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Original article

The role of survivin and Bcl-2 in zinc-induced apoptosis in prostate cancer cells☆

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Abstract

Objectives: To study the effects of zinc treatment on the gene expression levels of survivin and Bcl-2 in prostate cancer cells.

Materials and methods: The effects of zinc exposure on apoptosis were assessed using two human prostate cancer cell lines, LNCaP and PC-3. Zinc-induced apoptosis was measured by Annexin V staining. The direct effect of zinc on the expression levels of zinc transporters (ZnT-1 and ZnT-4) and apoptosis-related genes (Bax, Bcl-2, and survivin) was determined by RT-PCR analysis.

Results: When LNCaP and PC-3 cells were exposed to various concentrations of zinc sulfate for 48 hors, their growth was inhibited in a dose-dependent manner. The levels of zinc in both cell lines treated with zinc sulfate for 24 hours were higher than in untreated cells. Exposure to zinc induced apoptosis and necrosis in LNCaP and PC-3 cells. Apoptosis became more extensive as the treatment time with zinc increased. There was a significant increase in the gene expression levels of ZnT-1 and ZnT-4 in both cell lines treated with zinc sulfate compared with untreated cells. The expression of Bax mRNA was up-regulated, while the expression of Bcl-2 and survivin were decreased in both cell lines following zinc treatment.

Conclusions: Exposure to zinc sulfate in human prostate cancer cells increased intracellular levels of zinc, which resulted in increased apoptosis. The apoptogenic effect of elevated concentration of zinc could be due either to increased expression of zinc transporters and increased levels of Bax or decreased Bcl-2 and survivin expression.

Keywords: Prostate cancer; Zinc; Bcl-2; Survivin; Apoptosis

1. Introduction

Zinc is an essential trace element that serves as a cofactor for various enzymes involved in either macro molecule synthesis or protein metabolism. In human, highest levels of zinc accumulation in soft tissue can be found in the prostate gland under normal conditions. Zinc levels in prostate epithelial cells rapidly decline upon transformation to a malignant phenotype. The loss of the unique ability of the prostate to retain normal intracellular levels of zinc may be an important factor in the development and progression of prostate cancer. The exact mechanism underlying the reduced levels of zinc in prostate cancer is relatively unknown.

Increased levels of intracellular zinc have been shown to decrease proliferation of prostate cancer cells. The decrease in prostate cancer cell proliferation is due to the induction of apoptosis [1]. Zinc levels modulate key apoptosis-related proteins such as Bax, Bcl-2, and caspases [2–4]. Reduced levels of zinc could alter apoptotic signal transduction pathway.

In addition to the Bcl family, the inhibitor of apoptosis proteins (IAPs) has recently gained attention in cancer. In fact, IAPs may play a more significant role in apoptosis inhibition than any other family of apoptotic inhibitors including Bcl-2 [5]. Among the members of the IAP family, survivin directly inhibits caspase-3 and −7 activity. Survivin is associated with the microtubules of the mitotic spindle at the beginning of mitosis [6]. Functionally, Bcl-2 and survivin have been positioned in nonoverlapping anti-apoptotic pathways [7].

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Studies of the anti-apoptotic properties of zinc are important lines of research in disease states, such as cancer, where zinc homeostasis is often dysregulated. In the current study, we assessed the effects of zinc treatment on gene expression levels of survivin and Bcl-2 in human prostate cancer.

2. Materials and methods

2.1. Cells and culture conditions

Human prostate cancer cell lines, LNCaP and PC-3 cells, derived from a human adenocarcinoma of the prostate, were obtained from the Korean cell line bank. Cells were cultured in RPMI medium containing 10% heat-inactivated fetal bovine serum (FBS) and antibiotics. Cells were grown as monolayer cultures and maintained at 37°C under a humidified atmosphere of 5% CO_2 in air. Experiments were performed using cell lines with passage numbers within the range of 5–40.

2.2. Zinc treatment

Once the cells grew to 70%–80% confluence, the growth medium was replaced with fresh serum/supplement-free medium for 24 hours to synchronize cells. Following 24-hour incubation in serum-free media, cells were treated with various concentrations of zinc for variable times as indicated in the Results section.

2.3. Cell viability assay

Cell proliferation/cell viability was evaluated in 96-well microplates using the WST-1 cell proliferation assay kit according to the manufacturer's instructions. Briefly, cells were seeded at a density of 3,000 cells/well into 96-well plates. After adherence, cells were grown for 6 hours in serum-free medium. Serum-starved cells were incubated with 0, 25, 50, 75, 100, 150, 200, and 250 μM concentrations of $ZnSO_4$ (Sigma, St. Louis, MO). Cleavage of the tetrazolium salt (Boehringer-Mannheim GmbH, Mannheim, Germany) to formazan was determined after 24 and 48 hours. Ten μl of WST-1 reagent was added to 100 μl of sample in each well and incubated for 30 to 60 minutes at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The absorbance of the formazan product was measured at 430 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA).

2.4. Determination of cellular zinc

The relative intracellular zinc content was determined according to the manufacturer's instructions (Molecular Probes, Inc., Eugene, OR). Briefly, cells were grown in 60 mm dishes up to 80% confluence and then transferred to serum-free media. Cells were either grown in the presence of zinc for 24 hours or were left untreated. Harvested cell pellets were washed in PBS, resuspended in cell lysis buffer (Cell Signaling, no. 9803, Boston, MA), and homogenized over ice. The cells were centrifuged at 14,000 *g* for 10 minutes at 4°C. The nuclei and cell membranes were separated by centrifuging samples at 800 *g* for 10 min. The supernatants were collected and centrifuged at 10,000 *g* for 5 minutes, and the protein concentrations of the cleared supernatants were measured by the Bradford method. Each sample (200 μg of protein) was placed in a 96-well plate and mixed with 200 μl of TSQ buffer, which was made by dissolving 1.5 mg of TSQ in 91.354 μl of warmed ethanol and then diluting 50 μl of this solution to 10 ml dH_2O, pH 10. The fluorescence of zinc labeled by TSQ was detected using a Cary eclipse fluorescence spectrophotometer (Varian, Palo Alto, CA) with excitation at 360 nm and emission at 495 nm.

2.5. Annexin V assay

An Annexin V staining kit (Vibrant Apoptosis Assay kit no. 2; Molecular Probes, Inc., Eugene, OR) was used to determine the proportion of apoptotic cells. Cells were grown in 60 mm dishes up to 70% confluence. After 24 and

Table 1
Sequences of oligonucleotide primers for PCR

	Gene (sequence)	Annealing temperature (°C)	PCR product size (bp)
ZnT-1	5′-CCT GGG CTT CTT CTC TAG ATT G-3′ 5′-TTG TCT TGG AAA GGT TGT TCT G-3′	53	634
ZnT-4	5′-GGA GAA CTT GTA GGT GGA TAC-3′ 5′-TAT TAG CAC ACC AAC ACT C-3′	53	473
Bax	5′-CCC TTT TGC TTC AGG GTT TC -3′ 5′-GCC ACT CGG AAA AAG ACC TC-3′	55	250
Bcl-2	5′-TGT TGG CCT TCT TTG AGT TCG -3′ 5′-TCA CTT GTG GCC CAG ATA GG-3′	55	290
Survivin	5′-AAC AGT CTT CAG GCA AAA CG-3′ 5′-AAC AGT CTT CAG GCA AAA CG-3′	55	350
β-Actin	5′-CCA CAC CTT CTA CAA TGA GC-3′ 5′-TGA GGT AGT CAG TCA GGT CC-3′	59	292