

Differential response to zinc-induced apoptosis in benign prostate hyperplasia and prostate cancer cells[☆]

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Abstract

Zinc concentrations in the prostate are uniquely high but are dramatically decreased with prostate cancer. Studies have suggested that increasing zinc in the prostate may be a potential therapeutic strategy. The goal of this study was to evaluate the antiproliferative effects of zinc in prostate cancer cells (PC-3) and noncancerous benign prostate hyperplasia (BPH) cells (BPH-1) and to define possible mechanisms. PC-3 and BPH-1 cells were treated with zinc (0–250 μ M) for 24 and 48 h, and cell growth and viability were examined. Apoptosis was assessed by phosphatidylserine externalization, caspase activation and protein expression of B-cell CLL/lymphoma 2 (Bcl-2)-associated X protein (BAX):Bcl-2. BPH-1 cells were more sensitive to the antiproliferative effects of zinc compared to PC-3. The response to zinc in PC-3 and BPH-1 cells differed as evidenced by opposing effects on Bcl-2:BAX expression. Additionally, different effects on the nuclear expression and activity of the p65 subunit of nuclear factor kappa B were observed in response to zinc between the two cell types. The differential response to zinc in PC-3 and BPH-1 cells suggests that zinc may serve an important role in regulating cell growth and apoptosis in prostate cancer and hyperplasia cells.

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Keywords: Apoptosis; Benign prostate Hyperplasia; Cancer; Prostate; Zinc

1. Introduction

Prostate cancer is the second leading cause of cancer related deaths in American men [1]. Benign prostate hyperplasia (BPH) is another disease of the prostate that primarily results in symptoms in the lower urinary tract and can significantly impair quality of life [2]. Together, prostate cancer and BPH make up a considerable portion of health concerns for men in the United States. Recently, increased attention has been given to the contribution of dietary and lifestyle factors to prostate cancer risk. Several studies have shown an association between a variety of dietary compounds and decreased risk for BPH and prostate cancer [3–8]. Zinc is an essential mineral that plays multiple roles in the human body. Low zinc status has been observed in cancer patients, suggesting a possible link between zinc and cancer development [9–12]. Although data examining correlations between zinc and prostate cancer has been inconsistent, a study by Kristal et al. [13] suggested use of zinc supplements decreased risk for prostate cancer. Furthermore, a recent study by the same group

found an inverse correlation between zinc intake and BPH risk [8]. Thus, the function of zinc in the prostate and its possible protective role against BPH and prostate cancer have been of increasing interest.

The prostate contains uniquely high zinc levels compared to other soft tissues in the body. Although zinc concentrations are relatively high throughout the prostate, zinc accumulation mainly occurs in the peripheral zone. Zinc concentrations in this region can be 3–10 times greater than in other soft tissues [14]. Prostate cancer typically arises from the high zinc accumulating peripheral zone of the prostate, while BPH is thought to originate in the transition zone [14,15]. Importantly, prostate cancer cells (PC-3) lose the ability to accumulate zinc, resulting in significantly decreased zinc concentrations in malignant prostate cells. It is thought that this transformation occurs early on during prostate carcinogenesis, since malignant prostate cells have always been observed to have low zinc [14]. It has been hypothesized that increasing cellular zinc levels and reestablishing normal zinc concentrations could impair prostate cancer cell growth. Previous studies have shown that the treatment of androgen-dependent LNCaP cells and androgen-independent PC-3 with zinc resulted an inhibition of cell growth, cell cycle arrest, increased p21 mRNA levels and increased apoptosis [16]. Furthermore, zinc-induced apoptosis is only observed in cells in which there was intracellular zinc accumulation [17]. In vivo, zinc supplementation also increased Bcl-2-associated X protein (BAX):B-cell CLL/lymphoma 2 (Bcl-2) expression and inhibited tumor growth in nude mice inoculated with

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PC-3 [18]. These studies indicate that zinc may have a protective effect by inhibiting prostate tumor cell growth and inducing apoptosis. Although the role of zinc in limiting cell growth prior to malignancy is not clear, it has become apparent that zinc plays an essential role in prostate health and that there is a strong inverse correlation between intracellular zinc and uncontrolled cell proliferation in the prostate. Therefore, many studies have been focused on examining the regulation and specific functions of zinc in the prostate. Whether zinc supplementation could benefit both prostate cancer and BPH cells is unknown. Unlike PC-3, zinc concentrations in BPH vary and have been reported to be equal to or higher than in normal prostate cells [14]. Thus, the role of zinc and its association with the development of non cancerous BPH maybe regulated though different mechanisms compared to prostate cancer. The goal of this study was to examine the differences in the response to zinc between prostate cancer and BPH cells. Furthermore, possible mechanisms leading to zinc-induced apoptosis in each cell type were explored, including caspase induction, Bcl-2:BAX expression, nuclear factor kappa B (NF κ B) activation and p53 expression.

2. Materials and methods

2.1. Cell culture

Human androgen dependent PC-3 (American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI 1640 with glutamine plus 10% fetal bovine serum (FBS). Human BPH cells (BPH-1) (Kind gift from Dr. Simon Hayward, Vanderbilt University, Nashville, TN, USA) were cultured in RPMI 1640 with glutamine plus 5% FBS and 1% penicillin/streptomycin. Cells were maintained in 5% CO₂ at 37°C.

2.2. Cell growth, viability and cellular zinc

Equal numbers of PC-3 and BPH-1 cells were seeded and allowed to reach 70% confluency before being treated with 0–250 μ M zinc (as zinc chloride dissolved in deionized water) for 24 or 48 h. Zinc concentrations in control media was \sim 4 μ M (that of normal media). To evaluate cell growth, total cells were counted using Beckman Coulter Z1 Coulter Particle Counter (Fullerton, CA, USA). PC-3 and BPH-1 cells were seeded in 24-well plates at 1×10^5 cells per well and allowed to attach overnight. Cells were then treated with 0–250 μ M zinc for 24 h. Cellular viability was determined by detection of formazan product from methylthiazolyldiphenyl-tetrazolium bromide (MTT). MTT assay was performed as described by Mosmann [19]. Production of formazan product was detected at 580 nm using Molecular Devices SpectraMax (Molecular Devices, Sunnyvale, CA, USA).

Cellular zinc levels were determined using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES). Cells were harvested by trypsin and washed three times with Dulbecco's phosphate-buffered saline (DPBS) and cell pellets were collected for analysis. Cell pellets containing 2–5 million cells were vortexed with 1 ml 70% ultrapure nitric acid (EMD Omnitrace, Gibbstown, NJ, USA) overnight. After incubation, samples were diluted with chelex-treated nanopure water to a 7% acid solution, centrifuged and analyzed by the Prodigy High Dispersion ICP-OES instrument (Teledyne Leeman Labs, Hudson, NH, USA) against known standards.

2.3. Apoptosis

Annexin V staining and caspase activation were determined as indicators of apoptosis. Annexin V staining was conducted using the Nexin Kit from Guava Technologies (Hayward, CA, USA) as directed by manufacturer. Briefly, floating and adherent cells were collected, washed and stained with annexin V-phycoerythrin (annexin V-PE) and 7-amino-actinomycin-D (7-AAD). Annexin V-PE binds with high

affinity to phosphatidylserine, which is externalized during the early stage of apoptosis. The 7-AAD dye is an indicator of membrane structural integrity and is excluded from healthy live cells but permeates late stage apoptotic and necrotic cells. Percentage of cells staining for each dye was quantified using the Guava personal cell analyzer (PCA) (Hayward, CA). The Guava Multi Caspase Kit (Hayward, CA, USA) was used to determine caspase activation. Cells were harvested and exposed to sulforhodamine-valyl-alanyl-aspartyl-fluoromethyl-ketone (SR-VAD-FMK), a cell permeable fluorochrome which covalently binds to multiple activated caspases and also stained with 7-AAD. Percentage of cells staining for each of these dyes was quantified using the Guava PCA (Hayward, CA, USA).

2.4. Western blot and protein isolation

After zinc treatment, floating and adherent cells were collected for protein isolation. Whole cell lysates were obtained using IP lysis buffer (20 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM sodium pyrophosphate, 1 mM beta-glycerolphosphate, 1 mM sodium orthovanadate and 1 mg/L leupeptin) plus protease inhibitor (Complete, Mini; Protease Inhibitor Cocktail Tablets, Roche, Indianapolis, IN, USA). Nuclear and cytoplasmic fractionation was conducted using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL, USA). Total protein concentrations were determined using DC Protein Assay (Bio-Rad, Hercules, CA). Standard Western blot procedure was performed using 20 μ g protein loaded and separated on NuPAGE 4–12% Bis-Tris gels (Invitrogen, Carlsbad, CA, USA) and transferred onto nitrocellulose membrane (BioRad Laboratories, Hercules, CA, USA). Antibody dilutions were as follows: BAX 1:100, Bcl-2 1:1000, p53 1:1000 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) and β -actin 1:4000 (Sigma-Aldrich, St. Louis, MO, USA). Secondary antibodies raised against the corresponding host conjugated to horseradish peroxidase were used. Chemiluminescence was detected using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL, USA) on AlphaInnotech photodocumentation system. Quantification of signal intensity was determined using Image J 1.37v [National Institutes of Health (NIH), Bethesda, MD, USA] software. Triplicate samples for each treatment group were analyzed.

2.5. NF κ B binding activity

The binding activity of NF κ B subunit p65 in nuclear extracts was assessed using the TransAM NF κ B p65 Kit (Active Motif, Carlsbad, CA, USA) as directed by the manufacturer. TransAM is an enzyme-linked immunosorbent assay (ELISA)-based method which detects p65 binding to NF κ B consensus sequence from nuclear cell extracts. Nuclear extracts from PC-3 and BPH-1 cells treated with zinc were isolated as described above.

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 4.01 (San Diego, CA, USA). Data for total cell number (as percent of control) and relative viability for PC-3 and BPH-1 cells was natural log transformed. Then linear regression lines (and equations) were determined for each data set. These data were used to calculate the EC 50 for cell growth and viability. One-way analysis of variance (ANOVA) with Tukey's posttest was used to determine statistical differences between control and treatment groups for analyses except for the MTT assay. For MTT assay, 2-way ANOVA with Bonferroni posttests was conducted to determine differences in relative viability across cell type and zinc treatment. *P* value <.05 was considered significant.

3. Results

3.1. Cell growth, viability and cellular zinc

BPH-1 cells were more sensitive than PC-3 to growth inhibitory effects of zinc. Zinc concentrations $\geq 75 \mu\text{M}$ resulted in a significant decrease in total cells in BPH-1 cells, whereas higher concentrations of zinc ($150 \mu\text{M}$) were required to significantly decrease total cells in PC-3 (Fig. 1A). The natural log of total cell number (as percent of control) was plotted against zinc treatment (data was natural log-transformed to normalize distribution before linear regression analysis was

performed, and linear regression analysis was conducted to determine the EC 50 for cell growth (Fig. 1B). Data was natural log-transformed to normalize distribution before linear regression analysis was performed. The calculated EC 50 for cell growth was 141.1 and 55.1 μM for PC-3 and BPH-1 cells, respectively. Differences in cell viability between PC-3 and BPH-1 cells were also observed at zinc treatments $\geq 100 \mu\text{M}$. Cell viability in BPH-1 cells decreased significantly at $\geq 100 \mu\text{M}$ zinc, but higher concentrations ($250 \mu\text{M}$) were required to significantly decrease viability in PC-3 (Fig. 1C). The calculated EC 50 for cell viability was 190.8 and 95.9 μM for PC-3 and BPH-1 cells, respectively. Despite different responses to zinc, there

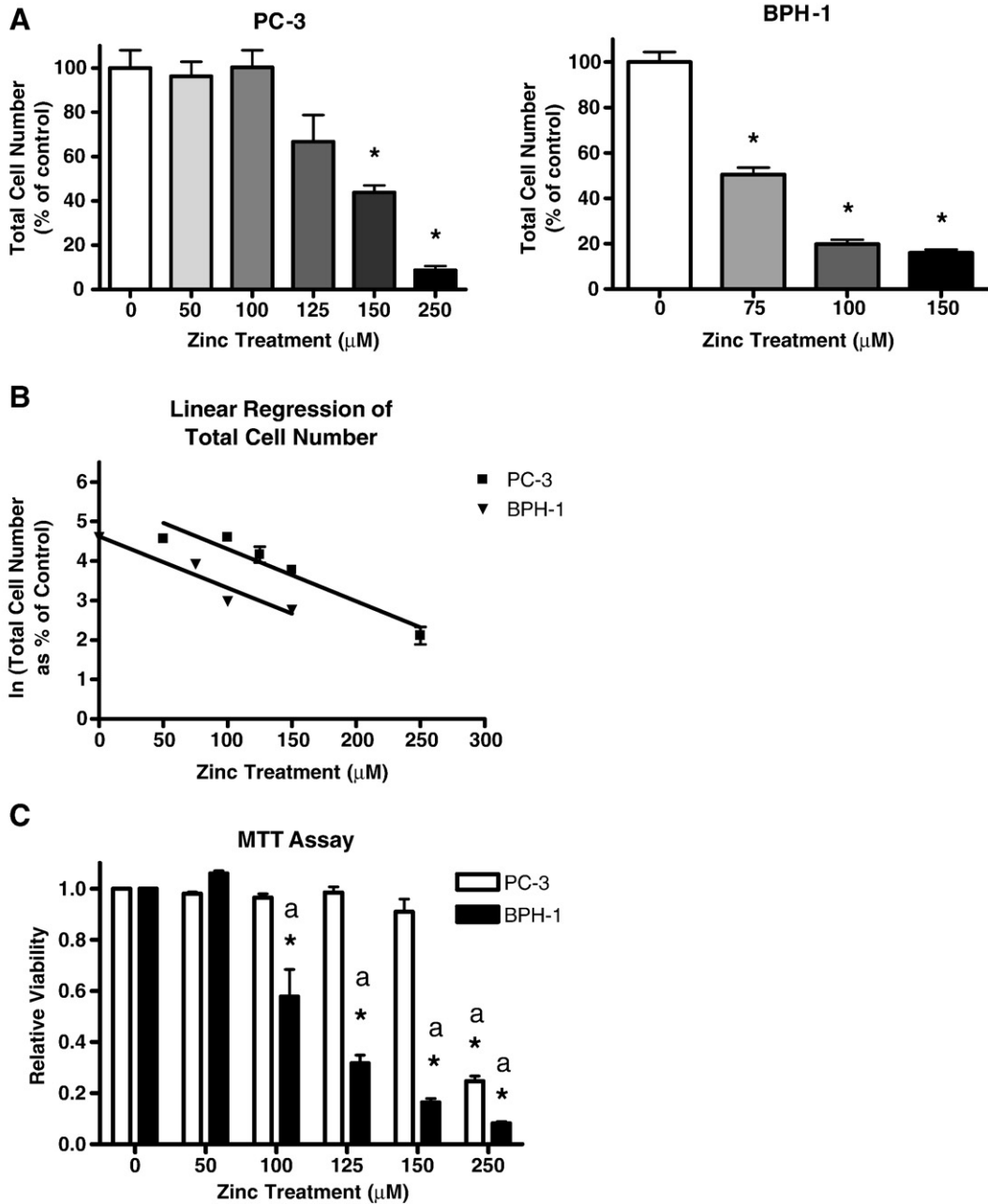


Fig. 1. BPH-1 cells were more sensitive to the antiproliferative effects of zinc compared to PC-3. Cells were treated with zinc for 48 h; total cell numbers were determined using Beckman Coulter Z1 Coulter Particle Counter and plotted as percent of control \pm S.E.M. (A) Higher concentrations of zinc ($150 \mu\text{M}$) were required to significantly decrease total cell number in PC-3 compared to BPH-1 cells ($75 \mu\text{M}$). (B) Linear regression lines were determined for PC-3 and BPH-1 cells, and data is shown as percent of total cells as a function of zinc treatment. (C) PC-3 and BPH-1 cells were treated with zinc for 24 h, and viability was determined by MTT assay. Higher concentrations of zinc were required to induce a significant decrease in cell viability in PC-3 compared to BPH-1 cells. * $P < .05$ versus control treatment (no zinc) within a cell type. "A" indicates significant difference between PC-3 and BPH-1 cells within treatment.

Table 1
Zinc concentrations in prostate epithelial cells following zinc treatment (48 h)

Cell line	Control (0 μM zinc)	100 μM zinc	150 μM zinc
BPH-1	0.084 \pm 0.019	0.297 \pm 0.014*	0.232 \pm 0.026*
PC-3	0.076 \pm 0.003	0.3098 \pm 0.020*	0.2801 \pm 0.056*

Data is represented as mean ($\mu\text{g}/\text{million cells}$) \pm S.D.
* $P<0.001$ from control.

were still significant increases in cellular zinc in both cell lines after $\geq 100 \mu\text{M}$ zinc treatment. Cellular zinc concentrations following 48h of 100 μM zinc treatment were 0.310 \pm 0.007 and 0.297 \pm 0.006 μg zinc/million cells for PC-3 and BPH-1 cells, respectively, and were not significantly different from each other (Table 1). Zinc treatment in androgen-dependent LNCaP cells also decreased cellular viability;

however, greater than 250 μM zinc was needed to reduce viability by 50% (data not shown).

3.2. Apoptosis

To further explore the mechanism of the zinc-induced decrease in cell number and viability in BPH-1 cells, we examined two markers of apoptosis, phosphatidylserine externalization and caspase activation. Zinc treatment at $\geq 100 \mu\text{M}$ resulted in apoptosis as evidenced by increased annexin V staining (indicated by positive annexin V and negative 7-AAD labeling). Increased annexin V staining was more dramatic after 48 h zinc treatment versus 24 h; however both time points showed a significant increase in annexin V staining (Fig. 2). A significant increase in caspase activation was observed at 24 h with 150 μM zinc and at 48 h with 100 μM zinc. At

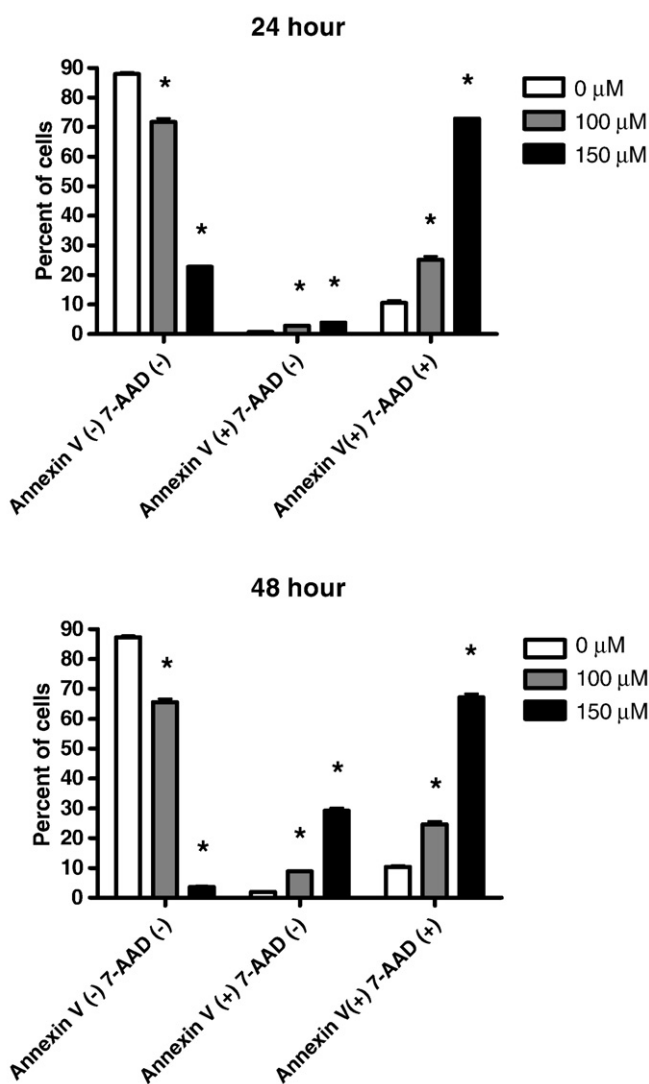


Fig. 2. Zinc increased annexin V staining, a marker of apoptosis, in BPH-1 cells. Cells were stained with annexin V and 7-AAD (an indicator of membrane integrity). Annexin V (-) 7-AAD (-) represents viable cells, annexin V (+) 7-AAD (-) represents early apoptotic cells and annexin V (+) 7-AAD (+) represents late apoptotic/necrotic cells. Both concentrations of zinc significantly decreased percentage of viable cells and increased apoptotic and necrotic cells. Statistical analysis was conducted using one-way ANOVA to compare zinc treatments with control (0 μM zinc) within each staining pattern [annexin V (-) 7-AAD (-), annexin V (+) 7-AAD (-) and annexin V (+) 7-AAD (+)] for each time point. Asterisk indicates $P<0.001$. Triplicate samples were analyzed and results plotted as mean \pm S.E.M.

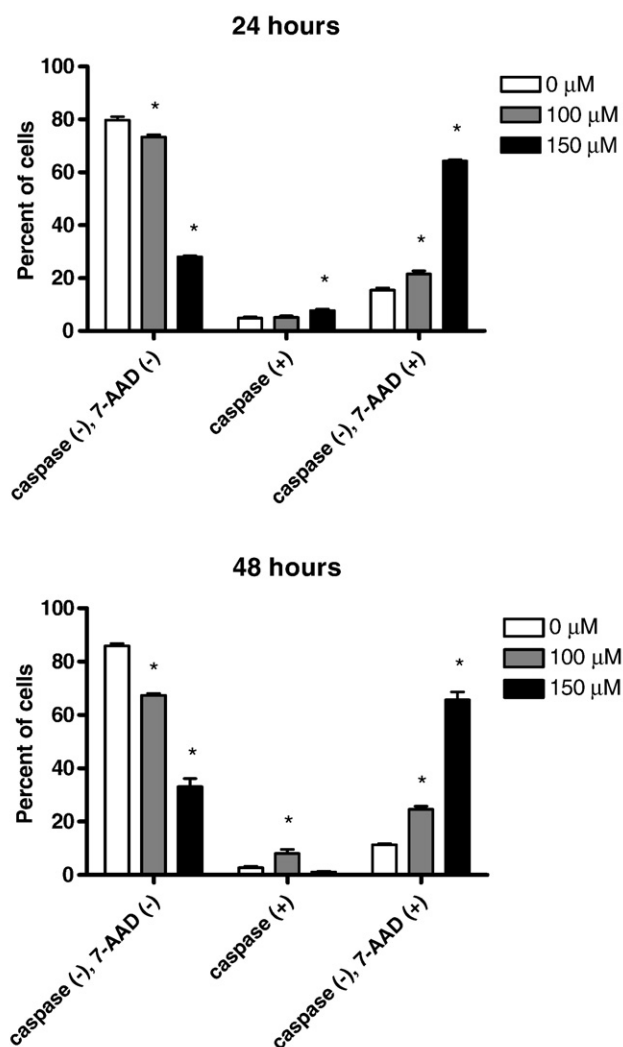


Fig. 3. Zinc induced caspase activation, decreased cell viability and increased cell death in BPH-1 cells. Cells were analyzed for caspase activation using the Guava Multi Caspase Kit. Cells were exposed to the SR-VAD-FMK fluorochrome (which covalently binds to activated caspases) and the cell impermeant dye 7-AAD (an indicator of cell membrane integrity). Caspase (-) 7-AAD (-) represents viable cells; Caspase (+) represents early and late apoptotic cells; and caspase (-), 7-AAD (+) represents late apoptotic/necrotic cells. Cultures treated with zinc have reduced cell viability, increased caspase activation and an increased population of apoptotic and necrotic cells. Statistical analysis was conducted using one-way ANOVA, $*P<0.05$ compared to 0 μM zinc within each treatment for each staining pattern for each time point. Triplicate samples were analyzed and results plotted as mean \pm S.E.M.

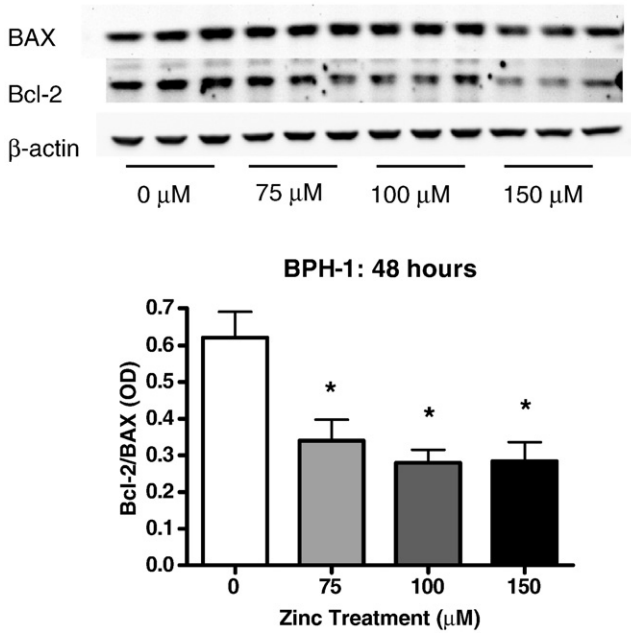


Fig. 4. Zinc caused a significant decrease in Bcl-2:BAX ratio after 48 h in BPH-1 cells. BAX and Bcl-2 protein expression was determined by Western blotting. B-actin was used as the loading control. Triplicate samples were analyzed. Densitometry was conducted to quantify protein expression and plotted as OD (normalized to actin) versus zinc treatment as mean±S.E.M. Statistical analysis was conducted using one-way ANOVA, comparing each treatment to 0 μM zinc treatment. Triplicate samples were analyzed. BAX expression was significantly decreased at ≥100 μM zinc. Bcl-2 expression was significantly decreased at ≥75 μM zinc. The Bcl-2:BAX ratio was significantly decreased at ≥75 μM zinc. Asterisk indicates $P<0.05$.

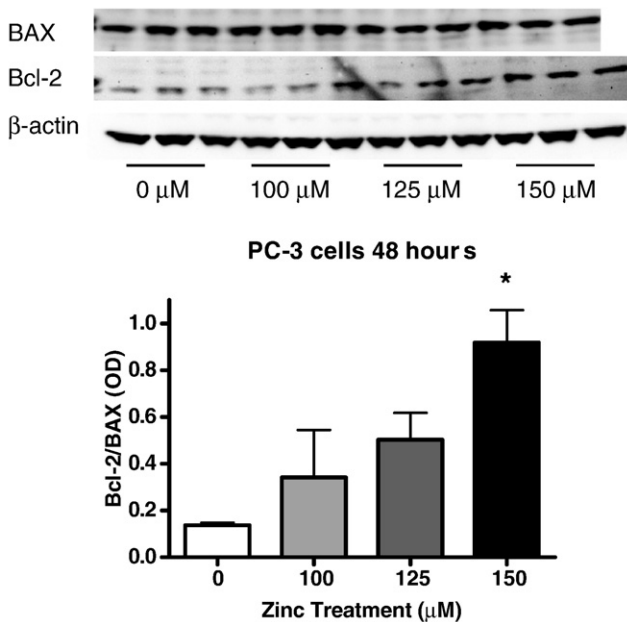


Fig. 5. Zinc induced a significant increase in Bcl-2:BAX ratio after 48 h in PC-3. BAX and Bcl-2 protein expression was determined by Western blotting. B-actin was used as the loading control. Triplicate samples were analyzed. Densitometry was conducted to quantify protein expression and plotted as OD versus zinc treatment as mean±S.E. Statistical analysis was conducted using one-way ANOVA, comparing each treatment to 0 μM zinc treatment. Triplicate samples were analyzed. There was no significant change in BAX or Bcl-2 alone. There was a significant increase in Bcl-2:BAX expression at 150 μM zinc. Asterisk indicates $P<0.05$.

both time points and treatment concentrations, there was a significant increase in 7-AAD positive cells (representing late apoptotic and/or necrotic cells) (Fig. 3). Caspase activation occurs early during apoptosis signaling, prior to loss of membrane integrity. It is possible that we did not observe stronger increases in caspase activation because it occurred earlier than 24 h, and the time points chosen did not represent the peak increases in caspase activation. No significant changes in Annexin staining or caspase activation were seen with PC3 cells treated with similar concentration of zinc (data not shown).

To explore possible mechanisms contributing to zinc-induced apoptosis, protein expression of BAX and Bcl-2 was examined. There was a significant decrease in Bcl-2:BAX expression ratio with ≥75 μM zinc treatment ($P<0.05$) in BPH-1 cells after 48 h (Fig. 4). In contrast, 48-h zinc treatment resulted in a trend of increasing Bcl-2:BAX expression ratio with increasing zinc treatment which reached significance at 150-μM zinc in PC-3 (Fig. 5).

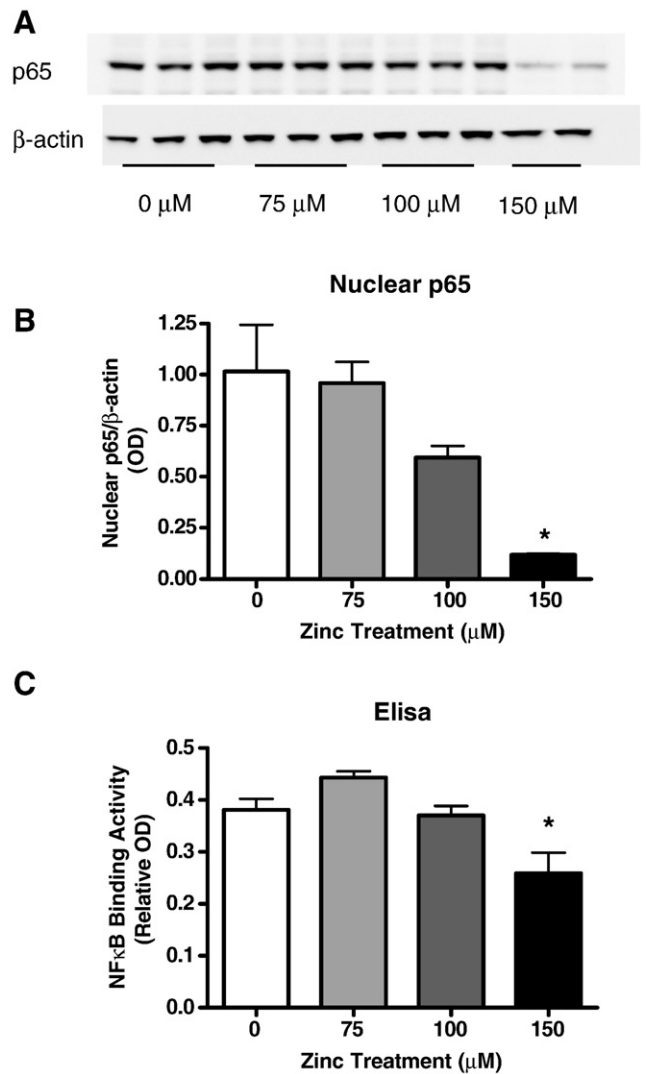


Fig. 6. Zinc decreased nuclear p65 expression and binding activity in BPH-1 cells. BPH-1 cells were treated with zinc for 48 h. Nuclear protein expression of p65 was determined by Western blotting (A), quantified by densitometry analysis and plotted as mean±S.E. (B). Beta-actin was used as the loading control; 150 μM zinc induced a significant decrease in p65 nuclear protein expression after 48 hours ($P<0.05$). (C) Accordingly, 150 μM zinc also decreased p65 binding activity as determined by ELISA ($P<0.05$).

3.3. NFκB expression and activity

NFκB is a well characterized redox-sensitive transcription factor that controls immune function as well as cell survival and apoptosis. Previous studies have shown that zinc effectively inhibits NFκB and promotes apoptosis; thus, NFκB binding activity and nuclear p65 protein expression were examined. After 48 h, 150 μM zinc decreased nuclear p65 protein expression and NFκB binding activity in BPH-1 cells (Fig. 6A-C). Although there was no change in nuclear p65 protein expression with zinc treatment in PC-3, there was a significant decrease in NFκB binding activity at ≥125 μM zinc (Fig. 7A-C).

3.4. p53 expression

Since p53 is another transcription factor involved in regulation of apoptosis, we examined the response of p53 due to zinc in BPH-1 cells only, given that PC-3 do not express p53. There was a significant

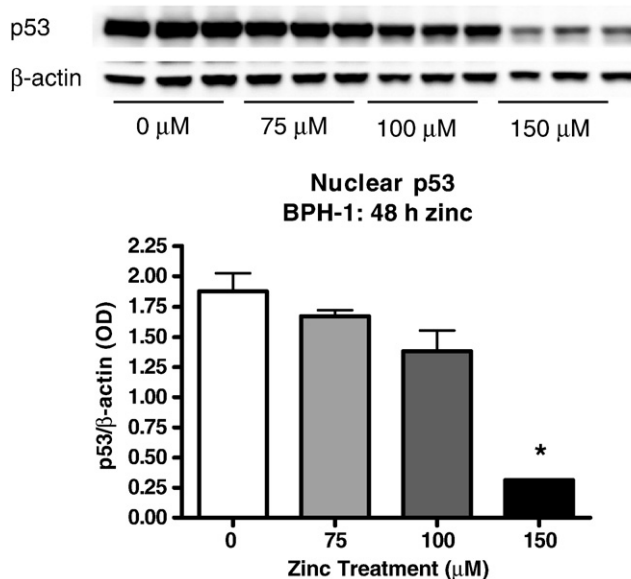


Fig. 8. Zinc decreases nuclear p53 expression in BPH-1 cells. BPH-1 cells were treated with 0–150 mM zinc for 48 h and then harvested for analysis. Nuclear p53 expression was determined by Western blotting, quantified by densitometry analysis and plotted as mean±S.E. Triplicate samples were analyzed. One-way ANOVA was conducted with Tukey's multiple comparison testing. Asterisk indicates $P<0.001$ compared to 0 μM zinc treatment.

decrease in nuclear p53 expression in BPH-1 cells treated with 150 μM zinc for 48 h (Fig. 8).

4. Discussion

Overall, these data suggest that zinc supplementation may play an important role in the prevention of prostate cancer and hyperplasia. In this study, BPH-1 cells appeared to be more sensitive to zinc compared to PC-3. In BPH-1 cells, zinc decreased cell number and viability, which was associated with increased apoptosis, decreased Bcl-2:BAX expression ratio and decreased NFκB (p65) activity and nuclear p65 protein expression. Conversely, in PC-3, higher concentrations of zinc were required to decrease total cell number and viability. Additionally, zinc increased Bcl-2:BAX protein expression ratios but did not alter nuclear p65 protein expression in PC-3. Although zinc treatment concentrations used in this study were higher than typical plasma concentrations found in prostate and seminal fluids that reach mM levels [21,22] and that found in the normal prostate (100–200 μg/g wet tissue weight). Overall, this study demonstrates that zinc exerts antiproliferative effects in both noncancerous prostate hyperplasia and PC-3 and suggests that the effects of zinc may be more potent prior to malignancy.

Studies indicate that the loss of zinc from prostate cells alters their metabolic function, resulting in increased m-aconitase activity, oxidation of citrate and increased production of adenosine triphosphate (ATP) [23]. Studies by Costello et al [20,24,25] suggest that the loss of zinc and citrate is an important metabolic change that occurs with prostate cancer development, creating an optimal environment for uncontrolled proliferation, impaired apoptosis, cancer progression and metastasis. Therefore, reestablishing high zinc levels in cancer cells may restore normal cellular conditions and possibly control proliferation and induce apoptosis. Zinc treatment in PC-3 in culture has been reported to inhibit cell growth, induce apoptosis and cause cell cycle arrest through the loss of mitochondrial membrane potential, release of cytochrome c and alterations in p21 expression

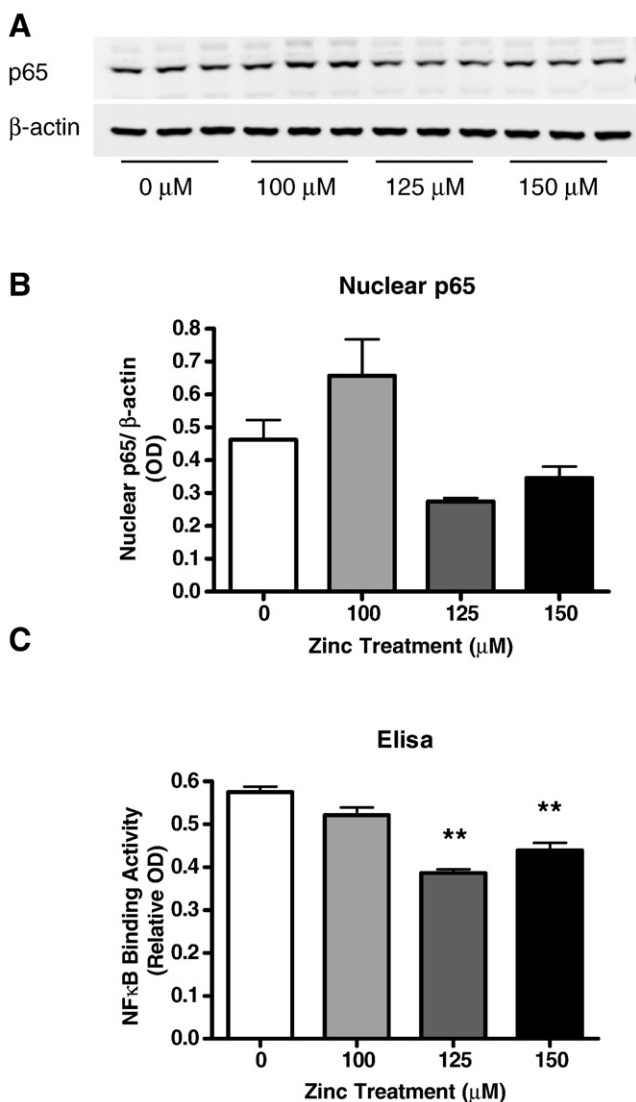


Fig. 7. Zinc did not change nuclear p65 expression in PC-3 cells. PC-3 were treated with zinc for 48 h. Nuclear protein expression of p65 was determined by Western blotting, quantified by densitometry analysis and plotted as mean±S.E. Beta-actin was used as the loading control. Zinc concentrations of ≥125 μM resulted in increased p65 binding activity as determined by ELISA ($P<0.01$).

[16,17]. Zinc has also been shown to decrease growth and induce morphological changes and DNA fragmentation characteristic of apoptosis in BPH-1 cells [26,27]. Our studies confirm these findings, and additionally show differential response to zinc supplementation in cancerous PC-3 versus noncancerous BPH-1 cells. Moreover, we find some of the differential response to zinc may be attributed to differential control of apoptotic regulators.

BAX and Bcl-2 are members of the Bcl-2 protein family which function to regulate apoptosis [28]. Although the precise mechanism by which Bcl-2 family proteins control apoptosis are still unclear, examining the Bcl-2:BAX protein expression ratio can be one indication of overall cell survival state [29]. Bcl-2:BAX expression was decreased in BPH-1 cells. In contrast, in PC-3, zinc increased Bcl-2:BAX expression despite an observed decrease in cell number and viability at higher zinc concentrations. The increase in Bcl-2:BAX appeared to be predominately due to increased Bcl-2, in combination with no change in BAX expression. In contrast to our findings, Feng et al [30] have reported zinc-induced BAX expression in PC-3 at concentrations as low as 15 μM . However, in these studies, PC-3 were serum-starved for 24 h prior to zinc treatment and may account for the divergent findings. The serum starvation for 24 h would likely sensitize prostate cells by “priming” cells to maximize zinc uptake and the synchronization of cells could also sensitize cells to apoptosis. The presence of serum could limit the availability of zinc, thereby needing higher concentrations for equivalent bioavailability. Importantly, the cellular levels of zinc seen with our treatments (Table 1) are similar to the cellular concentrations seen with the lower concentrations, but with serum starvation. We see a 4.1-fold increase in cellular zinc with 100 μM zinc treatment, whereas 15 μM zinc in serum-starved conditions resulted in a 4.3-fold increase in cellular zinc [17,26].

Regulation of Bcl-2 expression can occur transcriptionally or through posttranslational modifications [28,31]. Overexpression of Bcl-2 has been reported in certain types of cancers, including prostate cancer, and has been associated with chemoresistance, increased cell survival and impaired apoptosis [32–35]. The development of BPH is thought to be due partly to imbalances in cellular proliferation and apoptosis in the stroma and epithelia of the prostate [36]. Increased expression of Bcl-2, as well as altered expression of other regulators of apoptosis, is thought to be one mechanism by which apoptosis is impaired in BPH [37–39]. The decrease in Bcl-2 in BPH-1 cells suggests that zinc may attenuate Bcl-2 expression and ultimately decrease cell survival in BPH.

In cancer cells, high expression of Bcl-2 may create a prosurvival environment. Previously, zinc has been shown to decrease Bcl-2 expression in normal human prostate epithelial cells [27]. However, in nude mice, PC-3 cell-induced tumors had a small increase in Bcl-2 and significantly increased BAX expression with zinc treatment [18]. Thus, the effect of zinc on Bcl-2 expression and, possibly other members of the Bcl-2 family, may depend on the state of the cell. Despite decreased cell number and viability observed with high zinc treatment in PC-3, there was an observed increase in Bcl-2:BAX expression. It is possible that zinc treatment resulted in alterations in signaling molecules which are involved in posttranslational modifications of Bcl-2 or that there were changes in expression of other Bcl-2 family proteins. Studies from our lab and others have shown that following zinc treatment cause within an hour early alterations in zinc transporter and metallothionein levels and early mitochondrial defects that lead to apoptosis such as BAX expression and cytochrome c release. Thus, there appear to be early and direct effects on zinc on cellular zinc metabolism and the mitochondria that ultimately lead to DNA damage and apoptosis. Future studies examining these two factors would be useful in determining the role of zinc in the prostate and its effect on regulators of apoptosis.

The tumor suppressor protein p53 is known as the “guardian of the genome” and is responsible for the transcriptional regulation of

multiple downstream targets; two of which are BAX and Bcl-2 [40–42]. PC-3 have one copy of p53, which is mutated resulting in a truncated protein which can not be detected by immunocytochemistry [43,44]. BPH-1 cells do not contain mutations in the p53 gene but do express high basal levels of p53 [45]. To further examine the possible mechanistic differences in the response to zinc between PC-3 and BPH-1 cells, we examined nuclear p53 expression in BPH-1 cells only, since PC-3 do not express p53. Zinc resulted in a dose-dependent decrease in nuclear p53 expression which only reached significance at 150 μM . In contrast, other studies have shown increased nuclear p53 protein expression with zinc supplementation in normal human aortic endothelial and bronchial epithelial cell lines [46,47]. It is possible that the response of p53 to zinc may depend on cell type and state, and the response in hyperplasia cells of the prostate is different compared to normal cells originating from different organs. Since the change in nuclear p53 expression was not significant at concentrations of zinc which were associated with changes in BAX and Bcl-2 protein expression, it is likely that the response was primarily due to alterations in other regulators of apoptosis. NF κ B is an important transcription factor that also regulates cell survival and apoptosis. Due to the effect of zinc on NF κ B expression and activity observed in this study and previous studies establishing a link between NF κ B activation and prostate cancer, it is possible that zinc is a strong modulator of cell growth and survival through NF κ B pathway. In addition, other signaling molecules, such as those that control phosphorylation states of Bcl-2 family of proteins and other transcription factors like AP-1 may be responsible for the effect of zinc treatment in prostate cells.

An important area of future work is to delineate the mechanisms by which zinc response differs in various prostate epithelial cells. One possible candidate mechanism is a differential regulation of zinc flux in the cell. The ZnT and ZIP family of zinc transporters work together to regulate cellular and subcellular zinc levels. Differential gene and protein expression of zinc transporters in normal, BPH and PC-3 and tissues have been reported. Down-regulation of ZIP1 and ZnT1 gene expression has been observed in prostate cancer tissues compared to prostate hyperplasia tissue [48,49]. Henshall et al. [50] observed decreased protein expression of ZnT4 (by immunohistochemistry) in prostate cancer tissues compared to BPH tissues, suggesting decreasing expression of ZnT4 with progression to prostate cancer. In addition, normal and BPH tissues express ZIP2 and ZIP3 protein, while these proteins are undetectable in malignant tissues of the prostate. These data suggest that expression of various zinc transporters are uniquely altered during BPH and prostate carcinogenesis. An important future study would be to characterize changes in prostate zinc levels with zinc supplementation and possible differences in the regulation of zinc transporter expression in the prostate.

Overall, this study showed that BPH-1 cells were more sensitive to zinc-induced changes in cell viability, apoptosis markers and NF κ B expression compared to PC-3. Although changes in cellular zinc were identical in BPH-1 and PC-3, it is possible that differential regulation of apoptotic pathways or zinc transporters in these cells could account for the differential response. Regardless, proper zinc status is likely important for the prevention of prostate cancer but may also be beneficial for those with BPH. The effectiveness of zinc against prostate cancer is likely dependent on when it is given and may be more effective as a chemoprevention strategy rather than a chemotherapeutic agent.

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