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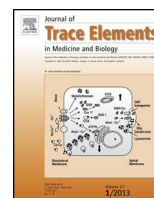


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Zinc deficiency induces apoptosis via mitochondrial p53- and caspase-dependent pathways in human neuronal precursor cells

Rohit Seth^{a,1,2}, Rikki S. Corniola^{a,b,1,3}, Shannon D. Gower-Winter^a, Thomas J. Morgan Jr.^a, Brian Bishop^a, Cathy W. Levenson^{a,b,*}

^a Department of Biomedical Sciences, College of Medicine, Florida State University, Tallahassee, FL, USA

^b Program in Neuroscience, Florida State University, Tallahassee, FL, USA

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ABSTRACT

Previous studies have shown that zinc deficiency leads to apoptosis of neuronal precursor cells in vivo and in vitro. In addition to the role of p53 as a nuclear transcription factor in zinc deficient cultured human neuronal precursors (NT-2), we have now identified the translocation of phosphorylated p53 to the mitochondria and p53-dependent increases in the pro-apoptotic mitochondrial protein BAX leading to a loss of mitochondrial membrane potential as demonstrated by a 25% decrease in JC-1 red:green fluorescence ratio. Disruption of mitochondrial membrane integrity was accompanied by efflux of the apoptosis inducing factor (AIF) from the mitochondria and translocation to the nucleus with a significant increase in reactive oxygen species (ROS) after 24 h of zinc deficiency. Measurement of caspase cleavage, mRNA, and treatment with caspase inhibitors revealed the involvement of caspases 2, 3, 6, and 7 in zinc deficiency-mediated apoptosis. Down-stream targets of caspase activation, including the nuclear structure protein lamin and polyADP ribose polymerase (PARP), which participates in DNA repair, were also cleaved. Transfection with a dominant-negative p53 construct and use of the p53 inhibitor, pifithrin- μ , established that these alterations were largely dependent on p53. Together these data identify a cascade of events involving mitochondrial p53 as well as p53-dependent caspase-mediated mechanisms leading to apoptosis during zinc deficiency.

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Introduction

Neural stem cells, capable of proliferating and differentiating into mature neurons, are important in both the developing and the adult CNS. In the developing embryo, asymmetric division of stem cells is followed by radial and tangential migration to sites that permit development of the notochord, neural tube, and neural crest. The process of developmental neurogenesis continues with

differentiation of stem cells into functional neurons, aggregation, synaptogenesis, and synaptic pruning [1]. Programmed cell death is an important part of these processes leading to normal brain growth and plasticity during development [2,3].

We have known for some time that the essential trace metal zinc plays a key role in the maintenance of neuronal stem cell populations in the developing CNS. For example, when pregnant female mice were fed diets either severely deficient (1 ppm) or marginally deficient (5 ppm) in zinc, the stem cell marker nestin was significantly reduced in the pups beginning on embryonic day 10 and persisting through post-natal day 10 [4]. Understanding the cellular and molecular events associated with impaired stem cell proliferation and survival is important because the CNS deficits associated with developmental zinc deficiency, such as learning and memory, cannot be reversed by subsequent addition of zinc to the diet after weaning [5,6].

The requirement for zinc in stem cell proliferation and neurogenesis is not limited to the developmental period. While largely isolated to specific brain regions, such as the subventricular zone (SVZ) that surrounds the rostral end of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus, stem cells in the

* Corresponding author at: Department of Biomedical Sciences, Florida State University College of Medicine, 1115W. Call Street, Tallahassee, FL 32306-4300, USA. Tel.: +1 850 644 4122; fax: +1 850 644 5781.

E-mail address: cathy.levenson@med.fsu.edu (C.W. Levenson).

¹ The first two authors contributed equally to this work and are thus co-first authors.

² Present address: Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G., India.

³ Present address: California Northstate University College of Medicine, Department of Medical Education, Elk Grove, CA, USA.

CNS continue to proliferate throughout adulthood [7]. Despite the limited number of these cells, it is clear that they are important for normal brain function. Neural stem cells that arise from the SVZ migrate along the rostral migratory stream where they undergo differentiation into interneurons in the olfactory bulb [8]. Neuronal precursor cells in the SGZ migrate into the granular cell layer of the dentate gyrus, undergo neuronal differentiation, and integrate themselves into the hippocampal circuitry [8,9]. The presence of these cells in the adult CNS is significant because not only are stem cells in the hippocampus known to participate in learning [10], but both the olfactory bulb and the hippocampus are also part of the limbic system that participates in the control of emotion [9,11,12].

In the mature CNS, zinc deficiency reduces the number of proliferating stem cells and neuronal precursors [13–15]. This appears to be the result of both a decrease in proliferation and an increase in apoptosis. For example, when adult rats were subjected to a zinc deficient diet for 3 weeks, there was an increase in terminal deoxynucleotidyl transferase dUTP nick end labeled (TUNEL-labeled) cells compared to pair-fed controls [13], a finding that was subsequently confirmed in mice fed a diet low in zinc for 5 weeks [14]. Analysis of the neuronal precursor cell lines NT-2 and IMR-32 grown under zinc deficient conditions has revealed a role for the tumor suppressor protein p53, which acts as a DNA-binding transcription factor to arrest the cell cycle and induce apoptosis [13,15]. In addition to the well-known nuclear and transcriptional roles of p53, there is new and mounting evidence suggesting that p53 may be acting via mitochondrial mechanisms to induce apoptosis [16–18]. In at least some cell types, p53 induces apoptosis via interaction with the pro-apoptotic Bcl-family member BAX that is associated with the outer mitochondrial membrane [16,17,19].

Together these data led us to hypothesize that in neuronal precursor cells zinc deficiency would result in the translocation of p53 to the mitochondria leading to mitochondrial alterations and apoptosis. We further hypothesized a role for p53-mediated caspase activation in zinc deficiency-induced apoptosis and thus examined the role of p53 in caspase cleavage leading to loss of neuronal precursor cells via apoptosis.

Materials and methods

Cell culture

The human neuronal precursor cell line, Ntera-2 (NT-2; Stratagene, La Jolla, CA) was grown in a humidified incubator at 37 °C with 5% CO₂ and 95% air [13]. Briefly, cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) and supplemented with 10% fetal bovine serum (Hyclone Laboratories, Logan, UT). To prepare zinc deficient media, FBS was mixed with 10% Chelex 100 at 4 °C overnight according to the method described in Ho et al. [20]. Final zinc concentrations were evaluated with atomic absorption spectrophotometry. Other divalent ions including calcium, magnesium, copper, and iron were also evaluated to ensure zinc specificity of Chelex 100. Zinc adequate (ZA) medium contained 2.5 μM zinc and zinc deficient (ZD) medium contained 0.4 μM zinc. All media were supplemented with 0.5 μg/ml gentamicin (GIBCO BRL, Rockville, MD), and 100 μg/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Mediatech Inc., Manassas, VA). Mitochondrial p53 was inhibited using 5 μM pifithrin-μ (μPFT) (Sigma Chemicals, St. Louis, MO) [18,21].

Immunocytochemistry

NT-2 cells were grown on glass 22 mm² coverslips in 35 mm² dishes to approximately 50% confluence. Cells grown in ZD or ZA media for 24 h (*n*=6 in two separate experiments) were

treated with 100 μM Mitotracker Deep Red 633 (Molecular Probes, Eugene, OR) for 45 min and then fixed with 4% formaldehyde and permeabilized with 0.2% Triton X-100 (5 min). After blocking with BSA, cells were incubated with a mouse anti-human monoclonal antibody for p53 (1:500, Santa Cruz) followed by fluorescently labeled secondary antibodies and nuclear staining with 4',6-diamidino-2-phenylindole (DAPI). Coverslips were then mounted onto microscope slides using anti-fade mounting medium (FluorSave Reagent, Calbiochem-Novabiochem, La Jolla, CA) and visualized by fluorescence microscopy (Olympus BX61 microscope).

Detection of mitochondrial ROS

In two separate experiments, the production of mitochondrial ROS was detected by incubation of live cultured NT-2 cells (*n*=3) in 10 μM dihydrorhodamine (DHR) in DMSO at 37 °C for 20 min following a 24 h incubation in ZA or ZD media with and without 5 μM μPFT. DHR is localized to the mitochondria and upon oxidation it is converted to rhodamine-123. Fluorescence was measured using Flx800 Microplate fluorescence plate reader (Winooski, Vermont) with excitation and emission spectra at 485/528 nm. KC4 v3.4 software was used for data analysis.

Western blot analysis

Cells were grown in 75 cm² flasks and treated with ZA or ZD media for 24 h. Mitochondrial fractions were isolated using a mitochondrial isolation kit (Pierce Biotechnology, Rockford, IL) following the manufacturer's protocol. Western blot analysis was performed using cytosolic and mitochondrial fractions which were added to sample buffer, heated to 95 °C for 10 min, and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 10% polyacrylamide gel. Samples were then transferred to a 0.2 μm nitrocellulose membrane on ice. The membrane was blocked with Tris-buffered saline containing Tween-20 (TBS-T) and nonfat dry milk for 1 h at room temperature followed by overnight incubation at 4 °C with antibodies to cleaved caspase-3, caspase-2, caspase-6, caspase-7, total p53, phosphor-p53 (Serine 33), PARP, lamin, and BAX (Cell Signaling Technology, Beverly, MA). Anti-histone H1 and AIF antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The COX IV antibody was obtained from Invitrogen Life Technology (Carlsbad, CA) and the mouse monoclonal β-actin antibody was obtained from Sigma Chemicals. The pan-caspase inhibitor, benzylxycarbonyl-Val-Ala-Asp-fluoromethylketone (ZVAD) was obtained from Peptides International (Louisville, KY). Reactive bands were visualized using Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, Rockford, IL) and then exposed to X-ray film (Kodak X-OMAT AR film, Eastman Kodak Co., Rochester, NY) for autoradiographic visualization. Densitometry was performed with ImageJ software from <http://rsbweb.nih.gov/ij/>.

Mitochondrial membrane permeability

In two separate experiments, NT-2 precursor cells were grown in 96-well plates and treated with ZA or ZD media with or without transfection of the dominant negative p53 construct as described in Corniola et al. [13]. Control cells were transfected with control plasmid. Cells were then incubated with 10 μM 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl benzimidazolylcarbocyanine iodide (JC-1, Biovision Inc., Mountain View, CA) for 10 min at 37 °C. JC-1 is normally localized to the mitochondria in aggregate form where it can be detected using an excitation wavelength of 520–570 nm and an emission of 570–610 nm. However, when membrane potential is compromised, JC-1 leaks into the

cytosol where it disaggregates to monomeric form with an excitation at 485 nm and emission at 535 nm. Fluorescence was measured using Flx800 Microplate fluorescence plate reader (Winooski, Vermont) using the KC4 v3.4 software for data analysis.

Quantitative real time PCR

Quantitative RT-PCR was performed using SA Biosciences (Valencia, CA) PCR Array kit for human apoptosis (Cat#PAHS-012A), using the manufacturer's protocol. Briefly, cDNA was prepared using RT² First Strand Kit with 1 µg of total cellular RNA. Reverse transcription was performed after degradation of genomic DNA. The reaction was stopped by incubation at 95 °C for 5 min before proceeding to the real-time PCR protocol. RT² SYBR Green Mastermix was added to cDNA mix followed by addition to each well of RT² Profiler PCR Array and mixed well using a multi-channel pipettor. Using a hot start protocol the plate was run on a Bio-Rad i-Cycler and analyzed according to the SA Biosciences protocol provided with the Array kit.

Subcellular fractionation

Mitochondrial and nuclear fractions were isolated using a previously published protocol [22]. Briefly, NT-2 cells were harvested, washed, and re-suspended in homogenization buffer containing 210 mM mannitol, 70 mM sucrose, 1 mM EDTA, 10 mM HEPES, pH 7.5, supplemented with protease inhibitor mixture (Sigma). After Dounce homogenization (Wheaton), samples were adjusted to a final concentration of 0.25 M sucrose and 0.35% Triton X-100 and layered on top of a discontinuous sucrose density gradient prepared with 0.32, 0.8, and 1.2 M sucrose for centrifugation at 40,000 × g for 2 h. Nuclei were recovered at the interface of 0.8 M and 1.2 M sucrose and stored at −80 °C.

Mitochondrial fractions were also isolated by sucrose density gradient centrifugation. The supernatant obtained after the isolation of nuclear fraction was centrifuged at 13,000 × g at 4 °C for 10 min. Pellets were re-suspended in homogenization buffer and layered on top of a discontinuous sucrose gradient consisting of 20 ml of 1.2 M sucrose, 10 mM HEPES, pH 7.5, 1 mM EDTA, and 0.1% BSA on top of 17 ml of 1.6 M sucrose, 10 mM HEPES, pH 7.5, 1 mM EDTA, and 0.1% BSA. Samples were centrifuged at 27,000 rpm for 2 h at 4 °C. Pure mitochondria, recovered at the 1.6–1.2 M sucrose interface, were washed and re-suspended in homogenization buffer. Contamination of mitochondria in the nuclear fraction was determined by immunoblotting for cytochrome oxidase subunit IV, an integral membrane protein of the mitochondria.

Statistical analysis

Data were analyzed using one way ANOVA with a Tukey's post hoc test. Data were considered significantly different at $p < 0.05$.

Results

Apoptosis

Confirming previous work [13,15], zinc deficiency produced changes in cultured neuronal precursor cells consistent with apoptotic cell death. Fig. 1 illustrates the finding that cells grown in zinc deficient media exhibited nuclear blebbing, the hall mark sign of apoptosis.

Activation and translocation of p53

While it did not significantly alter the total amount of p53 in NT-2 cells, Fig. 2 shows that growth of cells in zinc deficient

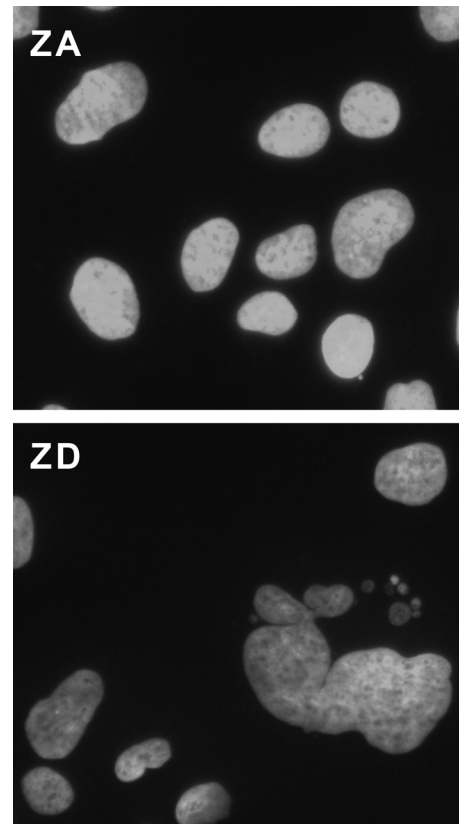


Fig. 1. Zinc deficiency induces apoptosis. Examination of nuclear morphology following DAPI staining of NT-2 cells grown for 48 h in zinc adequate (ZA) or zinc deficient (ZD) media revealed the presence of nuclear condensation and blebbing in ZD cells.

media resulted in the phosphorylation of this transcription factor. Immunohistochemistry not only confirmed previous reports showing that zinc deficiency induces the translocation of p53 to the nucleus, but also revealed co-localization of p53 with Mitotracker, a marker for mitochondria, in zinc deficient cells (Fig. 3).

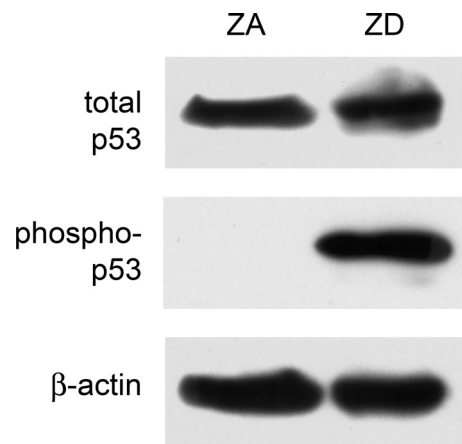


Fig. 2. Zinc deficiency induces phosphorylation of p53. Western blot analysis of total cellular and phosphorylated p53 shows that under zinc adequate control conditions p53 is maintained in the dephosphorylated state. Incubation in zinc deficient media (24 h) resulted in p53 phosphorylation. Figure is representative of cultured cells from three separate experiments.

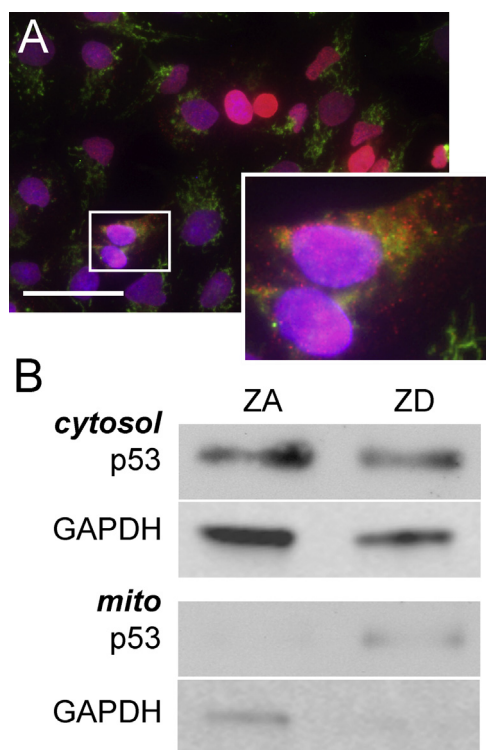


Fig. 3. Zinc deficiency results in nuclear and mitochondrial translocation of p53. Cells were treated with zinc adequate (ZA) or zinc deficient (ZD) media for 24 h. (A) Immunocytochemistry was used to localized p53 (red) in ZD cells. Nuclei were stained with DAPI (blue) and mitochondria were labeled with Mitotracker (green). Overlays of images show localization of p53 to the nuclei in purple and localization of p53 to mitochondria in yellow. (B) Western blot analysis of p53 confirmed the localization of p53 to the mitochondria in the ZD but not ZA conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Mitochondrial alterations

Zinc deficiency resulted in an approximately 2-fold (2.2 ± 0.5 , $p < 0.05$) increase in BAX mRNA that was consistent with an increase in BAX protein levels in zinc deficient cells. Inhibition of mitochondrial p53 with μ PFT prevented this increase in BAX (Fig. 4). Other zinc deficiency-induced mitochondrial changes included an approximately 40% decrease (n/s) in the mitochondrial abundance of the apoptosis inducing factor, AIF (Fig. 5). Fig. 5, a representative blot, shows that there were no detectable levels of nuclear AIF in ZA cells, but abundant nuclear AIF in ZD cells, suggesting translocation of AIF from the mitochondrial to the nuclear compartments. Furthermore, treatment of ZD cells with either ZVAD or μ PFT

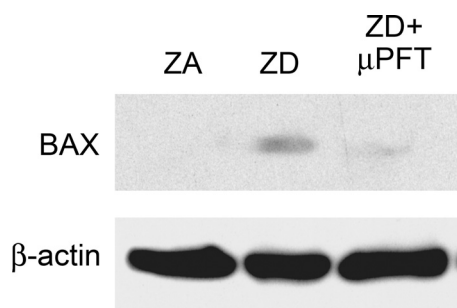


Fig. 4. Zinc deficiency increases BAX abundance. Representative Western blot of cells ($n = 3$) treated with zinc adequate (ZA) media, zinc deficient (ZD) media, or ZD media treated with μ PFT to inhibit mitochondrial p53, for 24 h. β -actin was used as a loading control.

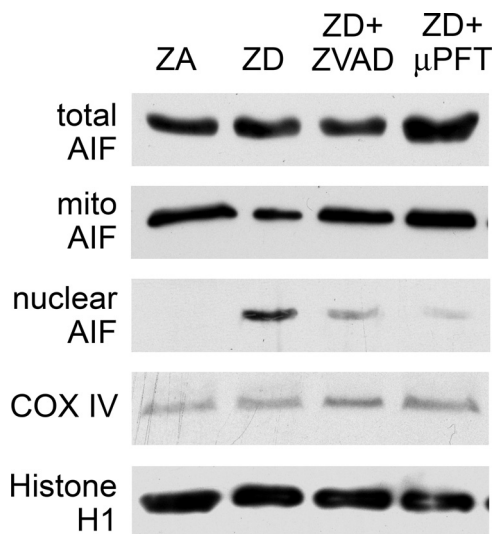


Fig. 5. Zinc deficiency induces translocation of mitochondrial Apoptosis Inducing Factor (AIF) to the nucleus. Representative blot of NT-2 cells treated with zinc adequate (ZA) or zinc deficient (ZD) media for 24 h and subjected to subcellular fractionation for Western blot analysis ($n = 6$). ZD treatment reduced the abundance of mitochondrial AIF (mito AIF). Reductions were prevented by treatment of ZD cells with the caspase inhibitor ZVAD (ZD + ZVAD). Nuclear AIF, which was not detected in ZA NT-2 cells, was increased in ZD cells. Treatment of ZD cells with ZVAD (ZD + ZVAD) or the mitochondrial p53 inhibitor (ZD + μ PFT) partially prevented nuclear accumulation of AIF. Mitochondrial COX IV and nuclear Histone H1 protein abundance served as controls.

partially prevented the nuclear accumulation of AIF (Fig. 5) with ZVAD reducing nuclear AIF by 50% and μ PFT reduction nuclear AIF by approximately 70% ($p < 0.05$).

Zinc deficiency also resulted in a decrease in mitochondrial membrane potential reflected in a 25% decrease in the red to green ratio of JC-1 (2.2 ± 0.1 vs 1.7 ± 0.6 , $p < 0.05$). Transfection of cells with the dominant negative p53 gene construct eliminated this decrease (2.5 ± 0.6). DHR fluorescence also suggested mitochondrial production of reactive oxygen species (ROS), with a 2-fold increase associated with zinc deficiency. ROS production was reduced to control levels when mitochondrial p53 was inhibited by treatment of cells with μ PFT (Fig. 6).

Caspase activation

Initial RT-PCR analysis suggested that zinc deficiency results in an increase in mRNA abundance for caspase 2 (7.6 \pm 1.7-fold increase, $p < 0.05$), caspase 3 (2.5 \pm 0.6-fold, $p < 0.05$), and caspase 7 (1.4 \pm 0.1-fold). Western and ImageJ analysis of caspases suggested a role for zinc deficiency in the relative increase in caspases 3, 6, and 7 abundance (Fig. 7). Caspase 3 abundance was increased by more than 6-fold ($p < 0.01$), caspase 6 was increased by 3-fold ($p < 0.001$), and caspase 7 was increased by 1.5-fold ($p < 0.05$). More importantly, caspases 2, 3, 6, and 7 were all cleaved under conditions of zinc deficiency (Fig. 7). Addition of the pan-caspase inhibitor ZVAD prevented cleavage-induced activation of these caspases (Fig. 7). Caspase activation in zinc deficient NT-2 cells was accompanied by cleavage of poly-ADP ribose polymerase (PARP) and lamin (Fig. 8). Cleavage of these down-stream caspase targets was also inhibited by the pan-caspase inhibitor ZVAD (Fig. 8).

Discussion

This work has not only confirmed the dependence of neuronal precursor cell survival on the essential trace element zinc, but has also reported data enabling us to build a model, illustrated in Fig. 9,

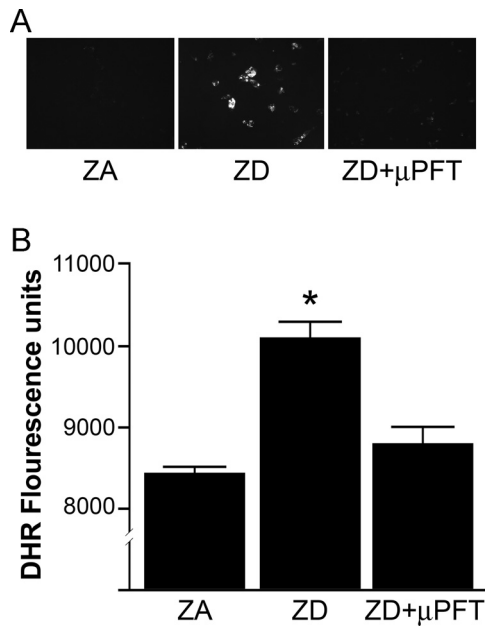


Fig. 6. Zinc deficiency induces ROS that is dependent on mitochondrial p53. NT-2 cells were treated with zinc adequate (ZA) or zinc deficient (ZD) media for 24 h. (A) Representative photomicrograph showing increased DHR fluorescence in ZD cells compared to ZA cells. Treatment of ZD cells with 5 μM μPFT (ZD + μPFT), that inhibits translocation of p53 to the mitochondria, abolished this signal. (B) Quantification (mean ± SD) of ROS in ZA, ZD, and ZD + μPFT cells. *Significantly different from ZA cells at $p < 0.001$.

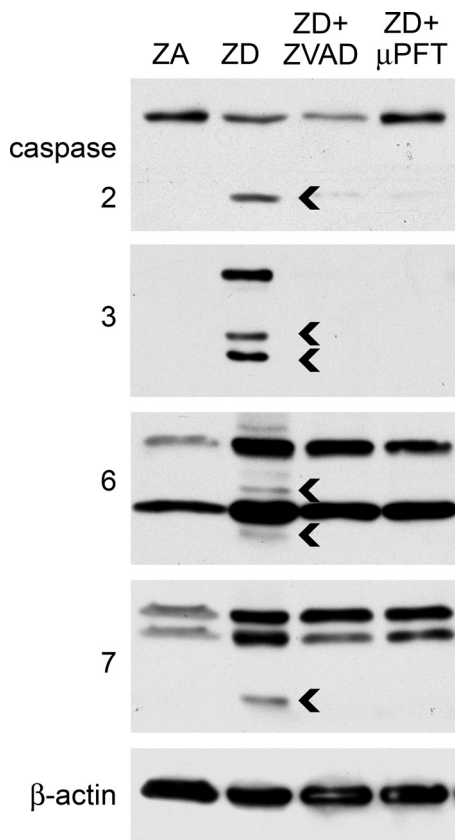


Fig. 7. Zinc deficiency induces caspase cleavage. NT-2 cells ($n = 6$) were treated with zinc adequate (ZA) or zinc deficient (ZD) media for 24 h and harvested for Western blot analysis to analyze activation of caspases 2, 3, 6, and 7. Separate dishes of zinc deficient cells were treated with the caspase inhibitor ZVAD (ZD + ZVAD). Arrows point to representative images of cleaved caspases in the zinc deficient condition.

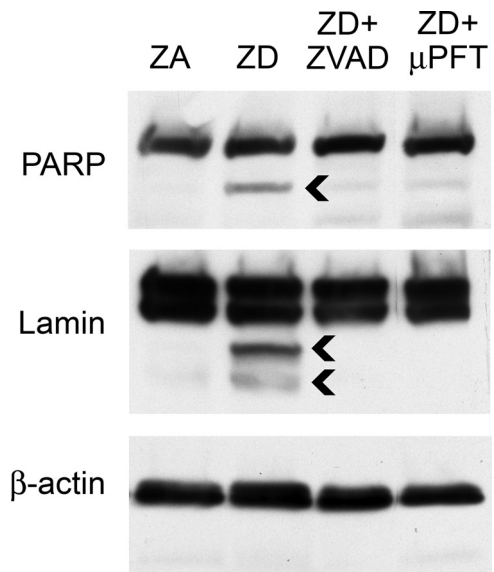


Fig. 8. Zinc deficiency induces PARP and lamin cleavage. NT-2 cells ($n = 6$) were treated with zinc adequate (ZA) or zinc deficient (ZD) media for 24 h and harvested for Western blot analysis to evaluate cleavage of PARP and lamin. Separate dishes of zinc deficient cells were treated with the caspase inhibitor ZVAD (ZD + ZVAD). Arrows point to representative images of cleaved substrates in the zinc deficient condition.

that describes our current understanding of p53-dependent mechanisms associated with zinc deficiency-induced apoptosis in this important cell type, and suggests a prominent role for mitochondrial p53 in these mechanisms.

p53-Mediated mechanisms

Previous work has identified p53 as a key regulator of apoptosis in zinc deficient neuronal precursor cells [13]. The finding, reported here, that zinc deficiency induces the phosphorylation of p53, is consistent with p53 activation and translocation to the nucleus [23] where it acts to regulate a wide variety of downstream gene targets [13,24]. Given that p53 has previously been shown to be phosphorylated under conditions of high zinc in other cell types such as human A549 pulmonary epithelial cells [25], these data suggest that p53 activation is under tight regulation by zinc and that optimal levels of zinc are needed for normal p53 function. When cells induce p53 in an intermittent fashion, alterations in gene expression arrest the cell cycle and increase the potential for cellular recovery. However, when active p53 levels are sustained, it induces apoptotic mechanisms that lead to cell death [26]. This is consistent with the finding that zinc deficiency initially regulates cell cycle arrest genes in a p53-dependent fashion, while sustained zinc deficiency leads to a p53-dependent increase in pro-apoptotic genes and a decrease in anti-apoptotic genes [13].

In addition to the important nuclear and transcriptional roles of p53, it has been recently recognized that the mitochondrial localization of p53 also participates in the induction of apoptosis [23,27] in part by interaction with Bcl-family proteins such as BAX [16,27,28]. Fig. 9 summarizes the findings from the current study showing the localization of p53 to the nucleus and the mitochondria as well as increases in the pro-apoptotic protein BAX and caspase activation. The interaction between BAX and p53, which has also been reported in staurosporine-treated cerebellar neuronal precursor cells and other cell types [16,29], leads to the direct activation of BAX [17,19,30] and BAX-pore formation at the outer mitochondrial membrane [18].

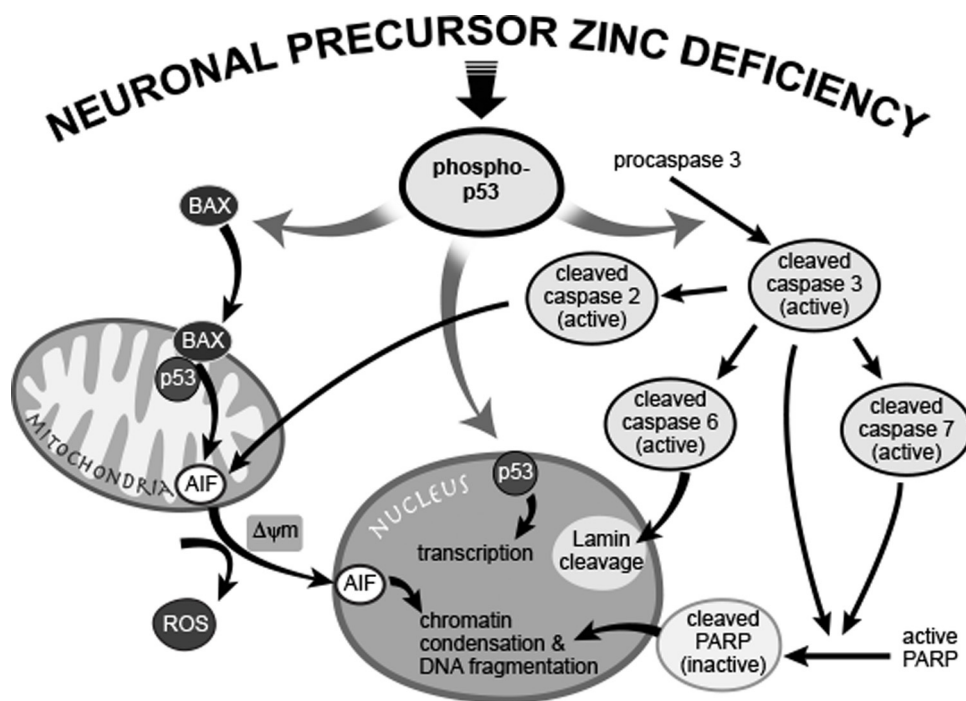


Fig. 9. Role of mitochondrial p53 and caspase activation in zinc deficient human neuronal precursor cells. Zinc deficiency induces p53 phosphorylation. Activated p53 is then translocated to the nucleus and mitochondria. Mitochondrial p53 interacts with the pro-apoptotic protein BAX, hypothesized to form pores in the outer mitochondrial membrane that reduces the mitochondrial membrane potential ($\Delta\psi_m$), increases reactive oxygen species (ROS), and permits the translocation of the apoptosis inducing factor, AIF, to the nucleus. Caspase cleavage results in caspase activation, lamin cleavage and inactivation of PARP. All of the above mechanisms contribute to apoptosis in zinc deficient neuronal precursor cells.

Our data suggest that this is likely to have multiple effects leading to apoptosis. First, as illustrated in Fig. 9, pore formation causes the release of mitochondrial proteins such as the apoptosis inducing factor, AIF. This is significant because not only has the release of AIF been linked to mitochondrial p53 in other cell types [22,31], but activation of AIF has also been reported in the hippocampus of rats fed a zinc deficient diet for 5 weeks [32]. Also consistent with BAX pore formation, we observed a loss of mitochondrial membrane potential as well as an increase in the production of ROS, that has previously been linked to mitochondrial p53 [33]. We provide two lines of evidence suggesting that these mechanisms of zinc deficiency are dependent on p53. First, treatment of cells with μ PFT, a drug that prevents the localization of p53 to the mitochondria [18], abolished the increase in BAX as well as the production of ROS in ZD cells. Secondly, transfection of deficient cells with a dominant negative p53 construct, previously used to reduce p53 activity in NT-2 cells [13], prevented the loss of mitochondrial membrane potential.

Caspase-mediated mechanisms

Consistent with data reported here for zinc deficiency, previous work using other stimulators of p53 phosphorylation has shown that activated p53 can induce the activation of caspase 3 [34]. Furthermore, while p53 appears to play a role in AIF translocation from the mitochondria to the nucleus during zinc deficiency, our data also provide evidence that mitochondrial release of AIF is mediated by p53-dependent caspase activation. We showed that caspase inhibition not only prevented the reductions in mitochondrial AIF, but also resulted in reduced nuclear accumulation of AIF associated with zinc deficiency. A role for caspases in AIF translocation is supported by previous work showing that caspase 2 activation leads to mitochondrial release of AIF in cisplatin-treated

renal tubular epithelial cells [22]. Our finding that caspase 2 mRNA abundance and caspase 2 cleavage were both induced also support a role for this enzyme in zinc deficient neuronal precursor cells.

Clearly, caspase activation and the caspase cascade are complex and not fully understood [35]. For example, while caspases 3 and 7 clearly have some redundant functions, such as the cleavage of PARP [36], they are also known to have distinct substrates as well [37]. In any case, the fact that caspases 3, 7, and PARP are all cleaved in zinc deficient neuronal precursor cells suggests that these members of the caspase cascade play a role in the p53-mediated mechanisms of apoptosis observed here. As illustrated in Fig. 9, caspase 3 activation has also been associated with caspase 6 cleavage and the subsequent cleavage of nuclear lamin [36] leading to the structural breakdown of the nuclear envelope [38]. Clearly caspase 3 is likely to have other downstream actions as well. Thus, while Fig. 9 represents only a single model that can occur when zinc deficiency induces caspase activation, it provides a framework for our understanding of the role of phosphorylated p53 and caspases in survival and apoptosis of neuronal precursor cells.

Conclusions

This work has identified zinc-regulated mechanisms that are responsible for the regulation of neuronal precursor cells. As the cell type that gives rise to neurons throughout the brain, neuronal precursor cells are important for brain development. Furthermore, we now appreciate the fact that, although limited, the adult brain contains neuronal stem cells and neuronal precursors that not only proliferate but have the potential to form new neurons. Thus, this work showing new roles for zinc in mitochondrial p53- and caspase-dependent mechanisms has implications for CNS health throughout the lifespan.

Conflicts of interest

The authors have no conflicts of interest to report.

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