and 90%, respectively) of p38 and JNK1/2, compared to controls (Fig. 3a–c). Although activated by multiple stimuli, p38 and JNK are particularly sensitive to oxidative stress, a condition frequently associated with zinc deficiency (Mackenzie et al. 2006). In fact, incubation of IMR-32 cells in zinc deficient media causes an increase in cellular oxidants that can be prevented by simultaneous treatment with the H₂O₂-metabolizing enzyme catalase, and with substances (α -lipoic acid and N-acetyl cysteine) that exert antioxidant effects through various mechanisms of action (Mackenzie et al. 2006). An increase in cellular oxidants occurs, at least partially, as a consequence of zinc deficiency-induced activation of the NMDAR, calcium influx, and associated activation of NADPH oxidase, an enzyme that generates superoxide anion (Aimo et al. 2010a). As expected, catalase, α -lipoic acid, and N-acetyl

cysteine prevent zinc deficiency-induced phosphorylation of p38 and JNK (Fig. 3c), which indicates an oxidant-triggered activation of these MAPK by zinc deficiency in neuronal cells (Mackenzie et al. 2006). However, antioxidants do not prevent zinc deficiency-associated apoptotic cell death (Adamo et al. 2010). This suggests that JNK, p38, and downstream transcription factor AP-1 do not play a major role in zinc deficiency-induced altered neuronal survival. On the other hand, the 40% decreased phosphorylation of ERK1/2 was not prevented by catalase (Fig. 3b) suggesting a lack of association between zinc deficiency-induced oxidative stress and ERK1/2 hypophosphorylation. Nevertheless, oxidative stress may affect other aspects of ERK1/2 modulation. In zinc deficient cells, tubulin oxidation and impaired polymerization affect the microtubule-dependent nuclear transport of transcription factor NF- κ B (Mackenzie et al. 2011). Given that ERK1/2 bind to microtubules (Reszka et al. 1995), zinc deficiency-induced alterations of the cytoskeleton could affect ERK1/2 activity and nuclear transport. A similar pattern of low ERK1/2 phosphorylation and high p38 and JNK phosphorylation was recently observed in E19 fetal brains when dams were fed a marginal zinc diet throughout gestation (Aimo et al. 2010b) (Fig. 3d). Thus, even under marginal dietary zinc levels, low levels of ERK1/2 phosphorylation are present in fetal brain. Given that a marginal zinc nutrition/availability can occur in human populations, the above findings stress the need to understand the relevance of this cascade in zinc deficiency-induced altered brain development.

A deficit in ERK1/2 could underlie the impaired cell proliferation during zinc deficiency. ERK1/2 plays a major role in the regulation of cell proliferation, and mutations in members of the Ras/Raf/MEK/ERK1/2 pathway are frequently found in neoplasia (McCubrey et al. 2008; Steelman et al. 2004). Several proteins involved in the regulation of cell proliferation are targets of ERK1/2 kinase activity, including RSK, c-Myc, Foxo3a, and ELK. Continuous ERK1/2 activation and translocation into the nucleus during the G1 phase, which downregulates anti-proliferative genes, are required for the progression of the cell cycle from G1 into S phase (Brunet et al. 1999; Yamamoto et al. 2006). Supporting a role for ERK1/2 in zinc deficiency-induced decreased cell proliferation, a decreased phosphorylation of ERK1/2 and cell cycle arrest at the G0/G1 phase were observed in zinc deficient IMR-32 cells induced to proliferate by serum starvation and repletion (Adamo et al. 2010). Growth factors promote a biphasic activation of ERK1/2, a first burst occurring within minutes, and a later activation sustained for hours (reviewed in Mebratu and Tesfagzi 2009). Although not tested at early time points, we observed that ERK1/2 phosphorylation in zinc deficient cells remains low within 6–24 h after growth factor stimulation (Adamo et al. 2010). Accordingly and as previously discussed, in the nervous system ERK1/2 promotes neurogenesis (Miller and Gauthier, 2007), and genetic ERK1/2 deficits are associated with impaired neurogenesis (Samuels et al. 2008).

Dysregulation of ERK1/2 might contribute to increased apoptosis during zinc deficiency. In cell cultures, zinc deficiency-induced ERK hypophosphorylation is associated with cell cycle arrest and apoptotic cell death (Adamo et al. 2010). In this regard, incubation of IMR-32 cells in zinc deficient media (0.5 and 5 μ M zinc) is associated with a decrease in cellular labile zinc levels, ERK1/2 phosphorylation, and cell viability (Fig. 3a). While ERK1/2 is inhibited, the pro-survival signal Akt is activated by decreased zinc availability (Adamo et al. 2010). Akt and ERK1/2 can prevent apoptosis in part by phosphorylating the

pro-apoptotic protein Bad, which prevents its translocation from the cytosol to the mitochondria. At the mitochondria Bad initiates a series of events that lead to mitochondrial cytochrome c release into the cytosol, caspase activation, and subsequently apoptosis. The permanence of Bad at the cytosol requires phosphorylation at three different serine residues (Zha et al. 1996). Zinc deficiency leads to decreased Bad phosphorylation at serine 75 (Adamo et al. 2010), which is catalyzed by protein kinase A (Yang et al. 2005) and the ERK1/2 target RSK (She et al. 2002; Zhu et al. 2002). Thus, a dysregulation of Bad phosphorylation due to impaired ERK1/2 activation may explain mitochondrial translocation of Bad, cytochrome c release, caspase 3 activation, and DNA fragmentation in zinc deficient human IMR-32 cells and RCN (Adamo et al. 2010).

ERK-mediated alterations in cell proliferation/apoptosis/differentiation patterns could underlie the nervous system, cardiac and craniofacial abnormalities, and cognitive alterations associated with genetic deficits of ERK1/2 in mice and humans (Samuels et al. 2009), and in developmental zinc deficiency (Hurley and Swenerton 1966). Furthermore, the craniofacial abnormalities found in Zip4 heterozygous embryos are similar to those found in dietary zinc deficiency (Hurley and Swenerton 1966) and in genetic ERK deficits (Samuels et al. 2009). In fact, both conditions affect a subset of progenitor cells, neural crest cells, that contributes to the formation of those tissues/structures (Lopez et al. 2008; Samuels et al. 2009).

Zinc Deficiency, ERK1/2, and Behavior

Zinc deficiency affects cognitive function and behavior in humans. Low plasma zinc levels are associated with altered cognition and emotional functioning in 3–5 year old children from low income families (Hubbs-Tait et al. 2007). Developmental zinc deficiency is associated with altered emotional behavior, food motivation (Arnold and DiSilvestro 2005; Golub et al. 2000), cognitive performance, and psychomotor development (Bentley et al. 1997; Gardner et al. 2005; Kirksey et al. 1994; Penland et al. 1997). Intervention trials have shown that zinc supplementation can improve neurological and psychological development in undernourished children (Bentley et al. 1997; Bhutta et al. 1999; Brown et al. 2002; Gardner et al. 2005; Penland et al. 1997; Sazawal et al. 2001). However, some studies have also reported null (DiGirolamo et al. 2010), or even negative effects of zinc supplementation on children's cognitive development (Hamadani et al. 2001; Hamadani et al. 2002). Some children may benefit from supplementation more than others making it difficult for these trials to reach statistical significance. Also, it is important to consider nutrient–nutrient interactions when interpreting intervention trials. For example, zinc supplementation could result in copper deficiency (Rowin and Lewis 2005). Future, interventions should try to carefully monitor the status of zinc and nutrients that may interact with zinc such as copper, iron, and vitamin A (Sandstrom 2001).

Zinc deficiency may contribute to the pathophysiology of attention deficit-hyperactivity disorder (ADHD). Patients with ADHD have lower serum zinc concentrations than age-matched controls, and serum zinc concentration correlates with parent and teacher scores of inattentiveness (Arnold et al. 2005; Toren et al. 1996). While this correlation does not provide evidence for a causal connection, zinc restriction in animal models can cause

attention deficits. Rhesus macaques fed moderate zinc deficient diet displayed impairments on a continuous performance task designed to evaluate vigilance of attention (Golub et al. 1996). Zinc supplementation has shown promise in some clinical trials, but results have been inconsistent. Two randomized double blind placebo controlled trials among school aged children in Turkey showed significant therapeutic effects of zinc supplementation on measures of ADHD (Bilici et al. 2004; Uckardes et al. 2009). A trial conducted in Iran found significant improvement when zinc supplementation was given with the psychostimulant methylphenidate (Akhondzadeh et al. 2004). However, a trial conducted with children in the United States was negative, except for decreasing the optimal dose of amphetamine (Arnold et al. 2011). Given that zinc deficiency is more prevalent in Turkey and Iran than in the United States, this discrepancy may reflect differences between the populations. Indeed, zinc supplementation increased serum zinc levels in the Turkish studies but not in the American trial (Arnold et al. 2011). Thus, further study is needed to understand the relationship between zinc status and ADHD.

Zinc deficiency during development can have behavioral consequences, such as impairments of learning and memory, which persist into adulthood. Zinc deficient animals display anorexia, impaired social behavior, increased aggression, anxiety, decreased activity, impaired learning, and memory deficits (Golub et al. 1995). To discriminate between the effects of protein/energy malnutrition and zinc deficiency it is often necessary to use pair-fed controls that consume the same amount of food as the zinc deficient animals (Golub et al. 1995; Halas et al. 1986b; Halas and Sandstead 1975; Keller et al. 2001). Compared to pair fed controls, severely zinc deficient dams fail to build a nest, or consume the afterbirth, suggesting an impairment of maternal behavior resulting specifically from zinc deficiency (Apgar 1968). In young mice 2 weeks of severe zinc deficiency increased aggression on the resident intruder test (Takeda et al. 2008). Compared to pair-fed animals, young rats fed zinc deficient diets display increased latency on the Morris water maze indicative of spatial memory impairment (Keller et al. 2001). While severe zinc deficiency during the last third of gestation also caused lasting memory impairments (active avoidance fear conditioning test and Morris water maze), gestational zinc supplementation improved performance on the Morris water maze (Halas and Sandstead 1975; Tahmasebi Boroujeni et al. 2009). Perhaps the most striking findings are the persistence of memory deficits resulting from transient or mild zinc deficiencies. Even a marginal zinc deficiency during development can cause learning and memory impairments in rats that persist into adulthood. Marginal zinc deficiency during gestation and lactation resulted in impairments of working memory (increased rate of errors on the seventeen-arm radial maze) later in life which correlated with alterations in hippocampal morphology (Halas et al. 1986; Hunt et al. 1984).

Similarities between mice with genetic ERK1/2 deficits and animals exposed to developmental zinc deficiency suggests that hypophosphorylation of ERK1/2 may contribute to the effects of developmental zinc deficiency on behavior. Satoh et al. recently demonstrated impaired social behaviors in mice with conditional deletion of ERK2 in neural progenitor cells (2011). Similar to zinc deficient animals, nest building behavior was impaired and aggression (measured with the resident intruder test) increased. Impairments of long term memory performance on the fear conditioning tests have been observed in mice that express a dominant negative MEK1 or have conditional deletions of ERK2 in neural

progenitor cells (Satoh et al. 2011; Shalin et al. 2004). Even a partial decrease in ERK2 expression (20–40%) in mice impairs learning and memory performance on the Morris water maze, eight-arm radial maze, and contextual/cued fear conditioning tests (Satoh et al. 2007). While ERK2 deficiencies disrupt social behavior, learning, and memory, ERK1 knockout mice present a subtle phenotype characterized by hyperactivity, and sensitivity to amphetamine- and cocaine-induced hyperlocomotion (Creson et al. 2009; Engel et al. 2009; Ferguson et al. 2006) that can be attenuated by antipsychotic agents such as lithium, valproate, and olanzapine (Engel et al. 2009). ERK1 knockout mice also have enhanced reward seeking behavior mediated by long term-potential at the nucleus accumbens, a target of striatal dopaminergic projections implicated in addiction. Suggesting that increased ERK2 signaling is responsible for reward seeking behavior of ERK1 knockout mice, injection of the MEK inhibitor U0126 attenuated ERK2 phosphorylation and restored long term potential at the nucleus accumbens (Mazzucchelli et al. 2002). Differences in the phenotypes of ERK1 and ERK2 are not well understood. However, in the brain ERK2 is more abundant than ERK1 and the severity of knockout phenotypes may reflect this difference. Further study is needed to understand the mechanisms responsible for these behavioral phenotypes, but it is clear that a disruption of ERK1/2 signaling can have adverse effects on behavior similar to those observed in developmental zinc deficiency.

Many drugs used to treat psychological disorders including schizophrenia (e.g., haloperidol) (Kim et al. 2008), bipolar disorder (e.g., lithium, valproate) (Creson et al. 2009; Hao et al. 2004), ADHD (e.g., amphetamine) (Pascoli et al. 2005), depression and anxiety (e.g., fluoxetine, imipramine, *Hypericum perforatum*) (Kim et al. 2011; Neary et al. 2001; Qi et al. 2008) activate the ERK1/2 pathway. Roles for zinc in the pathophysiology of schizophrenia (Andrews 1992), ADHD (Bilici et al. 2004), depression (Maes et al. 1997), and anxiety (Takeda et al. 2007), have all been hypothesized. Detailed analysis of these hypotheses is beyond the scope of this review, but the potential role of zinc and ERK1/2 in the pathophysiology and treatment of depression will be discussed to explore the potential therapeutic benefits of zinc supplementation.

The Role of Zinc and ERK1/2 in Depression

Zinc deficiency may contribute to the development of depression. Several investigators have reported low zinc status among patients with depression, and some have found a correlation between serum zinc concentrations and the severity of depression (Amani et al. 2010; Maes et al. 1994; McLoughlin and Hodge 1990). Furthermore, serum zinc status may be a predictor of resistance to treatment (Maes et al. 1997). In support of this hypothesis, fluoxetine improved forced swim test performance of zinc adequate and zinc supplemented, but not of zinc deficient rats. However, desipramine or *H. perforatum* can improve performance of zinc deficient rats on the forced swim test. It is possible that depression results in low serum zinc levels. Indeed, Maes et al. (2009) proposed a role for inflammation in the etiology of depression; and inflammatory processes can stimulate the expression of acute phase proteins, such as metallothioneins, that sequester zinc in the liver decreasing plasma zinc. On the other hand, Amani et al. found that dietary zinc intake correlated with serum zinc levels and with the severity of depression suggesting an etiological role for zinc deficiency (2010). Furthermore, rats fed zinc deficient diets exhibit depression-like

symptoms (Tassabehji et al. 2008; Whittle et al. 2009) and zinc supplementation has an antidepressant-like effect (Franco et al. 2008) suggesting that zinc deficiency contributes to the pathophysiology of depression. Randomized double blind placebo controlled trials have consistently shown that zinc supplementation can decrease measures of depression in humans (Nowak et al. 2003; Sawada and Yokoi 2010).

Low zinc availability may decrease ERK1/2 activity and suppress neural progenitor proliferation contributing to depression. Decreased hippocampal volume and levels of BDNF have been found in depressed patients and higher hippocampal volume and BDNF concentrations were associated with antidepressant therapy (Chen et al. 2001; Sheline et al. 2003). Furthermore, protocols designed to induce depression-like behavior in mice and rats lead to decreased BDNF expression and ERK phosphorylation (Qi et al. 2006; Smith et al. 1995). Not only does zinc have antidepressant-like properties, but antidepressant therapies (including imipramine and electroconvulsive therapy) also stimulate an increase in zinc and BDNF concentrations along with increased ERK1/2 phosphorylation, and neural progenitor proliferation in the rat brain (Ito et al. 2010; Nibuya et al. 1995; Reus et al. 2011; Schiavon et al. 2010). In mice, the behavioral effects of antidepressant drugs require activation of the TrkB receptor (Saarelainen et al. 2003). Like zinc, antidepressants are able to transactivate TrkB independent of neurotrophins (Rantamaki et al. 2011). Thus, antidepressant therapies may function by increasing zinc availability, and decreased zinc availability may contribute to the development of depression by suppressing ERK1/2 signaling and neural progenitor proliferation.

Conclusion

The development of animal models with genetic deficits of ERK1 and ERK2, as well as the identification of human disorders characterized by mutations in the genes encoding for ERK1/2, support a major role for these kinases in normal brain development and function. It has become clear that ERK1/2 signaling is critical for the balance of neurogenesis and apoptosis. Zinc can regulate ERK1/2 through various mechanisms. While zinc deficiency causes a decreased phosphorylation of ERK1/2 and neural progenitor proliferation, zinc supplementation can increase ERK1/2 phosphorylation and neural progenitor proliferation. Similarities in the structural and behavioral abnormalities associated with genetic ERK1/2 deficits and developmental zinc deficiency grant further research to understand the potential involvement of ERK1/2 kinases in the deleterious effects of decreased zinc availability on brain development, structure, and function.

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Abbreviations

ADHD	Attention deficit-hyperactivity disorder
BDNF	Brain-derived neurotrophic factor

CREB	cAMP-responsive element binding protein
Csk	C-terminal Src kinase
E	Embryonic day
ERK	Extracellular signal-regulated kinases
GFAP	Glial fibrillary acidic protein
GPR39	G-protein coupled receptor 39
JNK	c-Jun N-terminal kinases
MAPK	Mitogen activated protein kinase
MEK	Mitogen activated ERK kinase
MMP	Matrix metalloproteinases
NCFC	Neuro-cardio-facial-cutaneous syndromes
NMDAR	N-methyl-D-aspartate sensitive glutamate receptor
P	Postnatal day
RCN	Rat cortical neurons
SFK	Src family kinases
Sos	Son of sevenless
TrkB	Tropomyosin-receptor-kinase B

References

- Adamo AM, Zago MP, Mackenzie GG, Aimo L, Keen CL, Keenan A, Oteiza PI. The role of zinc in the modulation of neuronal proliferation and apoptosis. *Neurotoxic Res.* 2010; 17:1–14.
- Adlard PA, Parncutt JM, Finkelstein DI, Bush AI. Cognitive loss in zinc transporter-3 knock-out mice: a phenocopy for the synaptic and memory deficits of Alzheimer's disease? *J Neurosci.* 2010; 30:1631–1636. [PubMed: 20130173]
- Aimo L, Cherr GN, Oteiza PI. Low extracellular zinc increases neuronal oxidant production through NADPH oxidase and nitric oxide synthase activation. *Free Radic Biol Med.* 2010a; 48:1577–1587. [PubMed: 20211250]
- Aimo L, Mackenzie GG, Keenan AH, Oteiza PI. Gestational zinc deficiency affects the regulation of transcription factors AP-1, NF-kappaB and NFAT in fetal brain. *J Nutr Biochem.* 2010b; 21:1069–1075. [PubMed: 20092996]
- Akhondzadeh S, Mohammadi MR, Khademi M. Zinc sulfate as an adjunct to methylphenidate for the treatment of attention deficit hyperactivity disorder in children: a double blind and randomized trial [ISRCTN64132371]. *BMC Psychiatry.* 2004; 4:9. [PubMed: 15070418]
- Alessandrini A, Namura S, Moskowitz MA, Bonventre JV. MEK1 protein kinase inhibition protects against damage resulting from focal cerebral ischemia. *Proc Natl Acad Sci USA.* 1999; 96:12866–12869. [PubMed: 10536014]
- Amani R, Saeidi S, Nazari Z, Nematpour S. Correlation between dietary zinc intakes and its serum levels with depression scales in young female students. *Biol Trace Elem Res.* 2010; 137:150–158. [PubMed: 20013161]

- Andrews RC. An update of the zinc deficiency theory of schizophrenia. Identification of the sex determining system as the site of action of reproductive zinc deficiency. *Med Hypotheses*. 1992; 38:284–291. [PubMed: 1491625]
- Apgar J. Effect of zinc deficiency on parturition in the rat. *Am J Physiol*. 1968; 215:160–163. [PubMed: 5659329]
- Arnold LE, DiSilvestro RA. Zinc in attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol*. 2005; 15:619–627. [PubMed: 16190793]
- Arnold LE, Bozzolo H, Hollway J, Cook A, DiSilvestro RA, Bozzolo DR, Crowl L, Ramadan Y, Williams C. Serum zinc correlates with parent- and teacher- rated inattention in children with attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol*. 2005; 15:628–636. [PubMed: 16190794]
- Arnold LE, Disilvestro RA, Bozzolo D, Bozzolo H, Crowl L, Fernandez S, Ramadan Y, Thompson S, Mo X, Abdel-Rasoul M, Joseph E. Zinc for attention-deficit/hyperactivity disorder: placebo-controlled double-blind pilot trial alone and combined with amphetamine. *J Child Adolesc Psychopharmacol*. 2011; 21:1–19. [PubMed: 21309695]
- Azman MS, Wan Saudi WS, Ilhami M, Mutalib MS, Rahman MT. Zinc intake during pregnancy increases the proliferation at ventricular zone of the newborn brain. *Nutr Neurosci*. 2009; 12:9–12. [PubMed: 19178786]
- Bentley ME, Caulfield LE, Ram M, Santizo MC, Hurtado E, Rivera JA, Ruel MT, Brown KH. Zinc supplementation affects the activity patterns of rural Guatemalan infants. *J Nutr*. 1997; 127:1333–1338. [PubMed: 9202087]
- Besser L, Chorin E, Sekler I, Silverman WF, Atkin S, Russell JT, Hershfinkel M. Synaptically released zinc triggers metabotropic signaling via a zinc-sensing receptor in the hippocampus. *J Neurosci*. 2009; 29:2890–2901. [PubMed: 19261885]
- Bhutta ZA, Black RE, Brown KH, Gardner JM, Gore S, Hidayat A, Khatun F, Martorell R, Ninh NX, Penny ME, Rosado JL, Roy SK, Ruel M, Sazawal S, Shankar A. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc Investigators' Collaborative Group. *J Pediatr*. 1999; 135:689–697. [PubMed: 10586170]
- Bilici M, Yildirim F, Kandil S, Bekaroglu M, Yildirmis S, Deger O, Ulgen M, Yildiran A, Aksu H. Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004; 28:181–190. [PubMed: 14687872]
- Black MM. The evidence linking zinc deficiency with children's cognitive and motor functioning. *J Nutr*. 2003; 133:1473S–1476S. [PubMed: 12730446]
- Boulton TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenbesser SD, DePinho RA, Panayotatos N, Cobb MH, Yancopoulos GD. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell*. 1991; 65:663–675. [PubMed: 2032290]
- Brenton DP, Jackson MJ, Young A. Two pregnancies in a patient with acrodermatitis enteropathica treated with zinc sulphate. *Lancet*. 1981; 2:500–502. [PubMed: 6115249]
- Briefel RR, Bialostosky K, Kennedy-Stephenson J, McDowell MA, Ervin RB, Wright JD. Zinc intake of the US population: findings from the third National Health and Nutrition Examination Survey, 1988–1994. *J Nutr*. 2000; 130:1367S–1373S. [PubMed: 10801945]
- Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2002; 75:1062–1071. [PubMed: 12036814]
- Brunet A, Roux D, Lenormand P, Dowd S, Keyse S, Pouyssegur J. Nuclear translocation of p42/p44 mitogen-activated protein kinase is required for growth factor-induced gene expression and cell cycle entry. *EMBO J*. 1999; 18:664–674. [PubMed: 9927426]
- Charest DL, Mordret G, Harder KW, Jirik F, Pelech SL. Molecular cloning, expression, and characterization of the human mitogen-activated protein kinase p44erk1. *Mol Cell Biol*. 1993; 13:4679–4690. [PubMed: 7687743]

- Chen B, Dowlatsahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiatry*. 2001; 50:260–265. [PubMed: 11522260]
- Choi YS, Cho HY, Hoyt KR, Naegele JR, Obrietan K. IGF-1 receptor-mediated ERK/MAPK signaling couples status epilepticus to progenitor cell proliferation in the subgranular layer of the dentate gyrus. *Glia*. 2008; 56:791–800. [PubMed: 18338791]
- Chowanadisai W, Kelleher SL, Lonnerdal B. Maternal zinc deficiency reduces NMDA receptor expression in neonatal rat brain, which persists into early adulthood. *J Neurochem*. 2005; 94:510–519. [PubMed: 15998301]
- Cieslik K, Sowa-Kucma M, Ossowska G, Legutko B, Wolak M, Opoka W, Nowak G. Chronic unpredictable stress-induced reduction in the hippocampal brain-derived neurotrophic factor (BDNF) gene expression is antagonized by zinc treatment. *Pharmacol Rep*. 2011; 63:537–543. [PubMed: 21602609]
- Cole TB, Wenzel HJ, Kafer KE, Schwartzkroin PA, Palmiter RD. Elimination of zinc from synaptic vesicles in the intact mouse brain by disruption of the ZnT3 gene. *Proc Natl Acad Sci USA*. 1999; 96:1716–1721. [PubMed: 9990090]
- Cole TB, Martynova A, Palmiter RD. Removing zinc from synaptic vesicles does not impair spatial learning, memory, or sensorimotor functions in the mouse. *Brain Res*. 2001; 891:253–265. [PubMed: 11164830]
- Corniola RS, Tassabehji NM, Hare J, Sharma G, Levenson CW. Zinc deficiency impairs neuronal precursor cell proliferation and induces apoptosis via p53-mediated mechanisms. *Brain Res*. 2008; 1237:52–61. [PubMed: 18778698]
- Corona C, Masciopinto F, Silvestri E, Viscovo AD, Lattanzio R, Sorda RL, Ciavardelli D, Goglia F, Piantelli M, Canzoniero LM, Sensi SL. Dietary zinc supplementation of 3 × Tg-AD mice increases BDNF levels and prevents cognitive deficits as well as mitochondrial dysfunction. *Cell Death Dis*. 2010; 1:e91. [PubMed: 21368864]
- Coyle P, Tran N, Fung JN, Summers BL, Rofo AM. Maternal dietary zinc supplementation prevents aberrant behaviour in an object recognition task in mice offspring exposed to LPS in early pregnancy. *Behav Brain Res*. 2009; 197:210–218. [PubMed: 18793679]
- Creson TK, Hao Y, Engel S, Shen Y, Hamidi A, Zhuo M, Manji HK, Chen G. The anterior cingulate ERK pathway contributes to regulation of behavioral excitement and hedonic activity. *Bipolar Disord*. 2009; 11:339–350. [PubMed: 19500087]
- Davies SP, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J*. 2000; 351:95–105. [PubMed: 10998351]
- DiGirolamo AM, Ramirez-Zea M, Wang M, Flores-Ayala R, Martorell R, Neufeld LM, Ramakrishnan U, Sellen D, Black MM, Stein AD. Randomized trial of the effect of zinc supplementation on the mental health of school-age children in Guatemala. *Am J Clin Nutr*. 2010; 92:1241–1250. [PubMed: 20881069]
- Dufner-Beattie J, Langmade SJ, Wang F, Eide D, Andrews GK. Structure, function, and regulation of a subfamily of mouse zinc transporter genes. *J Biol Chem*. 2003; 278:50142–50150. [PubMed: 14525987]
- Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. I. Impaired acquisition of granule cells. *Brain Res*. 1983; 271:217–226. [PubMed: 6616175]
- Dvergsten CL, Fosmire GJ, Ollerich DA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. II. Impaired maturation of Purkinje cells. *Brain Res*. 1984a; 318:11–20. [PubMed: 6488049]
- Dvergsten CL, Johnson LA, Sandstead HH. Alterations in the postnatal development of the cerebellar cortex due to zinc deficiency. III. Impaired dendritic differentiation of basket and stellate cells. *Brain Res*. 1984b; 318:21–26. [PubMed: 6488052]
- Engel SR, Creson TK, Hao Y, Shen Y, Maeng S, Nekrasova T, Landreth GE, Manji HK, Chen G. The extracellular signal-regulated kinase pathway contributes to the control of behavioral excitement. *Mol Psychiatry*. 2009; 14:448–461. [PubMed: 18227838]

- Ferguson SM, Fasano S, Yang P, Brambilla R, Robinson TE. Knockout of ERK1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. *Neuropsychopharmacology*. 2006; 31:2660–2668. [PubMed: 16407894]
- Franco JL, Posser T, Brocardo PS, Trevisan R, Uliano-Silva M, Gabilan NH, Santos AR, Leal RB, Rodrigues AL, Farina M, Dafre AL. Involvement of glutathione, ERK1/2 phosphorylation and BDNF expression in the antidepressant-like effect of zinc in rats. *Behav Brain Res*. 2008; 188:316–323. [PubMed: 18191237]
- Fyffe-Maricich SL, Karlo JC, Landreth GE, Miller RH. The ERK2 mitogen-activated protein kinase regulates the timing of oligodendrocyte differentiation. *J Neurosci*. 2011; 31:843–850. [PubMed: 21248107]
- Gao HL, Zheng W, Xin N, Chi ZH, Wang ZY, Chen J. Zinc deficiency reduces neurogenesis accompanied by neuronal apoptosis through caspase-dependent and -independent signaling pathways. *Neurotoxic Res*. 2009; 16:416–425.
- Gao HL, Xu H, Xin N, Zheng W, Chi ZH, Wang ZY. Disruption of the CaMKII/CREB signaling is associated with zinc deficiency-induced learning and memory impairments. *Neurotoxic Res*. 2011; 19:584–591.
- Gardner JM, Powell CA, Baker-Henningham H, Walker SP, Cole TJ, Grantham-McGregor SM. Zinc supplementation and psychosocial stimulation: effects on the development of undernourished Jamaican children. *Am J Clin Nutr*. 2005; 82:399–405. [PubMed: 16087985]
- Golub MS, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. *J Nutr*. 1995; 125:2263S–2271S. [PubMed: 7623165]
- Golub MS, Takeuchi PT, Keen CL, Hendrickx AG, Gershwin ME. Activity and attention in zinc-deprived adolescent monkeys. *Am J Clin Nutr*. 1996; 64:908–915. [PubMed: 8942416]
- Golub MS, Keen CL, Gershwin ME. Moderate Zn–Fe deprivation influences behavior but not growth in adolescent Rhesus monkeys. *J Nutr*. 2000; 130:354S–357S. [PubMed: 10721905]
- Gonzalez FA, Raden DL, Davis RJ. Identification of substrate recognition determinants for human ERK1 and ERK2 protein kinases. *J Biol Chem*. 1991; 266:22159–22163. [PubMed: 1939237]
- Halas ES, Sandstead HH. Some effects of prenatal zinc deficiency on behavior of the adult rat. *Pediatr Res*. 1975; 9:94–97. [PubMed: 1118197]
- Halas ES, Hunt CD, Eberhardt MJ. Learning and memory disabilities in young adult rats from mildly zinc deficient dams. *Physiol Behav*. 1986; 37:451–458. [PubMed: 3749304]
- Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. *Am J Clin Nutr*. 2001; 74:381–386. [PubMed: 11522564]
- Hamadani JD, Fuchs GJ, Osendarp SJ, Huda SN, Grantham-McGregor SM. Zinc supplementation during pregnancy and effects on mental development and behaviour of infants: a follow-up study. *Lancet*. 2002; 360:290–294. [PubMed: 12147372]
- Hao Y, Creson T, Zhang L, Li P, Du F, Yuan P, Gould TD, Manji HK, Chen G. Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis. *J Neurosci*. 2004; 24:6590–6599. [PubMed: 15269271]
- He K, Aizenman E. ERK signaling leads to mitochondrial dysfunction in extracellular zinc-induced neurotoxicity. *J Neurochem*. 2010; 114:452–461. [PubMed: 20412391]
- Heffron DS, Landreth GE, Samuels IS, Mandell JW. Brain-specific deletion of extracellular signal-regulated kinase 2 mitogen-activated protein kinase leads to aberrant cortical collagen deposition. *Am J Pathol*. 2009; 175:2586–2599. [PubMed: 19893051]
- Holst B, Egerod KL, Schild E, Vickers SP, Cheetham S, Gerlach LO, Storjohann L, Stidsen CE, Jones R, Beck-Sickinger AG, Schwartz TW. GPR39 signaling is stimulated by zinc ions but not by obestatin. *Endocrinology*. 2007; 148:13–20. [PubMed: 16959833]
- Huang YZ, Pan E, Xiong ZQ, McNamara JO. Zinc-mediated transactivation of TrkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. *Neuron*. 2008; 57:546–558. [PubMed: 18304484]
- Hubbs-Tait L, Kennedy TS, Droke EA, Belanger DM, Parker JR. Zinc, iron, and lead: relations to head start children's cognitive scores and teachers' ratings of behavior. *J Am Diet Assoc*. 2007; 107:128–133. [PubMed: 17197281]

- Hunt CD, Halas ES, Sandstead HH. Mild perinatal zinc-deficiency affects brain hippocampal morphology and behavior. *Fed Proc.* 1984; 43:382.
- Hurley LS, Swenerton H. Congenital malformations resulting from zinc deficiency in rats. *Proc Soc Exp Biol Med.* 1966; 123:692–696. [PubMed: 6006233]
- Hwang JJ, Park MH, Choi SY, Koh JY. Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. *J Biol Chem.* 2005; 280:11995–12001. [PubMed: 15659400]
- Imamura O, Satoh Y, Endo S, Takishima K. Analysis of extracellular signal-regulated kinase 2 function in neural stem/progenitor cells via nervous system-specific gene disruption. *Stem Cells.* 2008; 26:3247–3256. [PubMed: 18818436]
- Imamura O, Pages G, Pouyssegur J, Endo S, Takishima K. ERK1 and ERK2 are required for radial glial maintenance and cortical lamination. *Genes Cells.* 2010; 15:1072–1088. [PubMed: 20825492]
- Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR. Cross talk between ERK and PKA is required for Ca^{2+} stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron.* 1998; 21:869–883. [PubMed: 9808472]
- Ito M, Seki T, Liu J, Nakamura K, Namba T, Matsubara Y, Suzuki T, Arai H. Effects of repeated electroconvulsive seizure on cell proliferation in the rat hippocampus. *Synapse.* 2010; 64:814–821. [PubMed: 20340175]
- Kanterewicz BI, Urban NN, et al. The extracellular signal-regulated kinase cascade is required for NMDA receptor-independent LTP in area CA1 but not area CA3 of the hippocampus. *J Neurosci.* 2000; 20(9):3057–3066. [PubMed: 10777769]
- Kawamoto, JC.; Halas, ES. Lasting morphologic effects of mild perinatal zinc deficiency in the adult hippocampal formation. In: Frederickson, CJ.; Howell, GA.; Kasarakis, EJ., editors. *The neurobiology of zinc B: deficiency, toxicity, and pathology.* New York: Alan R. Liss, Inc.; 1984. p. 33-48.
- Keller KA, Grider A, Coffield JA. Age-dependent influence of dietary zinc restriction on short-term memory in male rats. *Physiol Behav.* 2001; 72:339–348. [PubMed: 11274675]
- Ketterman JK, Li YV. Presynaptic evidence for zinc release at the mossy fiber synapse of rat hippocampus. *J Neurosci Res.* 2008; 86:422–434. [PubMed: 17847078]
- Kim SH, Seo MS, Jeon WJ, Yu HS, Park HG, Jung GA, Lee HY, Kang UG, Kim YS. Haloperidol regulates the phosphorylation level of the MEK-ERK-p90RSK signal pathway via protein phosphatase 2A in the rat frontal cortex. *Int J Neuropsychopharmacol.* 2008; 11:509–517. [PubMed: 18272021]
- Kim Y, Kim SH, Kim YS, Lee YH, Ha K, Shin SY. Imipramine activates glial cell line-derived neurotrophic factor via early growth response gene 1 in astrocytes. *Prog Neuropsychopharmacol Biol Psychiatry.* 2011; 35:1026–1032. [PubMed: 21354245]
- Kirksey A, Wachs TD, Yunis F, Srinath U, Rahmanifar A, McCabe GP, Galal OM, Harrison GG, Jerome NW. Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. *Am J Clin Nutr.* 1994; 60:782–792. [PubMed: 7942587]
- Koh JY, Suh SW, Gwag BJ, He YY, Hsu CY, Choi DW. The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science.* 1996; 272:1013–1016. [PubMed: 8638123]
- Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH Jr, Dobyns WB, Christian SL. Recurrent 16p11.2 microdeletions in autism. *Hum Mol Genet.* 2008; 17:628–638. [PubMed: 18156158]
- Liu H, Oteiza PI, Gershwin ME, Golub MS, Keen CL. Effects of maternal marginal zinc deficiency on myelin protein profiles in the suckling rat and infant rhesus monkey. *Biol Trace Elem Res.* 1992; 34:55–66. [PubMed: 1382522]
- Lopez V, Keen CL, Lanoue L. Prenatal zinc deficiency: influence on heart morphology and distribution of key heart proteins in a rat model. *Biol Trace Elem Res.* 2008; 122:238–255. [PubMed: 18224284]
- Mackenzie GG, Zago MP, Keen CL, Oteiza PI. Low intracellular zinc impairs the translocation of activated NF-kappa B to the nuclei in human neuroblastoma IMR-32 cells. *J Biol Chem.* 2002; 277:34610–34617. [PubMed: 12089148]

- Mackenzie GG, Zago MP, Erleijman AG, Aimo L, Keen CL, Oteiza PI. Alpha-lipoic acid and N-acetyl cysteine prevent zinc deficiency-induced activation of NF-kappaB and AP-1 transcription factors in human neuroblastoma IMR-32 cells. *Free Radic Res.* 2006; 40:75–84. [PubMed: 16298762]
- Mackenzie GG, Salvador GA, Romero C, Keen CL, Oteiza PI. A deficit in zinc availability can cause alterations in tubulin thiol redox status in cultured neurons and in the developing fetal rat brain. *Free Radic Biol Med.* 2011; 51:480–489. [PubMed: 21600978]
- Maes M, D'Haese PC, Scharpe S, D'Hondt P, Cosyns P, De Broe ME. Hypozincemia in depression. *J Affect Disord.* 1994; 31:135–140. [PubMed: 8071476]
- Maes M, Vandoolaeghe E, Neels H, Demedts P, Wauters A, Meltzer HY, Altamura C, Desnyder R. Lower serum zinc in major depression is a sensitive marker of treatment resistance and of the immune/inflammatory response in that illness. *Biol Psychiatry.* 1997; 42:349–358. [PubMed: 9276075]
- Maes M, Yirmiya R, Noraberg J, Brene S, Hibbeln J, Perini G, Kubera M, Bob P, Lerer B, Maj M. The inflammatory & neurodegenerative (I & ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis.* 2009; 24:27–53. [PubMed: 19085093]
- Mazzucchelli C, Vantaggiato C, Ciamei A, Fasano S, Pakhotin P, Krezel W, Welzl H, Wolfer DP, Pages G, Valverde O, Marowsky A, Porrazzo A, Orban PC, Maldonado R, Ehrengreuber MU, Cestari V, Lipp HP, Chapman PF, Pouyssegur J, Brambilla R. Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron.* 2002; 34:807–820. [PubMed: 12062026]
- McCubrey JA, Steelman LS, Abrams SL, Bertrand FE, Ludwig DE, Basecke J, Libra M, Stivala F, Milella M, Tafuri A, Lunghi P, Bonati A, Martelli AM. Targeting survival cascades induced by activation of Ras/Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways for effective leukemia therapy. *Leukemia.* 2008; 22:708–722. [PubMed: 18337766]
- McLoughlin IJ, Hodge JS. Zinc in depressive disorder. *Acta Psychiatr Scand.* 1990; 82:451–453. [PubMed: 2291414]
- Mebratu Y, Tesfaigzi Y. How ERK1/2 activation controls cell proliferation and cell death: is subcellular localization the answer? *Cell Cycle.* 2009; 8:1168–1175. [PubMed: 19282669]
- Miller FD, Gauthier AS. Timing is everything: making neurons versus glia in the developing cortex. *Neuron.* 2007; 54:357–369. [PubMed: 17481390]
- Minichiello L. TrkB signalling pathways in LTP and learning. *Nat Rev Neurosci.* 2009; 10:850–860. [PubMed: 19927149]
- Molnar P, Nadler JV. Synaptically-released zinc inhibits N-methyl-d-aspartate receptor activation at recurrent mossy fiber synapses. *Brain Res.* 2001; 910:205–207. [PubMed: 11489274]
- Nakashima AS, Dyck RH. Zinc and cortical plasticity. *Brain Res Rev.* 2009; 59:347–373. [PubMed: 19026685]
- Namura S, Iihara K, Takami S, Nagata I, Kikuchi H, Matsushita K, Moskowitz MA, Bonventre JV, Alessandrini A. Intravenous administration of MEK inhibitor U0126 affords brain protection against forebrain ischemia and focal cerebral ischemia. *Proc Natl Acad Sci USA.* 2001; 98:11569–11574. [PubMed: 11504919]
- Neary JT, Whittemore SR, Bu Y, Mehta H, Shi YF. Biochemical mechanisms of action of Hypericum LI 160 in glial and neuronal cells: inhibition of neurotransmitter uptake and stimulation of extracellular signal regulated protein kinase. *Pharmacopsychiatry.* 2001; 34(Suppl 1):S103–S107. [PubMed: 11518055]
- Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci.* 1995; 15:7539–7547. [PubMed: 7472505]
- Nowak G, Siwek M, Dudek D, Zieba A, Pilc A. Effect of zinc supplementation on antidepressant therapy in unipolar depression: a preliminary placebo-controlled study. *Pol J Pharmacol.* 2003; 55:1143–1147. [PubMed: 14730113]
- Pascoli V, Valjent E, Corbille AG, Corvol JC, Tassin JP, Girault JA, Herve D. cAMP and extracellular signal-regulated kinase signaling in response to d-amphetamine and methylphenidate in the

- prefrontal cortex in vivo: role of beta 1-adrenoceptors. *Mol Pharmacol.* 2005; 68:421–429. [PubMed: 15890841]
- Penland JG, Sandstead HH, Alcock NW, Dayal HH, Chen XC, Li JS, Zhao F, Yang JJ. A preliminary report: effects of zinc and micronutrient repletion on growth and neuropsychological function of urban Chinese children. *J Am Coll Nutr.* 1997; 16:268–272. [PubMed: 9176834]
- Qi X, Lin W, Li J, Pan Y, Wang W. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. *Behav Brain Res.* 2006; 175:233–240. [PubMed: 17050000]
- Qi X, Lin W, Li J, Li H, Wang W, Wang D, Sun M. Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress. *Neurobiol Dis.* 2008; 31:278–285. [PubMed: 18586506]
- Rantamaki T, Vesa L, Antila H, Di Lieto A, Tammela P, Schmitt A, Lesch KP, Rios M, Castren E. Antidepressant drugs transactivate TrkB neurotrophin receptors in the adult rodent brain independently of BDNF and monoamine transporter blockade. *PLoS One.* 2011; 6:e20567. [PubMed: 21666748]
- Reszka AA, Seger R, Diltz CD, Krebs EG, Fischer EH. Association of mitogen-activated protein kinase with the microtubule cytoskeleton. *Proc Natl Acad Sci USA.* 1995; 92:8881–8885. [PubMed: 7568036]
- Reus GZ, Stringari RB, Ribeiro KF, Ferraro AK, Vitto MF, Cesconetto P, Souza CT, Quevedo J. Ketamine plus imipramine treatment induces antidepressant-like behavior and increases CREB and BDNF protein levels and PKA and PKC phosphorylation in rat brain. *Behav Brain Res.* 2011; 221:166–171. [PubMed: 21397634]
- Rowin J, Lewis SL. Copper deficiency myeloneuropathy and pancytopenia secondary to overuse of zinc supplementation. *J Neurol Neurosurg Psychiatry.* 2005; 76:750–751. [PubMed: 15834043]
- Saarelainen T, Hendolin P, Lucas G, Koponen E, Sairanen M, MacDonald E, Agerman K, Haapasalo A, Nawa H, Aloyz R, Ernfors P, Castren E. Activation of the TrkB neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. *J Neurosci.* 2003; 23:349–357. [PubMed: 12514234]
- Samuels IS, Karlo JC, Faruzzi AN, Pickering K, Herrup K, Sweatt JD, Saitta SC, Landreth GE. Deletion of ERK2 mitogen-activated protein kinase identifies its key roles in cortical neurogenesis and cognitive function. *J Neurosci.* 2008; 28:6983–6995. [PubMed: 18596172]
- Samuels IS, Saitta SC, Landreth GE. MAP'ing CNS development and cognition: an ERKsome process. *Neuron.* 2009; 61:160–167. [PubMed: 19186160]
- Sandstrom B. Micronutrient interactions: effects on absorption and bioavailability. *Br J Nutr.* 2001; 85(Suppl 2):S181–S185. [PubMed: 11509108]
- Satoh Y, Endo S, Ikeda T, Yamada K, Ito M, Kuroki M, Hiramoto T, Imamura O, Kobayashi Y, Watanabe Y, Itohara S, Takishima K. Extracellular signal-regulated kinase 2 (ERK2) knockdown mice show deficits in long-term memory; ERK2 has a specific function in learning and memory. *J Neurosci.* 2007; 27:10765–10776. [PubMed: 17913910]
- Satoh Y, Endo S, Nakata T, Kobayashi Y, Yamada K, Ikeda T, Takeuchi A, Hiramoto T, Watanabe Y, Kazama T. ERK2 contributes to the control of social behaviors in mice. *J Neurosci.* 2011; 31:11953–11967. [PubMed: 21849556]
- Sawada T, Yokoi K. Effect of zinc supplementation on mood states in young women: a pilot study. *Eur J Clin Nutr.* 2010; 64:331–333. [PubMed: 20087376]
- Sazawal S, Black RE, Menon VP, Dighra P, Caulfield LE, Dhingra U, Bagati A. Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized, controlled trial. *Pediatrics.* 2001; 108:1280–1286. [PubMed: 11731649]
- Schiavon AP, Milani H, Romanini CV, Foresti ML, Castro OW, Garcia-Cairasco N, de Oliveira RM. Imipramine enhances cell proliferation and decreases neurodegeneration in the hippocampus after transient global cerebral ischemia in rats. *Neurosci Lett.* 2010; 470:43–48. [PubMed: 20036317]
- Shalin SC, Zirrgiebel U, Honsa KJ, Julien JP, Miller FD, Kaplan DR, Sweatt JD. Neuronal MEK is important for normal fear conditioning in mice. *J Neurosci Res.* 2004; 75:760–770. [PubMed: 14994337]

- She QB, Ma WY, Zhong S, Dong Z. Activation of JNK1, RSK2, and MSK1 is involved in serine 112 phosphorylation of Bad by ultraviolet B radiation. *J Biol Chem*. 2002; 277:24039–24048. [PubMed: 11983683]
- Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. *Am J Psychiatry*. 2003; 160:1516–1518. [PubMed: 12900317]
- Sindreu C, Palmiter RD, Storm DR. Zinc transporter ZnT-3 regulates presynaptic Erk1/2 signaling and hippocampus-dependent memory. *Proc Natl Acad Sci USA*. 2011; 108:3366–3370. [PubMed: 21245308]
- Smith MA, Makino S, Kvetnansky R, Post RM. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J Neurosci*. 1995; 15:1768–1777. [PubMed: 7891134]
- Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, McCubrey JA. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. *Leukemia*. 2004; 18:189–218. [PubMed: 14737178]
- Suh SW, Thompson RB, Frederickson CJ. Loss of vesicular zinc and appearance of perikaryal zinc after seizures induced by pilocarpine. *Neuroreport*. 2001; 12:1523–1525. [PubMed: 11388441]
- Suh SW, Won SJ, Hamby AM, Yoo BH, Fan Y, Sheline CT, Tamano H, Takeda A, Liu J. Decreased brain zinc availability reduces hippocampal neurogenesis in mice and rats. *J Cereb Blood Flow Metab*. 2009; 29:1579–1588. [PubMed: 19536073]
- Tahmasebi Boroujeni S, Naghdi N, Shahbazi M, Farrokhi A, Bagherzadeh F, Kazemnejad A, Javadian M. The effect of severe zinc deficiency and zinc supplement on spatial learning and memory. *Biol Trace Elem Res*. 2009; 130:48–61. [PubMed: 19183867]
- Takeda A, Tamano H, Kan F, Itoh H, Oku N. Anxiety-like behavior of young rats after 2-week zinc deprivation. *Behav Brain Res*. 2007; 177:1–6. [PubMed: 17166602]
- Takeda A, Tamano H, Kan F, Hanajima T, Yamada K, Oku N. Enhancement of social isolation-induced aggressive behavior of young mice by zinc deficiency. *Life Sci*. 2008; 82:909–914. [PubMed: 18374363]
- Takeda A, Fuke S, Ando M, Oku N. Positive modulation of long-term potentiation at hippocampal CA1 synapses by low micromolar concentrations of zinc. *Neuroscience*. 2009; 158:585–591. [PubMed: 18984033]
- Tassabehji NM, Corniola RS, Alshingiti A, Levenson CW. Zinc deficiency induces depression-like symptoms in adult rats. *Physiol Behav*. 2008; 95:365–369. [PubMed: 18655800]
- Taubeneck MW, Daston GP, Rogers JM, Keen CL. Altered maternal zinc metabolism following exposure to diverse developmental toxicants. *Reprod Toxicol*. 1994; 8:25–40. [PubMed: 8186621]
- Taubeneck MW, Daston GP, Rogers JM, Gershwin ME, Ansari A, Keen CL. Tumor necrosis factor- α alters maternal and embryonic zinc metabolism and is developmentally toxic in mice. *J Nutr*. 1995; 125:908–919. [PubMed: 7722694]
- Toren P, Eldar S, Sela BA, Wolmer L, Weitz R, Inbar D, Koren S, Reiss A, Weizman R, Laor N. Zinc deficiency in attention-deficit hyperactivity disorder. *Biol Psychiatry*. 1996; 40:1308–1310. [PubMed: 8959299]
- Uckardes Y, Ozmert EN, Unal F, Yurdakok K. Effects of zinc supplementation on parent and teacher behaviour rating scores in low socioeconomic level Turkish primary school children. *Acta Paediatr*. 2009; 98:731–736. [PubMed: 19133873]
- Uriu-Adams JY, Keen CL. Zinc and reproduction: effects of zinc deficiency on prenatal and early postnatal development. *Birth Defects Res B Dev Reprod Toxicol*. 2010; 89:313–325. [PubMed: 20803691]
- Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc: health effects and research priorities for the 1990s. *Environ Health Perspect*. 1994; 102(Suppl 2):5–46. [PubMed: 7925188]
- Wang FD, Bian W, Kong LW, Zhao FJ, Guo JS, Jing NH. Maternal zinc deficiency impairs brain nestin expression in prenatal and postnatal mice. *Cell Res*. 2001; 11:135–141. [PubMed: 11453545]
- Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi

- RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ. Association between microdeletion and microduplication at 16p11.2 and autism. *N Engl J Med*. 2008; 358:667–675. [PubMed: 18184952]
- Whittle N, Lubec G, Singewald N. Zinc deficiency induces enhanced depression-like behaviour and altered limbic activation reversed by antidepressant treatment in mice. *Amino Acids*. 2009; 36:147–158. [PubMed: 18975044]
- Xu H, Gao HL, Zheng W, Xin N, Chi ZH, Bai SL, Wang ZY. Lactational zinc deficiency-induced hippocampal neuronal apoptosis by a BDNF-independent TrkB signaling pathway. *Hippocampus*. 2011; 21:495–501. [PubMed: 20101602]
- Yamamoto T, Ebisuya M, Ashida F, Okamoto K, Yonehara S, Nishida E. Continuous ERK activation downregulates antiproliferative genes throughout G1 phase to allow cell-cycle progression. *Curr Biol*. 2006; 16:1171–1182. [PubMed: 16782007]
- Yang X, Liu L, Sternberg D, Tang L, Galinsky I, DeAngelo D, Stone R. The FLT3 Internal tandem duplication mutation prevents apoptosis in interleukin-3-deprived BaF3 cells due to protein kinase A and ribosomal S6 kinase 1-mediated BAD phosphorylation at serine 112. *Cancer Res*. 2005; 65:7338–7347. [PubMed: 16103085]
- Ye B, Maret W, Vallee BL. Zinc metallothionein imported into liver mitochondria modulates respiration. *Proc Natl Acad Sci USA*. 2001; 98:2317–2322. [PubMed: 11226237]
- Zambuzzi WF, Granjeiro JM, Parikh K, Yuvaraj S, Peppelenbosch MP, Ferreira CV. Modulation of Src activity by low molecular weight protein tyrosine phosphatase during osteoblast differentiation. *Cell Physiol Biochem*. 2008; 22:497–506. [PubMed: 19088431]
- Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14–3–3 not BCL-X(L). *Cell*. 1996; 87:619–628. [PubMed: 8929531]
- Zhang F, Wang S, Signore AP, Chen J. Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. *Stroke*. 2007; 38:2329–2336. [PubMed: 17600230]
- Zheng CF, Guan KL. Cloning and characterization of two distinct human extracellular signal-regulated kinase activator kinases, MEK1 and MEK2. *J Biol Chem*. 1993; 268:11435–11439. [PubMed: 8388392]
- Zhu Y, Yang GY, Ahlemeyer B, Pang L, Che XM, Culmsee C, Klumpp S, Kriegstein J. Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. *J Neurosci*. 2002; 22:3898–3909. [PubMed: 12019309]

Fig. 1.

The ERK signaling cascade in the regulation of brain development. Typically, ERK1/2 activation occurs downstream from the binding of a ligand (e.g., BDNF) to a receptor tyrosine kinase (e.g., TrkB) leading to receptor autophosphorylation. Subsequently, Shc binds to phosphorylated TrkB and recruits the Grb2-Sos complex to activate the G-protein Ras. Ras stimulates Raf to phosphorylate MEK1/2. Activated MEK1/2 phosphorylates and activates ERK1/2, which subsequently activates through phosphorylation multiple target proteins in the cell cytosol and nucleus. Several of these proteins are involved in the

regulation of cellular events central to brain development, including proliferation, differentiation, cell survival, and synaptic plasticity

Fig. 2.

Regulation by zinc of the ERK1/2 signaling cascade. Zinc could activate the ERK1/2 signaling pathway at various levels: 1 zinc inhibits phosphatases that are involved in ERK1/2 dephosphorylation, and zinc inhibits kinases that indirectly regulate the phosphorylation levels of receptors that subsequently activate the ERK cascade; 2 zinc can directly bind to and activate receptors (e.g., GPR39) which have ERK1/2 as a downstream signaling cascade; and 3 zinc can activate MMPs, which subsequently cleave and activate

substrates (e.g., pro-BDNF) releasing ligands (e.g. BDNF) that activate receptors upstream from the ERK1/2 cascade

Fig. 3.

MAPKs are differentially regulated by zinc deficiency in neuronal cells and E19 fetal rat brain. **a** IMR-32 cells were incubated for 24 h in media containing different concentrations of zinc (zinc 0.5–15 μ M). Intracellular labile zinc was measured with the probe 6-methoxy-(8-*p*-toluenesulfonamido) quinolone (TSQ), cell viability was evaluated measuring cellular ATP levels, and ERK phosphorylation levels were determined by Western blot. **b, c** IMR-32 cells were incubated for 24 h in control or zinc deficient medium (1.5 μ M Zn) in the absence (*empty bars*) or the presence of catalase (*full bars*), α -lipoic acid (LA) (*dashed bars*), or N-acetyl cysteine (NAC) (*gray bars*). Phosphorylation levels of ERK1/2, p38, and JNK1/2 (*right graph*) were measured by Western blot. **d** Rats were fed from conception until embryonic day 19 (E19) a control (C, 25 μ g of Zn/g of diet) or marginal zinc (MZD, 10 μ g of Zn/g of diet) diet. At E19 fetal brains were excised and phosphorylation levels of ERK1/2, p38, and JNK1/2 were measured in brain homogenates by Western blots. Results are expressed relative to control values (1, *dashed line*) and presented as means $\pm$ SE.

*Significantly different compared to controls. This figure was adapted from (Adamo et al. 2010; Aimo et al. 2010b; Mackenzie et al. 2006; Mackenzie et al. 2002)