

Profiling of zinc-altered gene expression in human prostate normal vs. cancer cells: a time course study^{☆,☆☆}

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Abstract

We have demonstrated that zinc exposure induces apoptosis in human prostate cancer cells (PC-3) and benign hyperplasia cells (BPH), but not in normal prostate cells (HPR-1). However, the mechanisms underlying the effects of zinc on prostate cancer cell growth and zinc homeostasis remain unclear. To explore the zinc effect on gene expression profiles in normal (HPR-1) and malignant prostate cells (PC-3), we conducted a time course study of Zn treatment with microarray analysis. Microarray data were evaluated and profiled using computational approach for the primary and secondary data analyses. Final analyses were focused on the genes (1) highly sensitive to zinc; (2) associated with zinc homeostasis, *i.e.*, metallothioneins (MTs), solute zinc carriers (ZIPs) and zinc exporters (ZnTs); (3) relevant to several oncogenic pathways. Zinc-mediated mRNA levels of MT isotypes were further validated by semi-quantitative RT-PCR. Results showed that zinc effect on genome-wide expression patterns was cell-type specific, and zinc appeared to have mainly down-regulatory effects on thousands of genes (1953 in HPR-1; 3534 in PC-3) with a threshold of ± 2.5 -fold, while fewer genes were up-regulated (872 in HPR-1; 571 in PC-3). The patterns of zinc effect on functional MT genes' expression provided evidence for the cell type-dependent zinc accumulation and zinc-induced apoptosis in prostate cells. In PC-3 cells, zinc significantly up-regulated the expression of *MT-1* isotypes *MT-1J* and *MT-1M*, denoted previously as "nonfunctional" MT genes, and now a depictive molecular structure of *MT-1J* was proposed. Examination of genes involved in oncogenic pathways indicated that certain genes, *e.g.*, *Fos*, *Akt1*, *Jak3* and *PI3K*, were highly regulated by zinc with cell-type specificity. This work provided an extensive database on zinc-related prostate cancer research. The strategy of data analysis was devoted to finding genes highly sensitive to Zn, and the genes associated with zinc accumulation and zinc-induced apoptosis. The results indicate that zinc regulation of gene expression is cell-type specific, and MT genes play important roles in prostate malignancy.

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1. Introduction

Zinc, as a structural, catalytic and regulatory ion, is essential for the proper function of numerous enzymes and proteins in all cells [1]. This trace element plays a role in a wide variety of physiological and pathological processes involved in cell proliferation, differentiation and oncogenesis; in prevention of oxidative stress; and in immune status and aging [2,3]. In the human prostate, the high level of zinc, up to 3- to 7-fold higher than that in other organs, has been well documented [4]. On the other hand, the loss of zinc accumulation, 60–70% lower than that of normal prostate epithelial cells, is the most consistent characteristic of

prostate malignancy [4]. Previous studies have demonstrated that accumulation of a high level of zinc in the prostate is associated with two major functions: citrate production and normal growth of prostate tissue [4]. However, to date, whether the malignancy causes the disruption of zinc accumulation or *vice versa* remains unknown. It has been demonstrated that zinc treatment at physiologic levels (15 μ M) inhibits the growth of prostatic cancer cells *via* cell cycle arrest and induction of cell apoptosis both *in vitro* and *in vivo* [5–7]. Also, zinc-induced apoptosis in human prostate malignant PC-3 cells occurs *via* mitochondrial pathways through Bax-involved pore formation in mitochondrial membranes [5,8–10]. In contrast, no apoptotic effect of zinc was detected in the normal human prostate cell line HPR-1 [8]. However, the genes involved in the pathways of zinc-induced prostate cancer cell apoptosis have yet to be revealed. At the present time, there is a lack of information regarding the molecular mechanisms for (a) the lost capacity to accumulate zinc, and (b) zinc-induced apoptosis in malignant prostate cells, which represent the major challenges for future study.

Regulation of zinc accumulation in cells involves several central components, *e.g.* metallothionein (MT) and zinc transporters (ZIP and ZnT), functionally associated with cellular zinc storage, uptake and efflux, respectively. MT is a family of low molecular weight (6–7 kDa), cysteine-rich, metal-binding proteins [11–13]. In humans, four MT isoforms (MT-1, 2, 3 and 4) and 17 isotypes (13 isotypes for MT-1; 2 isotypes for MT-2 and 1 isotype each for MT-3 and -4) have been identified [14]. Among these isotypes, the biological functions of MT-1C, -D, -I, -J, -K, -L and MT-2B have not been identified and have been currently named as pseudogenes. MTs, as receptor/donors of cellular zinc, are considered to be the major regulators for zinc accumulation, distribution and bioactivity. MTs are also involved in the homeostasis of other metals, including cadmium and copper, and protect cells against damage induced by oxidative stress and detoxify harmful metals [2,15]. In addition to MTs, zinc transporters, which all have transmembrane domains encoded by two solute-linked carrier gene families, ZIP (SLC39) and ZnT (SLC30), are also known to be major factors associated with cellular zinc homeostasis in many different types of cells [16,17]. The ZIP family that imports zinc is composed of 15 members, and the ZnT family that functions in releasing zinc or sequestering zinc internally is composed of 10 integral membrane proteins. With opposite roles, they provide high-affinity systems for a dynamic maintenance of cellular zinc homeostasis. Previously, researchers determined zinc regulation of MT and zinc transporter expression in human prostate cancer cells and tissues [18–20]. The suppressed endogenous levels of MT-1/MT-2 were detected in prostate cancer cell lines of PC-3, DU145 and LNCaP [21]. An inhibitory effect of zinc on ZIP1 expression in prostate cancer cells was demonstrated

[22]. However, due to the complexity of isoforms in the MT, ZIP and ZnT families, information about the differential effect of zinc on the expression of these genes in normal and malignant prostate was limited.

Advances in microarray technology, which provides a broad view of gene expression, have allowed us to discriminate possible biomarkers linked specifically to zinc accumulation and zinc-induced apoptosis in prostate cells. In this study, we employed microarray analysis and semi-quantitative RT-PCR to display the expression patterns of genes highly affected by zinc in human prostatic normal, hyperplastic and malignant cells. The results may lead to future elucidation of zinc-responsive genes in activation of the pathways involved in prostate malignancy.

2. Methods and materials

2.1. Human prostate cell lines and zinc treatment

The human prostatic cell line HPR-1, derived from normal prostate epithelial cells (provided by Dr. C.K. Choo, University of Hong Kong), was cultured in keratinocyte serum-free medium (Invitrogen) supplemented with EGF 0.005 mg/ml, bovine pituitary extract 0.05 mg/ml and 1 U/ml penicillin and streptomycin sulfate (Invitrogen). The human benign prostatic hyperplasia cell line (BPH) (provided by Dr. S. Haywood, UCSF), human prostate malignant androgen-dependent LNCaP and androgen-independent PC-3 cell lines (ATCC, Rockville, MD, USA) were maintained in RPMI 1640 medium (GIBCO, BRL) with 10% fetal bovine serum (5% for BPH) and 1 U/ml penicillin–streptomycin. Cells were maintained in a humidified incubator with 5% CO₂ atmosphere at 37°C. Time course studies of zinc effect were initiated with the cells at 70–80% confluence, and the cells were synchronized by hormone/serum depletion for 24 h before zinc treatment. Zinc (20 μ M) was added with fresh hormone/serum-free media, and the cells were then collected at 1, 3 and 6 h, respectively. Cells without zinc treatment were collected as the control. Cell pellets were immediately frozen and stored at –80°C for RNA preparation. Total RNA was extracted using Qiagen (Valencia, CA, USA) RNeasy Mini kit according to the manufacturer's protocol. The quality of RNA was monitored by 28S/18S ribosomal RNA ratios and 260/280-nm absorbance ratios. The time course studies were conducted in each aforementioned cell line and repeated three times, respectively. RNA samples prepared from these bioassays were used for cDNA microarray and semi-quantitative RT-PCR analyses.

2.2. cDNA microarray expression analysis

Zinc effects on gene expression of HPR-1 and PC-3 cells were evaluated using Applied Biosystems (Foster City, CA, USA) human genome-wide microarray chips V2.0. The ABI human genome survey microarray chip contained 32,878 of 60-mer oligonucleotide probes for interrogation

Table 1
The sequences of primers for MT isotypes

Gene name	Forward primer sequences	Reverse primer sequences	Expected size (bp)
GAPDH	CCACCATGGCAAATTCATGGCA	TCTAGACGGCAGGTCAGGTCCACC	598
MT-1A	CTCGAAATGGACCCCAACT	ATATCTTCGAGCAGGGCTGTC	219
MT-1E	GCTTGTTTCGTCTCACTGGTG	CAGGTTGTGCAGGTTGTCTA	284
MT-1F	AGTCTCTCCTCGGCTTGC	ACATCTGGGAGAAAGGTTGTC	232
MT-1G	CTTCTCGCTTGGGAACTCTA	AGGGGTCAAGATTGTAGCAAA	303
MT-1J	ACTGGTGGCTCCTGCACGTGCGCC	CCCACATCAGGCACAGCAG	168
MT-1M ^a	CTAGCAGTGCCTCCATTATCG	CAGCTGCAGTTCTCCAACGT	228
MT-1X	TCTCCTTGCCTCGAAATGGAC	GGGCACACTGGCACAGC	151
MT-2A	CCATGGATCCCAACTGCTC	TGGAAGTCGCGTTCTTTACA	232

^a Source of MT-1M primer sequences: Ref. [23].

of 29,098 known genes. For each chip, 2 µg total RNA from each sample was used for generation of cRNA. The synthesis of cRNA and a subsequent hybridization were completed by the Core Facility at the University of Maryland Biotechnology Institute (Baltimore, MD, USA) according to the ABI standard protocol. For each cell line, four groups of samples were employed including a control group and groups treated with zinc for 1, 3 or 6 h. The management of microarray images, algorithm raw data and primary data analysis including quantification and normalization was completed with the Applied Biosystems 1700 Chemiluminescent Microarray Analyzer and 1700 Gene Expression Microarray System. The primary data analysis was performed using the software provided with the Applied Biosystems Expression Array System. For secondary analysis, Spotfire (Somerville, MA, USA) was used to export microarray data in a flat-file format and to determine the differentially expressed genes. Each array is normalized by the median chemiluminescent signal. The fold change of zinc-treated sample vs. the control sample was analyzed by filtering the dataset using a threshold of ± 2.5 -fold and a signal-to-noise (background) ratio of >3 and without flags for determining a meaningful alteration. Final profile data management including sorting data and data mining procedures was conducted using Microsoft Office Excel (Redmond, WA, USA). The experimental procedures and data are available at <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE5590> according to the Minimum Information About a Microarray Experiment standards.

2.3. Semi-quantitative RT-PCR for MT isotypes

Semi-quantitative RT-PCR analyses were conducted to validate the alterations of gene expression induced by zinc obtained from microarray and also to further investigate zinc effects on the mRNA level of MT isotypes. cDNA was synthesized using 2 µg total RNA with reverse transcriptase (ABI, Foster City, CA, USA) for a total 20 µl reaction following the manufacturer's protocol. The sequences of primers for GAPDH and MT isotypes including MT-1A, MT-1E, MT-1F, MT-1G, MT-1J, MT-1K, MT-1X and MT-2A are listed in Table 1. A cDNA

template (0.6 µl) was used for a 25-µl reaction mixed with 0.5 µl of 10 mM dNTP, 1 µl of 50 mM MgCl₂, 2.5 µl 10× Taq reaction buffer and 0.1 µl of 5 U platinum Taq (Invitrogen). Thirty (25 for MT-1J) cycles at 94°C for 30 s, 55°C (62°C for MT-1J, 50°C for MT-1M) for 30 s and 72°C for 30 s were performed using a Perkin-Elmer thermocycler (GeneAmp PCR System 9600, Shelton, CT, USA). Glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*) used as a control to normalize the MT transcript level was subjected to the same thermal cycle conditions as the MT isotypes except with an annealing temperature of 55°C. PCR products were analyzed in 1.5% agarose gels, stained with ethidium bromide and visualized by UV fluorescence. Densities of bands were scanned and quantitated with an LKB Ultra Scan XL laser densitometer (Image Quant, Molecular Dynamics, Sunnyvale, CA, USA). The ratio of each target to its corresponding *GAPDH* control represents the normalized transcript abundance. The ratio of transcript abundance in zinc-treated samples vs. the corresponding untreated control represents the zinc effect on the transcripts of MT isotypes, which were statistically analyzed.

2.4. Statistical analysis

Differences among subject groups were assessed by analysis of variance (ANOVA) and mean separations by least significant difference (LSD) analyses (SAS Institute, Cary, NC, USA) performed using the Mixed procedure of the SAS 9.1 program package for semi-quantitative RT-PCR analysis. A 95% confidence interval ($P \leq .05$) was considered

Table 2
Summary of the number of genes differentially regulated by zinc detected by microarray

Expression pattern	Prostate cell lines		
	Normal (HPR-1)	Malignant (PC-3)	HPR-1 and PC-3
Increased	872	571	59
Decreased	1953	3534	538
Varied in time course	13	16	0
Total number	2838	4121	597

significant. Each time point of zinc treatment was considered as an independent variable.

3. Results

3.1. Zinc regulation of gene expression in HPR-1 AND PC-3 cells determined by microarray analysis

A total of 6959 genes were identified to exhibit altered expression patterns with at least a 2.5-fold change in

response to zinc (Table 2). Among these genes, only 597 were common to both cell lines, including 538 and 59 genes that were consistently down- or up-regulated by zinc, respectively. From the differentially expressed genes induced by zinc, we selected 20 genes in each cell line with the most pronounced alterations in response to zinc, *i.e.*, the top 10 list for up-regulated and down-regulated genes, which are presented in Table 3 (HPR-1) and Table 4 (PC-3). The genes associated with protein phosphorylation, cell differentiation, transcription factor and signal transduc-

Table 3
Genes with the most zinc-altered expression levels in HPR-1 cells

GenBank ID	Gene name	Ratio of zinc treated vs. control in gene expression level						Major biological function
		HPR-1			PC-3			
		1 h	3 h	6 h	1 h	3 h	6 h	
<i>Increased gene expression</i>								
NM_006301	Mitogen-activated protein kinase 12 (MAP3K12)	12.14	8.56	2.62	1.09	1.30	-1.51	Protein phosphorylation; embryogenesis
NM_148172	Phosphatidylethanolamine <i>N</i> -methyltransferase (PEMT)	9.45	4.25	5.00	-1.29	1.12	1.22	Phosphatidylcholine biosynthesis; lipid metabolism
NM_173621	Chromosome 17 open reading frame 44 (C17orf44)	8.42	2.62	1.54	- ^a	- ^a	- ^a	Unclassified
NM_148172	Zinc finger protein 223 (ZNF223)	8.14	5.18	1.32	-1.96	-5.00	-2.25	Transcription factor; KRAB box transcription factor
NM_013313	Yippee-like 1 (Drosophila) (YPEL1)	6.39	5.18	5.42	1.76	-1.03	-4.17	Regulation of cellular morphology
NM_138334	Josephin domain containing 2 (JOSD2)	4.37	5.92	2.79	-1.20	1.59	1.11	Unclassified
NM_032520	<i>N</i> -Acetylglucosamine-1-phosphate transferase, gamma subunit (GNPTG)	3.92	6.93	3.26	1.51	1.17	1.05	Transferase
NM_145803	TNF receptor-associated factor 6 (TRAF6)	3.83	5.29	4.60	-2.37	-1.59	1.09	Signal transduction
NM_006538	BCL2-like 11 (apoptosis facilitator) (BCL2L11)	2.88	5.01	4.49	1.54	1.58	2.00	Oncogenesis; induction of apoptosis
NM_000076	CDK inhibitor 1C (p57, Kip2) (CDKN1C)	1.99	3.13	6.18	2.55	1.61	1.77	Kinase modulator; select regulatory molecule
<i>Decreased gene expression</i>								
NM_152558	IQ motif containing E (IQCE)	-12.5	-4.55	-3.03	1.03	-1.42	-1.85	Unclassified
NM_145288	Zinc finger protein 342 (ZNF342)	-8.33	-2.70	-3.45	-1.22	-2.88	-2.34	mRNA transcription regulation; oncogenesis
NM_001452	Forkhead box F2 (FOXF2)	-6.67	-3.85	-8.33	- ^a	-1.13	2.82	Developmental processes; nucleic acid metabolism; mRNA transcription and its regulation
NM_172239	RNA exonuclease 1 homolog (<i>Saccharomyces cerevisiae</i>)-like 2 (REXO1L2)	-6.07	-4.63	-13.78	-3.38	-4.40	-3.71	Nucleic acid binding; oxidoreductase
NM_152540	Sec1 family domain containing 2 (SCFD2)	-5.84	-7.93	-4.42	1.10	-1.71	12.14	Molecular function unclassified
NM_002548	Olfactory receptor, family 1, subfamily D, member 2 (OR1D2)	-5.77	-5.26	-8.36	-1.99	-2.42	-7.27	Chemosensory perception; signal transduction
NM_002429	Matrix metalloproteinase 19 (MMP19)	-5.54	-24.01	-6.47	-1.09	-1.64	-1.08	Extracellular matrix; protease
XM_211291	Chromosome 17 open reading frame 52 (C17orf52)	-5.03	-13.16	-9.11	-1.76	-1.10	-1.28	Unclassified
NM_173590	Chromosome 11 open reading frame 36 (C11orf36)	-4.08	-6.43	-5.91	-1.11	-1.20	-1.92	Unclassified
NM_019013	Family with sequence similarity 64, member A (FAM64A)	-2.75	-5.38	-20.98	-1.91	-1.14	-2.60	Unclassified

The genes were listed in a descending order according to the ratio of 1-h zinc-induced expression vs. the control in HPR-1 cells. Corresponding results in PC-3 cells were listed aside for the comparison.

^a Signal intensities were flagged as unavailable.

Table 4
Genes with the most zinc-altered expression levels in PC-3 cells

GenBank ID	Gene name	Ratio of zinc treated vs. control in gene expression level						Major biological function
		PC-3			HPR-1			
		1 h	3 h	6 h	1 h	3 h	6 h	
<i>Increased gene expression</i>								
NM_152857	Wilms' tumor 1 associated protein (WTAP)	37.14	29.41	31.21	-1.29	-1.05	-1.14	Transcriptional and posttranscriptional regulation of certain cellular genes
NM_002155	Heat shock 70-kDa protein 6 (HSP70B)	6.18	11.92	14.50	1.10	-1.19	-1.06	Ubiquitous protein; chaperon
NM_175622	Metallothionein 1J (<i>MT-1J</i>)	4.44	5.74	4.21	- ^a	- ^a	- ^a	Unclassified
NM_145276	Zinc finger protein 563 (ZNF563)	3.72	6.35	4.90	- ^a	- ^a	- ^a	Transcription factor; KRAB box transcription factor
NM_005252	V-fos FBJ murine osteosarcoma viral oncogene homolog (FOS)	3.10	19.70	13.59	2.06	-1.08	-1.35	Nucleic acid binding; transcription factor
NM_005527	Heat shock 70-kDa protein 1-like (HSPA1L)	1.18	6.15	5.33	- ^a	- ^a	- ^a	Hsp 70 family protein; chaperon
NM_172365	Chromosome 14 open reading frame 50 (C14orf50)	-1.13	3.42	11.17	1.12	1.08	-2.65	Unclassified
NM_018602	DnaJ (Hsp40) homolog, subfamily A, member 4 (DNAJA4)	0.74	9.56	18.51	3.96	1.42	-1.14	Chaperone
NM_001885	Crystalline, alpha B (CRYAB)	0.74	7.30	9.19	1.64	-1.18	1.05	Structural protein; miscellaneous function
NM_032961	Protocadherin 10 (PCDH10)	0.70	4.67	14.05	-1.02	1.06	-1.72	Cadherin; cell adhesion molecule
<i>Decreased gene expression</i>								
NM_014212	Homeobox C11 (HOXC11)	-14.25	-9.38	-7.25	- ^a	- ^a	- ^a	DNA-binding protein; homeobox transcription factor
NM_152637	MGC17301 (MGC17301)	-10.73	-3.78	-4.44	- ^a	2.18	1.53	Methyltransferase
NM_023070	Zinc finger protein 643 (ZNF643)	-10.19	-6.32	-5.16	-1.09	-1.08	-1.73	Transcription factor; KRAB box transcription factor
NM_014562	Orthodenticle homolog 1 (Drosophila) (OTX1)	-9.18	-8.59	-3.30	-1.93	-1.73	-1.98	Nucleic acid-binding protein; transcription factor
NM_032208	Anthrax toxin receptor 1 (ANTXR)	-8.43	-5.22	-3.71	-1.19	-1.19	1.05	Receptor
NM_032563	Late cornified envelope 3D (LCE3D)	-6.62	-6.84	-3.66	-1.21	1.20	-1.25	Structural protein; miscellaneous function
NM_002498	NIMA-related kinase 3 (NEK3)	-6.44	-5.30	-4.74	1.90	1.24	-1.07	Protein kinase; transferase
NM_001818	Aldo-keto reductase family 1, member C4 (AKR1C4)	-5.94	-6.20	-4.41	-2.06	-1.75	-5.70	Oxidoreductase
NM_015396	Armadillo repeat containing 8 (ARMC8)	-4.53	-3.54	-11.53	-1.84	1.13	-1.40	Unclassified
NM_005693	Nuclear receptor subfamily 1, group H, member 3 (NR1H3)	-4.30	-3.58	-10.83	2.43	1.31	1.25	Unclassified

The genes were listed in a descending order according to the ratio of 1-h zinc-induced expression vs. the control in PC-3 cells. Corresponding results in HPR-1 cells were listed aside for the comparison.

^a Signal intensities were flagged as unavailable.

tion, such as *MAP kinases*, *zinc finger protein 223*, *TNF receptor-associated factor 6* and *Bcl-2-like 11*, were markedly induced by zinc in HPR-1, but not in PC-3 cells (Table 3). In contrast, the expression of candidate genes for cell growth and apoptosis, such as heat shock 70-kDa protein 6 (HSP70B), heat shock 70-kDa protein 1-like and *Fos*, was dramatically increased by zinc as much as 4- to 20-fold in PC-3 cells. Yet no significant zinc effect on these genes was observed in HPR-1 cells. MGC17301, encoding for methyltransferase, and several candidate genes for transcriptional DNA binding proteins were down-regulated in PC-3 cells (Table 4).

3.2. Zinc regulation of its homeostasis-related genes in HPR-1 and PC-3 cells

To further understand the effect of zinc on genes associated with cellular zinc accumulation in normal and malignant prostate cells, the results of the expression of MTs and zinc transport genes (ZIPs and ZnT) are summarized in Table 5. Our microarray data showed a dramatic zinc induction of the expression of *MT-1J* and *MT-1M* genes in PC-3 and HPR-1 cells, respectively. In PC-3 cells, the increase in transcriptional levels of the *MT-1J* gene induced by zinc was approximately 4- to 5.7-fold and was listed as

Table 5
Expression patterns for MT-isotypes and zinc transporters in response to zinc

GenBank ID	Gene name	Ratio of zinc treated vs. control in gene expression level					
		HPR-1			PC-3		
		1 h	3 h	6 h	1 h	3 h	6 h
BC029475	Metallothionein 1A (<i>MT-1A</i>)	1.11	-1.11	1.05	-1.31	-1.45	-1.14
NM_005947	Metallothionein 1B (<i>MT-1B</i>)	-1.25	-1.03	-1.40	-1.08	-1.01	1.13
NM_175617	Metallothionein 1E (<i>MT-1E</i>)	-1.01	-1.21	-1.65	-1.07	1.07	1.03
NM_005949	Metallothionein 1F (<i>MT-1F</i>)	-1.34	-1.40	-1.24	-1.14	-1.12	1.08
NM_005950	Metallothionein 1G (<i>MT-1G</i>)	1.18	-1.03	1.04	-1.42	-1.23	-1.41
NM_175622	Metallothionein 1J (<i>MT-1J</i>)	– ^a	– ^a	– ^a	4.44	5.74	4.21
BC070351	Metallothionein 1L (<i>MT-1L</i>)	-1.07	-1.31	-1.31	-1.79	-1.10	-1.85
BF973961	Metallothionein 1M (<i>MT-1M</i>)	1.20	2.26	13.35	-1.97	2.43	2.21
NM_005952	Metallothionein 1X (<i>MT-1X</i>)	1.08	-1.01	– ^a	-1.06	-1.37	– ^a
NM_005954	Metallothionein 3 (growth inhibitory factor) (MT3)	– ^a	– ^a	– ^a	-2.26	2.35	5.62
NM_032935	Metallothionein 4 (MT4)	1.01	-1.51	1.11	1.11	1.22	1.24
BG181336	MTM	2.11	2.21	1.07	1.31	3.38	1.15
NM_005955	Metal-regulatory transcription factor 1 (MTF1)	1.01	-1.09	1.03	-2.57	-1.82	-1.24
NM_021194	Zinc transporter, SLC30A1 (ZnT-1)	-1.07	1.15	1.27	-1.11	1.16	-1.07
NM_003459	Zinc transporter, SLC30A3 (ZnT-3)	1.10	-2.78	-1.73	-2.30	-1.97	-2.35
NM_022902	Zinc transporter, SLC30A5 (ZnT-5)	-1.19	-1.21	-1.05	-1.21	-1.52	-1.65
NM_024055	Zinc transporter, SLC30A5 (ZnT-5)	1.05	1.11	1.27	-1.20	-1.03	-1.13
NM_017964	Zinc transporter, SLC30A6 (ZnT-6)	-1.94	-1.32	-1.76	1.21	-1.08	-2.03
NM_133496	Zinc transporter, SLC30A7 (ZnT-7)	-2.90	-1.39	-1.59	-2.30	-1.10	-2.55
NM_006345	Zinc transporter, SLC30A9 (ZnT-9)	-1.89	-1.64	-1.59	-1.87	-1.29	-2.10
NM_014437	Zinc transporter, SLC39A1 (ZIP1)	-1.14	-1.17	-1.40	-1.07	-1.12	-1.08
NM_014579	Zinc transporter, SLC39A2 (ZIP2)	– ^a	– ^a	– ^a	– ^a	– ^a	– ^a
NM_144564	Zinc transporter, SLC39A3 (ZIP3)	– ^a	– ^a	– ^a	– ^a	– ^a	– ^a
NM_130849	Zinc transporter, SLC39A4 (ZIP4)	-1.04	1.04	-1.30	-1.03	-1.10	-1.11
NM_173596	Zinc transporter, SLC39A5 (ZIP5)	-1.22	-2.53	-2.05	-2.22	-1.43	-3.57
NM_012319	Zinc transporter, SLC39A6 (ZIP6)	-1.16	-1.08	-1.35	1.28	1.30	1.20
NM_006979	Zinc transporter, SLC39A7 (ZIP7)	-1.59	-2.35	-1.51	-1.68	-1.29	-2.65
NM_022154	Zinc transporter, SLC39A8 (ZIP8)	-1.14	-1.28	-1.14	-1.28	-1.18	-1.45
NM_018375	Zinc transporter, SLC39A9 (ZIP9)	-3.10	-2.31	-3.41	-1.81	-1.26	-1.12
NM_020342	Zinc transporter, SLC39A10 (ZIP10)	-1.44	1.12	-1.18	-1.62	-1.19	-1.99
NM_139177	Zinc transporter, SLC39A11 (ZIP11)	-1.21	-1.06	-1.16	-1.06	-1.14	-1.67
NM_152725	Zinc transporter, SLC39A12 (ZIP12)	– ^a	– ^a	– ^a	-3.04	– ^a	-3.97
NM_152264	Zinc transporter, SLC39A13 (ZIP13)	1.12	1.28	1.30	-1.15	1.13	1.14
NM_015359	Zinc transporter, SLC39A14 (ZIP14)	– ^a	– ^a	– ^a	-4.09	– ^a	-2.63

^a Signal intensities were flagged as unreliable.

one of the top three among all zinc up-regulated genes during the time course study (Table 4). Moreover, the zinc up-regulation of the *MT-3* gene, recognized as a growth inhibitory factor [24], was also detected with a 2- to 5.6-fold increase in PC-3 cells. However, the level of *MT-1J* transcripts could not be detected validly by microarray analysis in HPR-1 cells, but was determined by RT-PCR as presented in Fig. 1. The up-regulation of *MT-1M* transcripts with a greater than 2-fold increase was observed in both HPR-1 and PC-3 cells, and a remarkable enhancement of *MT-1M* (13-fold) after longer durations of zinc exposure (6 h) was found in HPR-1 cells. Transcriptional levels of other MT isotypes revealed unremarkable effects of zinc in both cell lines (Table 5).

In addition to MTs, zinc transporters are known to play a pivotal role in maintenance of a dynamic homeostasis of cellular zinc. Correspondingly, we observed the inhibitory effect of zinc, in general, on the expression of the majority of zinc transporter genes (Table 5). The zinc down-regulation of

ZnT expression with greater than twofold decrease, such as *ZnT 3* and *ZnT 7* genes, was observed in both HPR-1 and PC-3 cells. Furthermore, zinc also appeared to inhibit most *ZIP* gene expression and zinc suppression with about a 3.5-fold reduction of *ZIP5* gene expression observed in both cell lines. However, the inhibitory effect of zinc with an approximately 2- to 3.4-fold decrease in *ZIP9* gene transcripts was only detected in HPR-1 cells.

3.3. Zinc regulation of *MT-1J* and *MT-1M* expression in HPR-1, BPH, LNCaP and PC-3 cells detected by semi-quantitative RT-PCR

Marked zinc-induced *MT-1J* gene expression observed in PC-3 cells via microarray analyses (Tables 4 and 5) was further validated using semi-quantitative RT-PCR with two additional human prostate cell lines, BPH and LNCaP cells (Fig. 1). In malignant PC-3 cells, the significant zinc-induced *MT-1J* mRNA levels were detected at 3 h and reached even higher at 6 h by 5.7- and 7.6-fold increase compared to

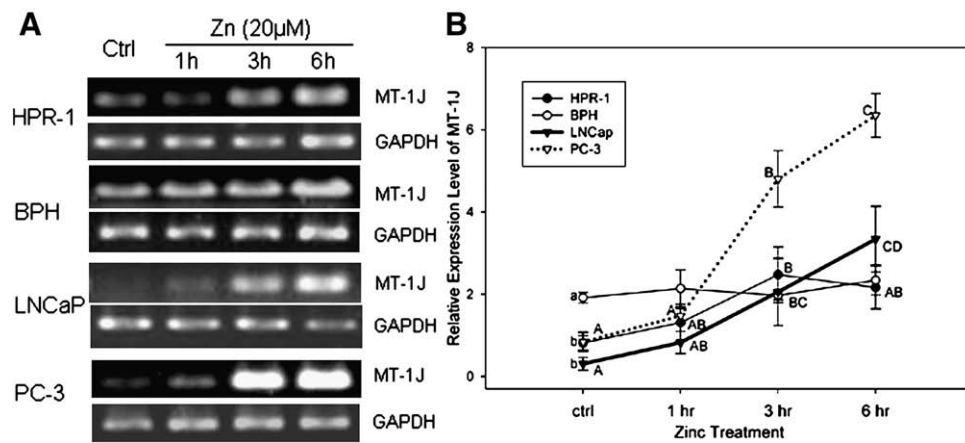


Fig. 1. Time course study of zinc-induced *MT-1J* mRNA levels in prostate cells detected by semi-quantitative RT-PCR. Cells were incubated with or without zinc (20 μ M) for different times, as indicated. (A) RT-PCR products of *MT-1J* and *GAPDH* were analyzed by gel electrophoresis. (B) *MT-1J* mRNA levels are expressed by the *MT-1J* relative optical densities normalized by related *GAPDH* signals. Each denoted point and bar represent the mean \pm S.E. ($n=3$). A significant difference ($P \leq 0.05$, LSD) between samples from different time point in each cell line is indicated with different capitalized letters (A, B, C, D). A significant difference ($P \leq 0.05$, LSD) of the endogenous levels of MT isotypes among four cell lines is denoted with different lowercase letters (a, b, c). The same illustration for the statistical significance analysis is used in Figs. 2 and 3.

endogenous *MT-1J* mRNA level, respectively. A remarkable zinc induction of *MT-1J* transcripts was also observed in malignant LNCaP cells with about 6.6- and 10.8-fold increase at 3 and 6 h, respectively. However, no zinc effect on *MT-1J* expression was observed either in BPH or HPR-1 cells. Our results indicate that zinc regulation of *MT-1J* gene expression is cell-type specific and time dependent. Zinc appeared to be more effective on *MT-1J* gene transcription in malignant PC-3 and LNCaP cells than in normal and benign hyperplastic BPH cells.

In order to confirm the inducible effect of zinc on *MT-1M* gene expression obtained from microarray analysis (Table 5), and to further investigate whether the zinc regulation of *MT-1M* is cell-type specific, we conducted a time course study of zinc using RT-PCR with the primers targeting the *MT-1M* gene in four cell lines (Fig. 2). The results showed an extremely lower amount of endogenous *MT-1M* mRNA

found in LNCaP and HPR-1 cells ($P \leq 0.05$). Except for LNCaP cells, a significant zinc induction of *MT-1M* gene expression was observed in the cell lines with 3- to 5-fold increases compared to their controls observed in the remaining three cell lines. Seemingly, the time course of *MT-1M* induction by zinc differs in the cells, being fast in PC-3 and delayed in HPR-1 and BPH cells. This early activation of gene transcription in PC-3 cells was not observed for *MT-1J* (Fig. 1).

3.4. Zinc regulation of functional MT-isotype expression in prostate cells

To further understand the roles of MTs in cellular zinc accumulation in prostate normal, BPH and malignant cells, we evaluated the zinc effect on functional *MT* gene expression, including five isotypes of *MT-1* (1A, 1E, 1F, 1G and 1X) and *MT-2A* by semi-quantitative RT-PCR with

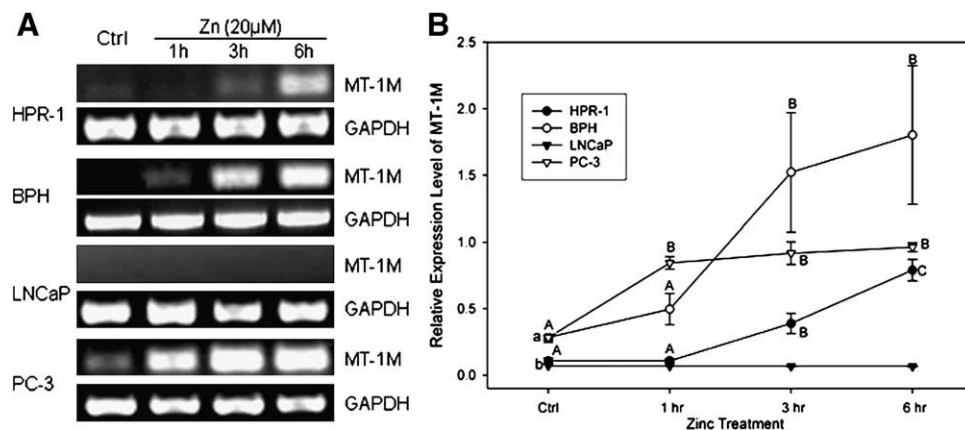


Fig. 2. Zinc-induced *MT-1M* mRNA levels in prostate cells detected by semi-quantitative RT-PCR. The experimental conditions are the same as in Fig. 1. (A) RT-PCR products of *MT-1M* and *GAPDH* were analyzed by gel electrophoresis. (B) *MT-1M* mRNA levels are expressed as the *MT-1J* relative optical densities normalized by related *GAPDH* signals. The points and bars represent the mean \pm S.E. ($n=3$).

samples obtained from the time course studies (Fig. 3). Among these four cell lines, the significant higher endogenous mRNA levels for *MT-1E* and *MT-2A* were found in BPH and PC-3 cells, and higher endogenous *MT-1G* transcripts were detected in BPH and LNCaP cells. No difference of endogenous mRNA for *MT-1X* was detected among these cell lines. Within these functional *MT*-isotype

genes, the endogenous mRNA levels for *MT-1E*, *MT-1F* and *MT-1G* and their responses to zinc appeared to correspond to their cellular zinc levels reported previously [19]. The *MT*-isotype genes, which are highly sensitive to zinc induction, were determined in multiple cell lines with the probes targeting *MT-1A* and *MT-1G* (Fig. 3B). The distinct up-regulation of *MT-1F* and *MT-1X* gene expression was only

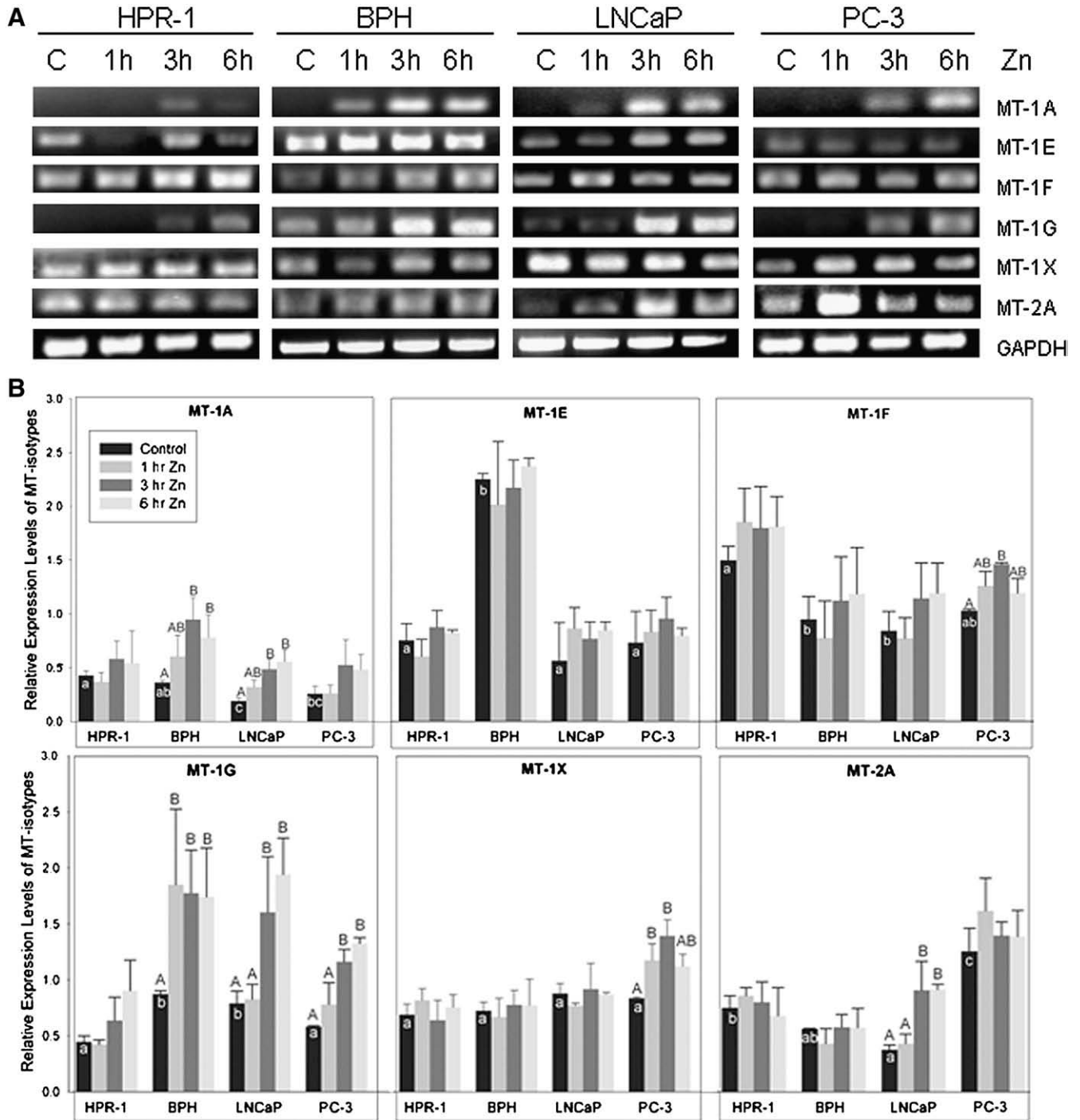


Fig. 3. Time course studies of zinc effect on *MT* isotype mRNA levels in prostate cells by RT-PCR. (A) Gel electrophoresis of *MT* isotype RT-PCR products in prostate cells. *GAPDH* signals were used as internal controls. (B) The quantitative results of RT-PCR were plotted as the ratio of the densities from each *MT* isotype relative to optical densities normalized by related *GAPDH* signals. The columns and bars represent the mean±S.E. ($n=3$).

recognized in PC-3 cells with a similar finding of *MT-2A* in LNCaP cells (Fig. 3B).

3.5. Zinc effect on the genes involved in oncogenesis pathways in HPR-1 and PC-3 cells

The 40 genes listed in Tables 3 and 4, which we identified by microarray, were greatly altered at their transcriptional levels in response to zinc. Many of their associated proteins are involved in the critical biological processes of these cells. In order to further understand the mechanism of the inhibitory effect of zinc on prostate tumor cell growth, we examined the expression patterns of the genes associated with oncogenesis. Oncogenes and tumor suppressor genes

have been known to interact and most form a biological network as illustrated by the Pathway Architect (Cell Signaling Technology) (Table 6). Interestingly, many genes were either highly induced or suppressed by zinc or distinctly altered in the opposite direction in response to zinc depending on the cell type examined. Among these genes, *Fos*, which codes for a transcription factor associated with AP-1, was dramatically up-regulated by zinc in PC-3 cells. In HPR-1 cells, expression of the *Akt1*, *JAK3* and *PIK3* genes, associated with the Akt pathway, was dramatically induced with transcriptional levels up to 6-, 8- and 7-fold higher, respectively, in response to zinc. In contrast, zinc had no significant effect on *AKT1* and *PIK3*

Table 6
Summary of zinc-altered oncogenes and tumor suppressor genes

Pathway	Protein	GenBank ID	Gene symbol	Ratio of gene expression in the cells of zinc treated vs. control					
				HPR-1			PC-3		
				1 h	3 h	6 h	1 h	3 h	6 h
TGF β	BMPR	NM_001204	BMPR2	-1.64	1.01	-1.20	-1.67	-1.39	-4.35
Wnt	SOX	NM_005634	SOX3	1.22	1.11	2.46	-1.69	-2.22	-3.85
		NM_003107	SOX4	1.35	1.15	-1.82	-1.30	-1.43	-2.50
		NM_017508	SOX6	4.50	2.31	2.25	2.86	— ^a	— ^a
		NM_006943	SOX12	5.34	1.88	3.00	2.57	-1.37	-2.13
		NM_004189	SOX14	-1.25	2.55	1.95	-2.17	-1.69	-3.13
		NM_022454	SOX17	1.38	1.53	— ^a	-2.17	-2.56	-2.94
	APC	NM_000038	APC	-1.16	-1.39	-1.72	-1.72	-3.45	-8.33
Ras	Fos	NM_005252	FOS	2.06	-1.09	-1.35	3.10	19.70	13.59
		NM_006732	FOSB	— ^a	— ^a	— ^a	1.48	13.15	3.33
	Jun	NM_002228	JUN	1.47	1.00	-1.30	-1.37	1.08	-1.20
	ILK	NM_004517	ILK	-1.69	-1.61	-2.63	-1.32	-1.06	-1.09
	Integrin	NM_003637	ITGA10	-1.27	1.06	-1.43	1.30	-1.43	-2.70
		NM_002203	ITGA2	-2.38	-1.61	-2.86	-2.27	-1.43	-2.33
		NM_002204	ITGA3	-2.00	-1.41	1.16	-1.85	-2.78	-2.08
		NM_005353	ITGAD	1.31	-2.38	1.65	1.39	3.09	2.31
		NM_000632	ITGAM	1.55	1.30	2.03	-1.52	-1.05	1.62
		NM_002210	ITGAV	-2.00	-2.33	-3.85	-1.25	-1.96	-1.30
		NM_000887	ITGAX	— ^a	3.44	2.40	-2.38	-1.05	— ^a
		NM_000211	ITGB2	-2.00	-2.94	-1.89	-1.61	-1.30	-1.49
		NM_000888	ITGB6	— ^a	-5.00	-2.38	-2.13	-1.04	— ^a
	NF1	NM_000267	NF1	-1.79	-1.96	-2.56	1.22	-2.78	-3.23
	VHL	NM_000551	VHL	1.08	1.80	1.58	-1.35	-2.27	-1.49
Akt	Akt	NM_005163	AKT1	6.38	2.73	5.00	-1.05	2.02	1.46
	JAK	NM_000215	JAK3	8.40	5.49	2.33	— ^a	-2.50	-4.00
	PI3K	NM_002645	PIK3C2A	-2.78	-1.59	-5.00	-2.13	-2.04	-3.70
		NM_002647	PIK3C3	1.10	-1.37	-1.19	-1.23	-1.54	-3.33
		NM_003629	PIK3R3	-2.94	-2.70	-2.17	-2.08	-1.35	-1.82
		NM_014308	PIK3R5	3.73	— ^a	7.18	— ^a	-1.11	1.35
	TSC1/TSC2	NM_000368	TSC1	1.27	-1.11	-1.85	-2.70	-3.33	-1.79
		NM_000548	TSC2	5.32	1.59	3.67	-1.20	-1.08	-1.22
Hedgehog	Smo	NM_005631	SMO	1.60	3.56	2.33	-2.22	2.29	-1.22
Cell cycle	Cyclin D	NM_053056	CCND1	1.89	3.59	-1.39	-1.28	-1.37	-1.69
	Cyclin E	NM_001238	CCNE1	2.03	-1.01	-1.09	-1.06	-1.41	1.12
		NM_057735	CCNE2	-2.17	-1.96	-3.23	1.07	1.18	-1.61
	CDK2	NM_001798	CDK2	— ^a	1.02	-1.27	-3.57	-1.08	-2.08
	ARF	NM_001662	ARF5	-1.33	-1.08	-1.85	-1.49	-1.37	-2.13
		NM_001663	ARF6	-1.00	1.25	-2.22	-1.03	-2.63	-2.63
	ATM	NM_000051	ATM	-1.12	-3.33	-4.55	-1.08	-3.03	— ^a
	ATR	NM_001184	ATR	-1.03	-1.18	-2.00	-2.22	-1.39	-1.69
	BRCA1	NM_007305	BRCA1	1.33	-1.41	-1.33	-1.28	-1.03	-2.50

^a Signal intensities were flagged as unreliable.

mRNA levels and had an inhibitory effect (up to 75% lower) on *JAK3* gene expression in PC-3 cells (Table 6). It has also been noticed that a tumor suppressor, *TSC2*, was induced up to fivefold higher after 1 h of exposure to zinc in HPR-1 cells, but no zinc effect on this gene was observed in PC-3 cells. Our data revealed that the genes involved in signaling pathways that are likely to be highly responsive to zinc are cell-type specific, which may lead to future studies on zinc targeting genes in prostate malignancy.

4. Discussion

In this study, we surveyed the effect of zinc on genome-wide gene expression profiles using microarray assays in normal and cancer prostate cell lines with multiple exposure times. Microarray results indicated that zinc exposure appeared to have (1) major down-regulatory effects on thousands of genes, while less than 900 genes were up-regulated in both prostate cell lines; and (2) cell-type specific effects on gene expression patterns. We previously reported that zinc treatment inhibits the growth of prostate malignant (PC-3) and benign hyperplasia (BPH) cells, mostly due to zinc-induced apoptosis through the mitochondrial pathway [5,8–10]. In contrast, no growth inhibitory effect of zinc was observed in normal prostate cells, which contain higher endogenous zinc levels and whose mitochondria do not respond to zinc with the release of cytochrome *c* [10]. The microarray assay provided us enormous information of zinc effect on the gene expression profiles which are involved in many biological and molecular events. The main challenge in this study is how to develop a strategy for sorting the microarray data. Considering our major goal in studying the mechanism of zinc effect on prostate cancer cell death, we developed a plan to determine and to sort the genes (1) highly sensitive in response to zinc treatment, (2) relevant to zinc homeostasis and (3) involved in cell growth and apoptosis, and related signaling pathways.

It is well recognized that *MTs* play major biological roles in the cells as metal binding/donors. The *MT* genes are highly inducible by heavy metals, and zinc is the primary physiological inducer in mammalian cells [13]. Using human prostate cells characterized as highly relevant to cellular zinc accumulation as a model system, we observed a specific zinc induction of *MTs*. Among 14 *MT-1* isotypes, *MT-1J* was significantly induced by zinc detected *via* microarray analysis and verified by semi-quantitative RT-PCR, and this induction was observed specifically only in PC-3 and LNCaP, but not in HPR-1, BPH cells (Fig. 1). Schmidt et al. [25] indicated that *MT-1J*, previously regarded as a nonfunctional pseudogene, is a *MT*-related gene and has an exon/intron structure consistent with that of a functional *MT* gene. However, unlike the currently characterized functional *MT* genes, *MT-1J* has been predicted to be incapable of encoding a complete typical *MT* protein due to the presence of a truncated structure and atypical aromatic amino acid

substitutions, which have never been found universally in any of the sequenced vertebrate *MTs* to date [25]. In addition, the overexpression of *MT-1J* (ψ hMT-I) in mouse cells failed to protect against cadmium (10 μ M) toxicity [25]. With limited functional studies on *MT-1J*, it is still labeled as a “nonfunctional pseudogene” [25,26]. The *MT* genes are highly conserved in DNA sequences within both coding and regulatory regions [25]. In particular, the positions of the cysteine residues, which serve to chelate heavy metal ions via thiolate complexes, are invariant and bind to three Zn ions in the N-terminal β domain and four Zn ions in the C-terminal α domain. Zn ions bound to thiolates (apo *MT*) of the β domain are more labile than those of the α domain. It has been suggested by Cousins et al. [27] that the β domain is physiologically relevant, while the α domain may be related to metal detoxification. Based on the predicted peptide sequence, *MT-1J* may encode a truncated protein which possesses a complete β domain with a tyrosine substitution of a conserved cysteine at Position 5 and a coil region with four cysteine residues and one phenylalanine replacement at Position 35 as proposed in Fig. 4. With this molecular structure, it could be highly possible that *MT-1J* might carry a reduced capability to bind metals (up to four zinc ions instead of a total of seven zinc ions), but retain the function to release the zinc from the β domain in response to cellular demands. Interestingly, our results revealed that in PC-3 cells, *MT-1J* transcripts showed the highest sensitivity to zinc treatment with a time-dependent increase. Previously, endogenous levels of *MT-1J* were reported to be down-regulated by an average ratio of 0.22 in 19 pancreatic carcinoma cell lines in comparison to the immortalized human pancreatic ductal epithelial cell line (HPDE) [28]. In their study, the authors suggested that the biological function of *MT-1J* was to “modulate apoptosis,” which would be the first report regarding the biological functions of *MT-1J* other than “metal ion binding” or “unclassified.” However, at the present time, no detailed information is available regarding the biological function of *MT-1J* in apoptosis, which emphasizes the importance of zinc’s effect on prostate cancer for future studies.

In addition to *MT-1J*, *MT-1M*, also labeled as a pseudogene and previously denoted as *MT-1K*, has been reported to be suppressed in pancreatic carcinoma cell lines in comparison to the immortalized HPDE cell line [28] and to be up-regulated by zinc with an 81-fold change in bronchial epithelial cells [29]. In this study, a significantly higher level of endogenous *MT-1M* transcripts, which was further rapidly induced by zinc with a 3- to 6-fold increase, was detected in PC-3 and BPH cells. In contrast, normal HPR-1 cells contain lower endogenous *MT-1M* mRNA and only a late induction by zinc was detected. In fact, the zinc-induced expression of *MT-1J* and *MT-1M* was detected in both PC-3 and BPH cell lines, which were demonstrated to be sensitive to zinc-induced apoptosis [8,10]. This correlation appears to lead to the apoptotic function of *MT-1J* and *MT-1M*, which was illustrated by Missiaglia et al. [28]. As

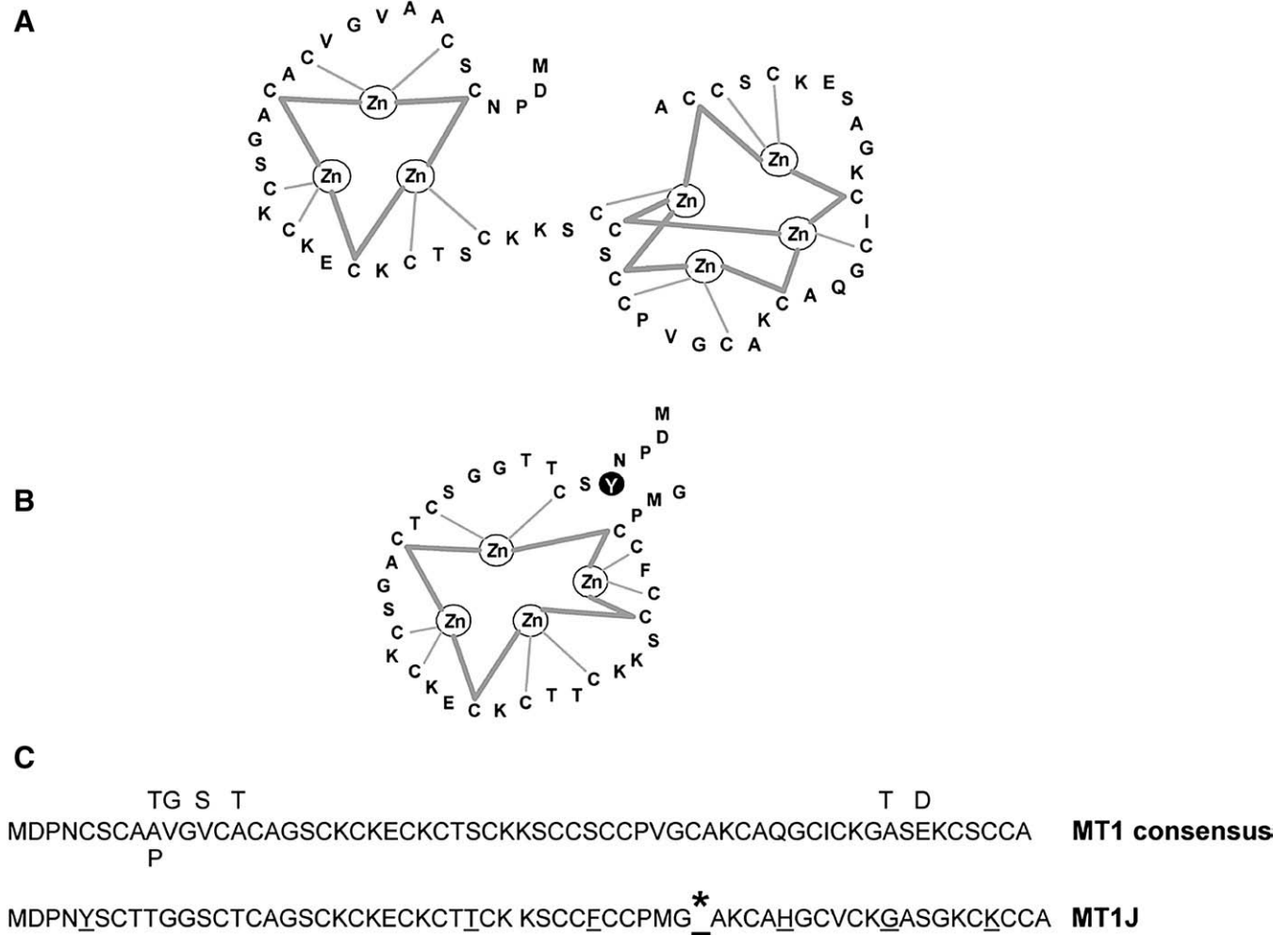


Fig. 4. Predicted protein structure of *MT-1J*. According to the protein sequences, *MT-1J* only possesses a β domain of the MT-1 molecule with a tyrosine substitution at Position 5 and a coil residue with four cysteine residues and one phenylalanine replacement at Position 35. *MT-1J* might carry a reduced metal ion binding capability down to four zinc ions compared to seven zinc ions in a normal MT-1 molecule. * indicates the presence of a TGA stop codon.

to the gene for *MT-1M*, Stennard et al. [26] noted the presence of a histidine residue and the loss of a lysine residue, a highly conserved residue among mammalian MTs. Thus, whether these genes and their protein products serve as natural detoxifiers or as acceptors/donors for zinc [30] in prostate cancer cells requires further study, including (1) the characterization of *MT-1J* and *MT-1M* translated products, (2) the identification of the capability and capacity of zinc binding in *MT-1J* and/or *MT-1M*, and (3) the relationship of *MT-1J* and *MT-1M* expression and zinc-induced cell apoptosis.

The expression of *MT-1/2* isotypes was reported to occur in a tissue-specific pattern [15], and their protein products may exert specific functions in human normal and/or malignant cells and tissues [31]. Previously, *MT-2A* gene expression had been connected with cell proliferation [32] and tumor invasiveness [33] in breast cancers. Our results showed that the higher abundance of *MT-2A* transcripts was only detected in PC-3 cells (Fig. 3), and

this analogous expression pattern across different cancer types (37, 38) suggested a possible candidacy of *MT-2A* as a biomarker for potentially malignant cells and tumor tissues. Moreover, our data also showed that among six *MT-1/2* isotypes (Fig. 3), the higher endogenous mRNA levels of *MT-1A* and *MT-1F* were detected in normal HPR-1 cells; likewise, those of *MT-1E* and *MT-1G* were found in BPH cells. The cell type-specific expression of MTs highly corresponds to the levels of their protein products which were determined by Western blot analysis [34] and also to the capacity of cellular zinc accumulation in different prostate tissues described previously [1].

Our data indicate that zinc highly induced the expression of a group of genes functionally related to cell proliferation and apoptosis, including *HSP70B* (14-fold increase), *heat shock 70kDa protein 1-like* (6-fold increase), *Hsp40 homolog* (18-fold increase) as well as *MT-1J* (5.7-fold increase) in the PC-3 cell line, while no or lesser expression alteration by zinc was detected in HPR-1. This finding in PC-

3 cells corresponds to the reports of Koizumi's group [35,36]. They demonstrated that human genes coding for *HSPs* (40, 60, 70, 90) and *MTs* (1E, 1B, 3) were up-regulated by cadmium in *HeLa* cancer cells. The expression of HSP70 is also induced by a variety of heavy metals including Zn, Cu, Hg and Ag [35,37]. The regulation of *MT* and *HSP* gene expression is mediated by metal-activated transcription factors, MTF-1 (metal-responsive element-binding transcription factor 1) and HSF1 (heat shock factor 1), respectively. Up-regulation of HSP70 inhibits cell apoptosis induced by a wide range of insults [38], and does so through mechanisms involving the interactions with Apaf-1, thereby preventing the recruitment of procaspase-9 to the apoptosome [39]. Altogether, zinc-induced HSP70 overexpression in prostate cancer cells can be considered as a cell self-defensive reaction. Moreover, the biological effect of zinc-induced HSP70 on prostate cancer cell growth should be further studied.

Another notable gene whose expression is regulated by zinc is the *fos* gene, which encodes the transcriptional factor Fos, which dimerizes with members of Jun and CREB/ATF families, forming the AP-1 complex [40]. AP-1 is activated in the regulation of genes involved in a variety of cellular events including normal development, neoplastic transformation and apoptosis [41]. Fos has been shown to be associated with apoptosis in photoreceptor cells and hepatocytes [42]. Fos-related molecular events in prostate malignancy have not yet been elucidated. Our microarray assays revealed a dramatic zinc induction of *fos* mRNA expression in PC-3 cells, but not in HPR-1. It would be important to further elucidate whether this zinc induction of *fos* gene expression is involved in regulation of zinc-induced apoptosis pathways in PC-3 cells. If such roles are identified as cell-type specific, *fos* could be a potential candidate used as a target gene for oncogenetic screening. This is the first report to address the zinc effect on increased *fos* transcripts in prostate cells. Further studies focused on the relationship of zinc-induced *fos* and zinc-induced tumor cell apoptosis will provide crucial information for the implementation of zinc in the clinical treatment of prostate malignancy.

5. Conclusions

This is the first report on the roles of zinc in differential regulation of gene expression profile in human prostate normal and malignant cell lines. A strategy for microarray data analysis was developed and led to distinguishing the genes which are (a) highly sensitive to zinc, (b) associated with zinc homeostasis AND (c) relevant to several oncogenic pathways. Our microarray data indeed provide evidence on zinc-altered gene transcripts which are involved in cellular zinc accumulation and cell apoptosis with oncogenic cell-type specificity. Among *MT-1* isotypes, *MT-1J* and *MT-1M*, previously considered as pseudogenes, were found to be

significantly up-regulated at the transcriptional level by zinc in malignant PC-3 and LNCaP cells only. We believe that this study delivered important insight to a new field of research on zinc and its roles in the prevention and intervention of prostate cancer.

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