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Human ZIP1 is a major zinc uptake transporter for the accumulation of zinc in prostate cells

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Abstract

The prostate gland of humans and other animals accumulates a level of zinc that is 3–10 times greater than that found in other tissues. Associated with this ability to accumulate zinc is a rapid zinc uptake process in human prostate cells, which we previously identified as the hZIP1 zinc transporter. We now provide additional evidence that hZIP1 is an important operational transporter that allows for the transport and accumulation of zinc. The studies reveal that *hZIP1* (SLC39A1) but not *hZIP2* (SLC39A2) is expressed in the zinc-accumulating human prostate cell lines, LNCaP and PC-3. Transfected PC-3 cells that overexpress *hZIP1* exhibit increased uptake and accumulation of zinc. The V_{max} for zinc uptake was increased with no change in K_m. Along with the increased intracellular accumulation of zinc, the overexpression of *hZIP1* also results in the inhibition of growth of PC-3 cells. Down-regulation of hZIP1 by treatment of PC-3 cells with hZIP1 antisense oligonucleotide resulted in a decreased zinc uptake. Uptake of zinc from zinc chelated with citrate was as rapid as from free zinc ions; however, the cells did not take up zinc chelated with EDTA. The cellular uptake of zinc is not dependent upon an available pool of free Zn^{2+} ions. Instead, the mechanism of transport appears to involve the transport of zinc from low molecular weight ligands that exist in circulation as relatively loosely bound complexes with zinc.

Keywords

Zinc; Zinc transporter; Prostate cells; ZIP transporter

1. Introduction

The prostate gland accumulates high levels of zinc. The tissue level of zinc in prostate is 3–10 times greater than that found in other tissues. It is this ability of the prostate epithelial cells to accumulate high zinc levels that results in the major prostate functions of citrate accumulation and secretion. Citrate accumulation results from inhibition of mitochondrial (m)-aconitase by zinc that prevents the oxidation of citrate via the Krebs cycle [1,2]. In addition, the accumulation of zinc also results in the inhibition of growth of prostate cells through its stimulation of mitochondrial apoptogenesis [3]. Consequently, it is evident that a significant component of the high intracellular level of zinc is in the form of reactive zinc.

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While zinc is an important co-factor for many enzymes and cellular proteins and is essential for the normal function of all cells, only recently have we begun to understand the mechanisms of cellular zinc uptake by mammalian cells. The existence of a rapid zinc-uptake transport process has been reported for the human prostate cell lines, LNCaP and PC-3 [4]. We identified *hZIP1* (SLC39A1) as a possible zinc transporter responsible for zinc uptake and accumulation in these cells. Transporters that transport zinc across the plasma membrane from the extracellular fluid have been identified in *Saccharomyces cerevisiae* [5,6] and *Arabidopsis* [7]. These transporters are members of the ZIP (Zrt/Irt-like proteins) family. In *Arabidopsis* three genes have been identified, *Zip1*, *Zip2* and *Zip3*. Four members of the *Zip* family, *ZIP1*, *ZIP2*, *ZIP3* and *ZIP4* have also been identified in mammalian cells [7–9]. Gaither and Eide [10] indicated that hZIP1 is the likely endogenous uptake transporter in mammalian cells. In the present report we provide compelling evidence that hZIP1 is an important functional transporter that is responsible for the rapid uptake and accumulation of physiologically effective zinc in prostate cells and suggest a mechanism of cellular zinc transport.

2. Experimental

2.1. Cell culture methods and transfection

PC-3 cells (from ATCC) were grown in complete DMEM medium supplemented with 10% fetal bovine serum (Gibco). Cells were plated in T 75 flasks, incubated in a humidified atmosphere of 5% CO_2 and air and transfected with 10 μg of pRc-CMV or pCMV-hZIP1 vectors [10] using Effectene reagents (Qiagen). Stably transfected clones were selected by the dilution plating technique in 350 μg/ml of G418. The stable transfectant cell lines were maintained in 350 μg/ml G418.

2.2. ^{65}Zn uptake assay

Cells were grown to 70–75% confluence, harvested by trypsin digestion from the flasks, washed once in cold Hanks Balanced Salt Solution (HBSS) and counted by hemacytometer. The cells were resuspended in cold uptake-buffer (HBSS, 50 mM HEPES, pH 7.4) and maintained on ice. Uptake assays were initiated by addition of 100 μl of cell suspension to 150 μl of pre-warmed uptake-buffer containing the specified concentration of $ZnCl_2$ and a constant specific activity of ^{65}Zn (Amersham Pharmacia Biotech, Inc.). The cell suspension was incubated at 37 °C unless otherwise indicated. Assays were terminated by addition of four volumes (1 ml) of stop-buffer (50 mM HEPES, 250 mM sucrose, 1 mM EDTA, pH 7.2). Cells were collected by filtration through glass fiber filters (Whatman GF/C) on a Brandel cell harvester. The filters were washed six times with wash-buffer (1×PBS with 1 mM EDTA) and counted by liquid scintillation.

2.3. RT-PCR analysis

hZIP and *GAPDH* cDNA were synthesized from total mRNA isolated from human prostate cells using 1.0 μg of total RNA, reverse transcriptase and random primers (TaqMan7 reagents, Perkin Elmer). *hZIP1*, *hZIP2* and *GAPDH* fragments were amplified from the cDNA using 1.0 μM forward and reverse primers and 35 cycles. These conditions were shown to be in the quantitative detection range based on the concentration of template DNA.

The cloned cDNA for *hZIP1* and *hZIP2* was used as the template DNA in control reactions to determine the specificity of the PCR reactions. The RT-PCR products were analyzed by agarose gel electrophoresis with ethidium bromide staining and photographed under UV light. No products were detected without reverse transcriptase. The primers for *hZIP1* were 5′-TCAGAGCCT-CCAGTGCCTGT-3′ and 5′-GCAGCAGGTCCAGGA-GACAA-3′; the primers for *hZIP2* were 5′-TGGTTCCAG-ATTGATGCAGC-3′ and 5′-CTGATCTGTTCTGCAC-CATG-3′; and the GAPDH primers were 5′-GAAGGT-GAAGGTCGGAGTC-3′ and 5′-GAAGATGGTGATGGG-ATTTC-3′.

2.4. Anti-hZIP1 antibody production and immunoblotting

Chicken polyclonal antibodies were raised against a peptide corresponding to residues 133–146 and affinity purified using the same peptide. Antibody depleted and preimmune preparations were used to verify the specificity of the antibody. Total protein extracts were prepared from cells in lysis buffer (25 mM HEPES, 5 mM KCl, 0.5 mM $MgCl_2$, 1 mM DTT, 1 mM PMSF, 1.0% NP-40) and the protein concentration determined using the BioRad protein assay based on the Bradford procedure [11]. Equal amounts of protein were subjected to SDS–PAGE gel electrophoresis according to the method of Laemmli [12], transferred to nitrocellulose membranes and incubated overnight with anti-hZIP1 antibody. Protein-bound antibody was detected using enhanced chemiluminescence detection reagents from the Amersham Corporation. The membranes were then stripped of antibody and re-probed with anti-β-actin antibody.

2.5. Antisense oligonucleotide inhibition of hZIP1 expression

Sense and antisense oligodeoxyphosphorothionucleotides were designed to bind to regions of hZIP1 and hZIP2 mRNA that include the start codon. The sequences for *hZIP1* and *hZIP2* were 17 bases long and were from location −5 to 12 relative to the ORF. The sense sequences were 5′-GCATCATGGGGCCCTGG-3′ and 5′-CAGAGATGGAGCAACTA-3′ and the antisense sequences were 5′-CCAGGGCCCCATGATGC-3′ and 5′-TAGTTGCTCCATCTCTG-3′ for *hZIP1* and *hZIP2*, respectively.

2.6. Assessment of intracellular zinc

CMV and *hZIP1* transfected PC-3 cells were cultured under normal culture conditions in medium containing 350 μg/ml of G418. After the cells reached 75% confluence, the medium was removed, the cells washed with 1×PBS (pH 7.4) and incubated in 20 μM Zinquin Ester for 30 min. The dye solution was removed and the cultures washed three times with 1×PBS. The cultures were viewed and digital images captured using an inverted fluorescence microscope and UV filters.

3. Results

3.1. hZIP1 expression in prostate cells

We previously reported that the rapid uptake of zinc by LNCaP cells was greater than that of PC-3 cells and that both cell lines expressed hZIP1 [4]. Therefore, we wanted to determine if differences in the expression of a zinc transporter could account for this difference in zinc uptake. Fig. 1 shows the results of RT-PCR reactions for *hZIP1* and *hZIP2* using total RNA

isolated from LNCaP and PC-3 cells. The results show a higher level of *hZIP1* expression in LNCaP compared with PC-3 cells. In addition, these results also show that expression of *hZIP2* was undetectable under these conditions. To assess the specificity of the PCR primers we used the cloned cDNAs for *hZIP1* and *hZIP2* as templates in PCR reactions carried out in the same microwell plates as the RNA samples. Results of these reactions showed that the primers amplified fragments that corresponded to the appropriate *ZIP* transporter. More importantly, the results showed that the lack of amplification of *hZIP2* from mRNA was not due to the primers or PCR conditions.

3.2. Overexpression of hZIP1 in pc-3 cells

PC-3 cells were transfected with pCMV-hZIP1 or pRc-CMV (control) as described in the Experimental section. *hZIP1* expression was assayed by RT-PCR and by Western blots and the accumulation of ^{65}Zn in hZIP1 transfected cells and CMV vector-only control cells measured. Fig. 2a shows the expected 212-bp fragments from mRNA samples and the overexpression of *hZIP1*. Fig. 2b shows hZIP1 protein levels in cells overexpressing hZIP1 compared with CMV controls.

Fig. 3 shows that overexpression of hZIP1 resulted in an increase in the uptake of ^{65}Zn. The kinetics of zinc transport showed an increase in V_{max} for cells overexpressing hZIP1 compared with the CMV control (105±7.8 and 46 ± 1.9 pmol/min per 10^6 cells, respectively) with no significant change in the K_m (7.6±2.3 and 6.6±2.3 μM). These kinetics are consistent with an increase in the abundance of hZIP1.

To determine if the overexpression of *hZIP1* resulted in the increased accumulation of cellular zinc, we incubated over-expressing PC-3 cells and CMV control cells with ^{65}Zn for various periods. Cells transfected with *hZIP1* accumulated more zinc than the CMV transfected controls (Fig. 4). In the same experiments we also measured ^{65}Zn accumulation at 4 °C. The accumulation of ^{65}Zn at the lower temperature was greatly decreased in both the CMV controls and the hZIP1 over-expressing cells indicating that the uptake process was temperature-dependent and likely a transport process. To ensure that the accumulated zinc was intracellular and not zinc that was bound to the cell membrane we used Zinquin to visualize intracellular zinc. Cells transfected with *hZIP1* showed an intracellular increase in Zinquin fluorescence that indicated an increase in cellular zinc (Fig. 5).

3.3. Antisense inhibition of hZIP1 expression

Since RT-PCR assays showed that *hZIP1* was expressed in LNCaP and PC-3 cells and that *hZIP2* was undetectable, we determined if inhibition of *hZIP1* expression would decrease the accumulation of zinc by wt PC-3 cells. Sense and antisense oligonucleotides were used to inhibit *hZIP1* expression and ^{65}Zn uptake determined. Fig. 6A shows that cells incubated for 3 days in the presence of antisense *hZIP1* oligonucleotide exhibited a decrease in ^{65}Zn uptake compared with cells incubated in the presence of the sense oligonucleotide. To ensure that the treatment with the oligonucleotide decreased *hZIP1* expression, hZIP1 mRNA was measured by RT-PCR and hZIP1 protein was measured by Western blot after incubation with the sense and antisense oligonucleotides. Results showed that the anti-sense oligonucleotide was effective in decreasing the expression of *hZIP1* (Fig. 6b–d).

3.4. ^{65}Zn uptake in the presence of citrate and EDTA

The K_m value for the hZIP1 transporter is approximately 7 μM. The concentration of total zinc in blood plasma is 15–20 μM; but the concentration of free Zn^{2+} ion is estimated to be in the pM to nM range. Therefore, virtually all of the zinc in circulation is bound to various ligands (i.e. Zn ligands) [13]. We wanted to determine if the PC-3 cells would take up zinc from Zn ligands as effectively as free Zn^{2+} ions. To achieve this, ^{65}Zn uptake was determined when zinc was chelated with citrate (ZnCit) and with EDTA (ZnEDTA) as compared to the uptake in the presence of $ZnCl_2$. Citrate or EDTA was added to 20 μM $ZnCl_2$ at a ligand/Zn ratio of 3:1, which provides an excess of ligand that ensures the virtual absence of any free Zn^{2+} ions with EDTA and greatly reduced Zn^{2+} ions with citrate. The results (Fig. 7a) reveal that the transport of zinc in the presence of ZnCit was as effective as the transport from $ZnCl_2$. In contrast, no zinc transport occurred from ZnEDTA. The 3:1 citrate/Zn ratio significantly decreased the concentration of Zn^{2+} ion; however, based on the formation constant (log K_f ~5) for Zn citrate the concentration of free Zn^{2+} ion would still be approximately 4.0 μM under these conditions. To determine zinc transport at lower concentrations of free Zn^{2+} ion in the presence of ligand, we determined ^{65}Zn accumulation with increasing ratios of citrate/Zn. Under these conditions the total zinc concentration remained constant at 20 μM while the free Zn^{2+} ion concentration decreased with increasing citrate/Zn ratios. The results (Fig. 7b) show that the rate of zinc accumulation was not strongly dependent on the free Zn^{2+} ion concentration. Fig. 7c shows the uptake of zinc expressed as a percent of the maximum uptake rate for $ZnCl_2$ and $ZnCl_2$ in the presence of citrate. In the presence of citrate the concentration of total zinc remains constant as the concentration of free Zn^{2+} ion decreases with increasing citrate. The figure shows that the rate of zinc uptake at low concentrations of free Zn^{2+} ion, when free Zn^{2+} ions represent the total pool of zinc, is much lower than that at a comparable concentration of free Zn^{2+} ion in the presence of ZnCit complex. To determine if the uptake of zinc from ZnCit was due to transport of citrate rather than zinc transport, we determined the uptake of citrate when zinc was chelated with ^{14}C-citrate (Zn^{14}C-citrate). The results (Fig. 7d) showed no uptake of ^{14}C-citrate while the transport of zinc was again as effective as the transport from $ZnCl_2$. Therefore, it is evident that the transport of zinc is not dependent upon the availability of a free Zn^{2+} ion pool, and that the transporter can effectively transport zinc from a Zn ligand pool. However, since the formation constant is much higher for ZnEDTA (log K_f ~16) than for ZnCit, the availability of transportable zinc appears to be dependent upon the relative binding affinities of the Zn ligand and the hZIP1 transporter.

3.5. Effect of hZIP1 overexpression on pc-3 cell growth

We reported that zinc inhibited the growth of PC-3 cells [14,15]. Therefore, we wanted to determine if overexpression of *hZIP1* and the resultant higher rate of zinc uptake would affect the growth of PC-3 cells. Fig. 8 shows the cell number with time of culture for the CMV controls and the *hZIP1* overexpressing cells. Cells that overexpress hZIP1 had slower growth rates than the CMV control. We also determined the effect of zinc added to the culture medium on cell number for CMV controls and cells overexpressing *hZIP1*. Fig. 9 shows that treatment with 15 μM $ZnCl_2$ resulted in a decrease in cell number of approximately 50% for the CMV control in 24 h and the loss of cells increased to

approximately 70% by 72 h. The effect of zinc on cell number in cells overexpressing hZIP1 was significantly greater at each time point compared with the CMV control with a maximum decrease of approximately 90% by 72 h.

4. Discussion

We previously reported that the PC-3 and LNCaP prostate cell lines, which are zinc-accumulating cells, take up zinc with kinetics consistent with the presence of a plasma membrane rapid zinc uptake transporter [4]. Here we provide additional evidence that the transporter is the hZIP1 human zinc transporter. *ZIP1* and *ZIP2* are two members of the ZIP family identified in mammalian cells. In our earlier report we showed *hZIP1* expression in prostate cell lines by Northern blot. However, in that report we did not measure *hZIP2*. Here we measured the expression of hZIP1 and hZIP2 mRNA in LNCaP and PC-3 cell lines. The results showed that hZIP2 mRNA was not detectable in either LNCaP or PC-3 cells. Moreover, the expression of *hZIP1* in LNCaP cells was greater than that in PC-3 cells. These results are consistent with kinetic data that show a greater V_{max} for zinc uptake in LNCaP cells than PC-3 cells [4].

Additional evidence that hZIP1 is the endogenous zinc uptake transporter in prostate cells is obtained by the overexpression studies and the inhibited expression studies. Overexpression of *hZIP1* results in an increase in the zinc uptake rate and zinc accumulation by the prostate cells. This is accompanied by a corresponding increase in V_{max} but no change in the K_m value from that of the CMV control cells. Moreover, the increase in the level of hZIP1 expression in overexpressing cells was twofold, which corresponded to the increase in V_{max} of overexpressing cells compared with the CMV controls. Little zinc accumulation was observed at 4 °C, which is consistent with it being a transport effect. Exposure of wild type PC-3 cells to *hZIP1* antisense results in down-regulation of expression of *hZIP1* and a corresponding decrease in the transport of zinc.

There also exists a correlation between the increased expression of *hZIP1* and the cellular effects of the uptake and accumulation of zinc. The accumulation of zinc in prostate cells results in mitochondrial apoptogenesis due to zinc-induced release of cytochrome *c* [3]. Therefore, the negative relationship between the overexpression of zinc transport protein and the growth of the cells is consistent with an increase in the hZIP1-mediated zinc accumulation. We have shown that exposure of prostate cells to zinc-supplemented medium results in inhibition of growth due, in part, to increased mitochondrial apoptogenesis [3,15]. In accordance with this effect, the cells that overexpress *hZIP1* and transport more zinc also exhibit a greater zinc inhibition of growth.

Gaither and Eide [10] transfected K562 human erythroleukemia cells with *hZIP1* and showed that the characteristics of zinc transport in the transfected cells were identical to those in wild type cells. They concluded that hZIP1 is the endogenous plasma membrane transporter in K562 cells. Milon et al. [16] using immunofluorescence reported that hZIP1 was localized to the plasma membrane in K562 cells, in contrast the transporter was localized to intracellular vesicles in epithelial cells, including PC-3. They concluded that there is differential subcellular localization of hZIP1 in adherent and non-adherent cells.

They proposed that hZIP1 is not likely to be involved with the uptake of zinc although they conducted no transport studies. Based on our previous report and the studies presented in this report, it is evident that hZIP1 is associated with the rapid net uptake of zinc from the medium by prostate cells, resulting in a net increase in the intracellular level of zinc. Therefore, hZIP1 operates as a rapid zinc-uptake transporter and the increase in cellular zinc could not be the result of intracellular zinc translocation and sequestration.

The issue of the physiological importance of the hZIP1 transporter for zinc accumulation in prostate cells needs to be addressed. It must be recognized that the plasma membrane of the cell is subjected to the conditions of the interstitial fluid (ISF), which is an ultrafiltrate of the blood plasma. Therefore, zinc is present in ISF bound to low molecular weight (LMW) zinc ligands and as free Zn^{2+} ions with very little protein bound zinc. Since the free Zn^{2+} ion concentration is 1000-fold lower than the hZIP1 K_m (approx. 7 μM), it is most unlikely that free Zn^{2+} is the source of zinc for rapid transport and cellular accumulation. Nevertheless, Gaither and Eide [10] proposed that hZIP1 transport involved the transport of zinc from a free Zn^{2+} ion pool as a result of the high capacity of the transporter. The current results (Fig. 7a) demonstrate that the transport of zinc from ZnCit is as effective as the transport from free zinc ions. The fact that zinc was not transported from ZnEDTA (Fig. 7a) under the same conditions as with ZnCit indicates that the binding affinity of the Zn ligand is an important determinant in the transportability of zinc by hZIP1. This suggests that, functionally, hZIP1 transports zinc from the LMW Zn ligand pool (e.g. amino acids, citrate, others) that exists in plasma and ISF. The concentration of this zinc pool would approximate the K_m value of the hZIP1 transporter.

Since free Zn^{2+} ion is such a small fraction of the total zinc present in plasma, others have suggested that LMW Zn ligands are the transported forms of the metal [13]. However, the results reported here demonstrate that the citrate component of the ZnCit complex was not transported into the cells. Thus, the current results indicate that zinc was not transported as a LMW complex. These observations indicate that the mechanism of zinc transport involves an intermolecular exchange of zinc between Zn ligand and the hZIP1 transporter. We are now extending these studies to establish the validity of this proposed mechanism and to define the structural–functional relationship of hZIP1.

In summary this report, coupled with our previous studies, demonstrates three important features of zinc uptake and accumulation in prostate cells. First, hZIP1 is an important functional zinc uptake transporter in prostate cells. Second, the hZIP1-mediated uptake results in the cellular accumulation of zinc that alters the growth and metabolic activities of the cell. Third, the transporter is not limited for transport to a pool of free Zn^{2+} ions; but, more likely, transports zinc from the available low molecular weight zinc-ligand pool that exists in plasma and ISF through an hZIP1–Zn ligand intermolecular exchange of zinc.

Acknowledgments

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5. Abbreviations

hZIP	human ZIP
RT-PCR	reverse transcription-polymerase chain reaction
PBS	phosphate-buffered saline
CMV	cytomegalovirus
ORF	open reading frame
DMEM	Dulbecco's Modified Eagle medium
EDTA	ethylenediaminetetraacetic acid
GAPDH	glyceraldeyde-3-phosphate dehydrogenase
G418	geneticin selective antibiotic
HEPES	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
PMSF	phenylmethylsulfonyl fluoride
SDS–PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

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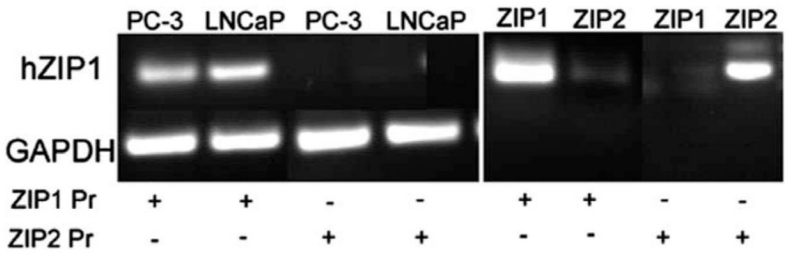

Fig. 1.

Expression of endogenous *hZIP* transporter in LNCaP and PC-3 cells. ZIP1 and ZIP2 lanes show PCR products where cloned ZIP1 or ZIP2 cDNA was the template with each primer pair. PC-3 and LNCaP lanes show RT-PCR products using total RNA from cell extracts. ZIP1 Pr and ZIP2 Pr indicate the primers used in each PCR reaction. GAPDH was assayed to ensure approximately equal template concentrations. No PCR products were obtained without reverse transcriptase.

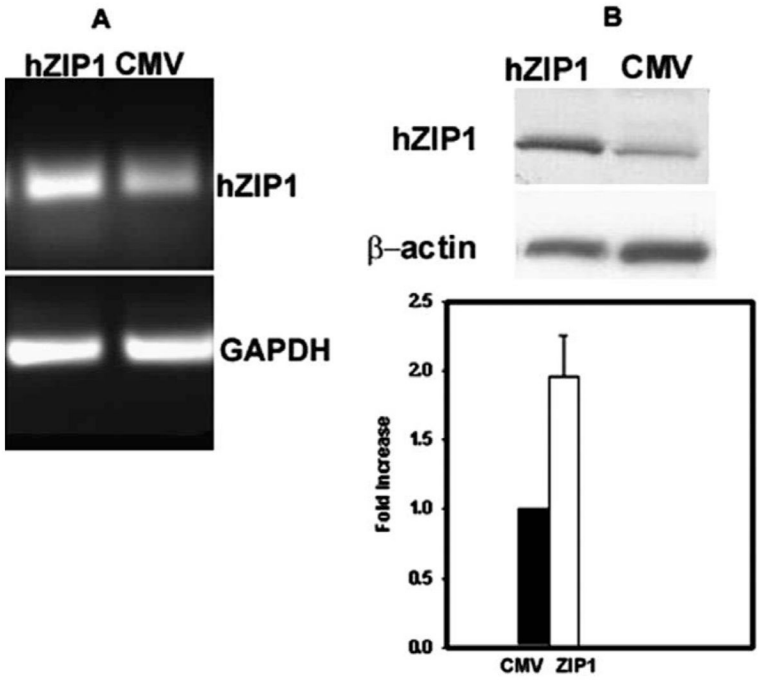

Fig. 2.

Overexpression of hZIP1 mRNA and protein. (A) RT-PCR analysis of hZIP1 and GAPDH mRNA extracted from PC-3 cells stably transfected with hZIP1 or the CMV control vectors. (B) Upper panel shows Western blot analysis of hZIP1 and β-actin from cell extracts of hZIP1 transfected and CMV control PC-3 cells. Bottom panel shows quantification of Western blot. hZIP1 transfected cells had approximately two fold more hZIP1 than the CMV control.

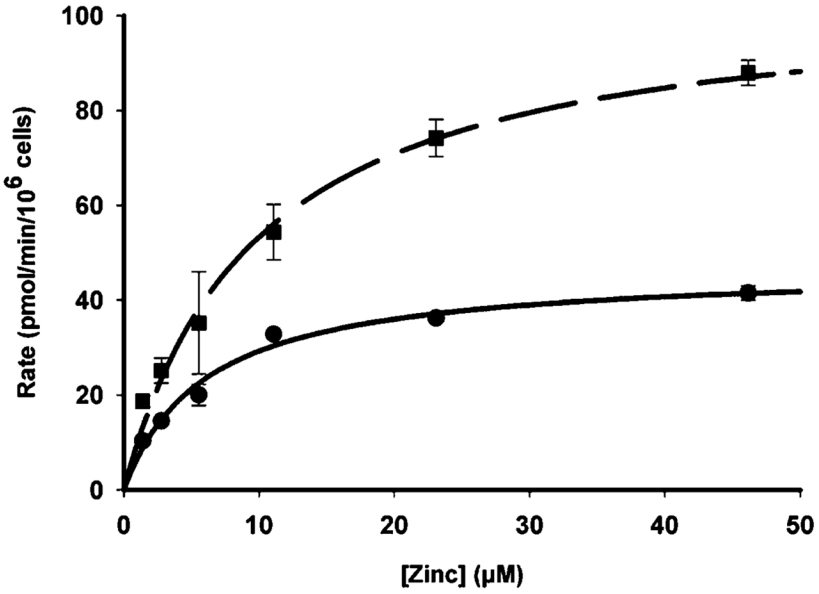

Fig. 3.

Concentration dependent zinc uptake. Zinc uptake kinetics of CMV control (circles) and hZIP1 transfected (squares) cells. Control and hZIP1 cells were cultured under identical conditions, collected and uptake of ^{65}Zn assayed as described in the Experimental section.

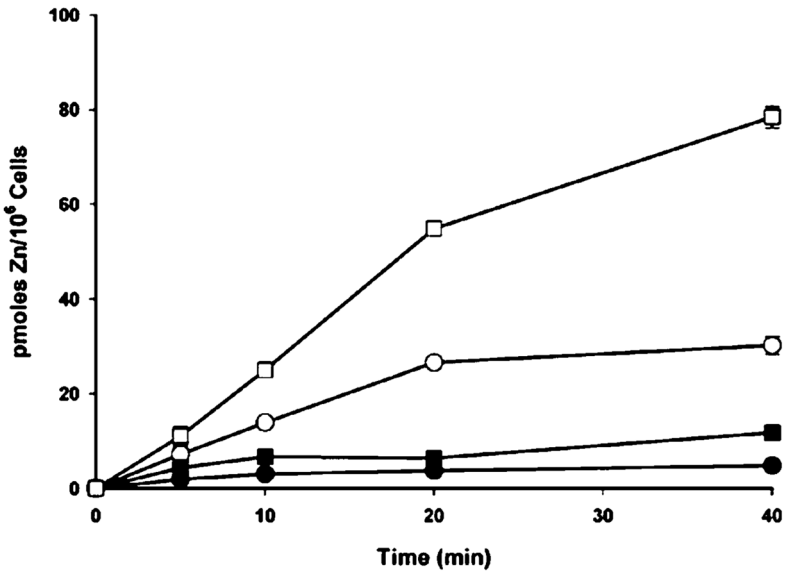

Fig. 4.

Time dependent zinc uptake. Zinc accumulation by hZIP1 (squares) and CMV control transfectants (circles) measured at 37 °C (open symbols) and at 4 °C (closed symbols). Cells were cultured under identical conditions, collected and zinc accumulation measured from 0 to 40 min at a medium zinc concentration of 15 μM $ZnCl_2$. Values are means±S.E.M. of a representative experiment (*n*=3).

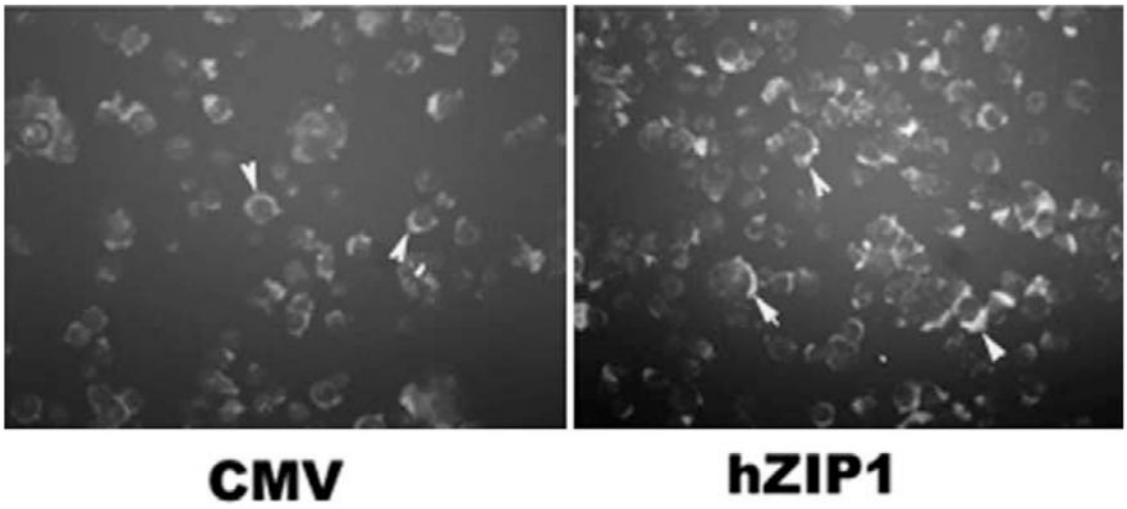

Fig. 5.

Intracellular zinc in CMV (control) and hZIP1 PC-3 cells. Cells were cultured in complete medium, washed with PBS, incubated for 30 min with 20 μM Zinquin, washed again with PBS and viewed on an inverted fluorescence microscopy. Zinquin detectable zinc is localized in the cytoplasm around the nuclei. Arrowheads show the difference in cytosol florescence intensity in CMV compared to hZIP1 overexpressing cells.

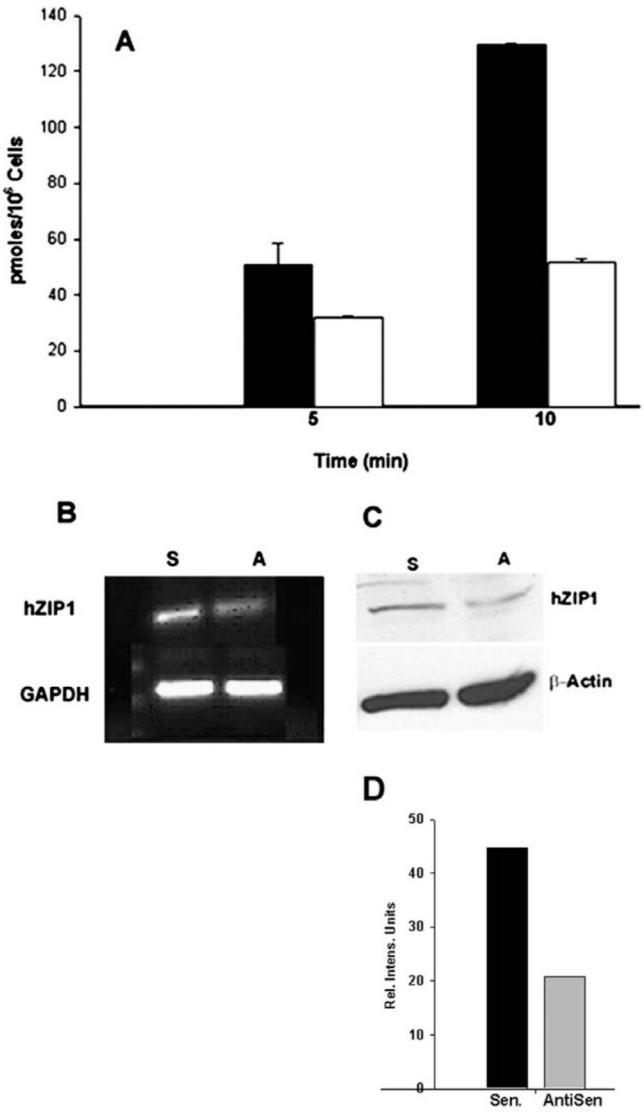

Fig. 6.

Antisense oligonucleotide inhibition of hZIP1 expression and zinc accumulation. A shows zinc accumulation at 5 and 10 min by non-transfected PC-3 cells treated for 3 days with *hZIP1* sense (closed bar) and antisense (open bar) oligonucleotides. Each bar is the mean and S.E.M. for combined data from two experiments (n=6). B shows RT-PCR analysis of hZIP1 mRNA from cells cultured and treated under identical conditions as in A. C shows the Western blot analysis of hZIP1 protein levels of cells cultured and treated under identical conditions as A. D shows the quantification of the Western blot.

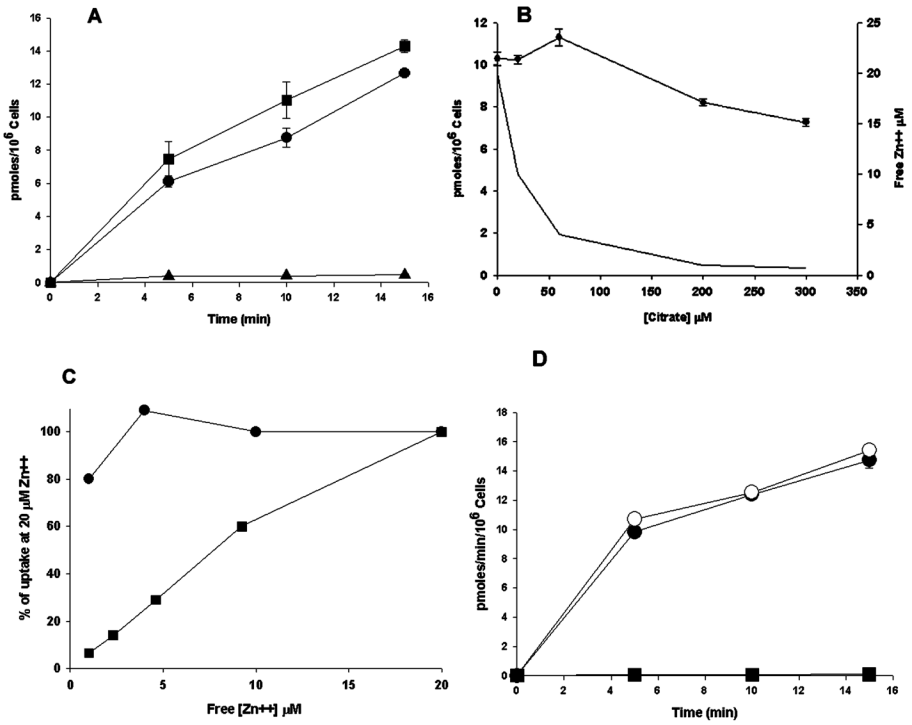

Fig. 7.

Zinc accumulation in the presence of citrate and EDTA. A shows ^{65}Zn accumulation by PC-3 cells measured in the presence of $ZnCl_2$ (circles), ZnCit, (squares) and ZnEDTA (triangles). The concentration of zinc was 20 μM in the presence of 60 μM citrate or EDTA. Each point is the mean±S.E.M. for a representative experiment (*n*=3). B shows ^{65}Zn accumulation (circles) by PC-3 cells measured in the presence of increasing ratios of citrate/Zn. The concentration of Zn is constant at 20 μM while the citrate concentration increases from 0 to 300 μM. Each point is the mean±S.E.M. for a representative experiment (*n*=3). The solid line is the calculated concentration of free Zn^{2+} ion at each citrate to zinc ratio. C shows zinc uptake as a percent of the maximum uptake in the presence of $ZnCl_2$ alone (squares) and $ZnCl_2$ (20 μM) with increasing concentrations of citrate added (circles). Free [Zn^{2+}] on the *x*-axis presents the total available zinc with $ZnCl_2$ alone and the calculated free [Zn^{2+}] with citrate added. D shows the accumulation of ^{65}Zn (circles) and ^{14}C-citrate (squares) in the presence of $^{65}ZnCl_2$ (closed circles) and $^{65}Zn^{14}C$-citrate (open circles). Each point is the mean±S.E.M. for a representative experiment (*n*=3).

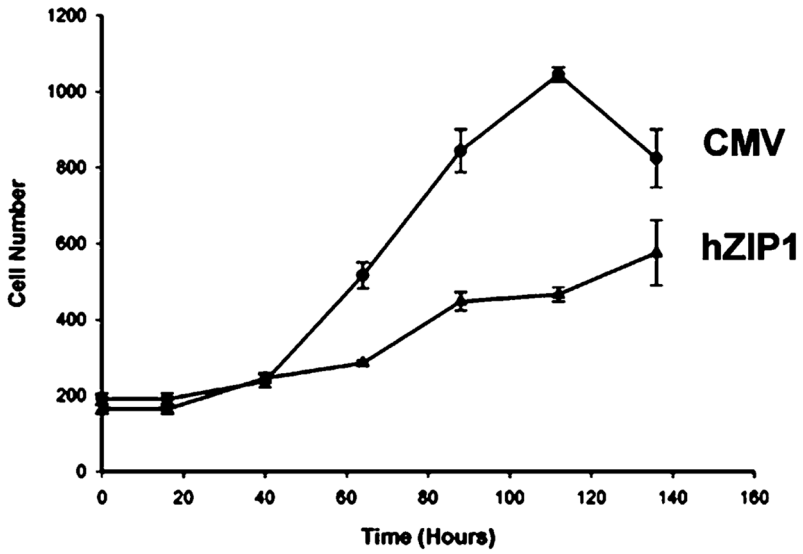

Fig. 8.

hZIP1 over expression inhibits growth of PC-3 cells. CMV control and CMV-hZIP1 transfected cells were plated in multi well plates. The cells were cultured under standard conditions, collected at various times over a 136-h interval and the cell number determined. Each point is the mean±S.E.M. for combined results from two experiments (*n*=6).

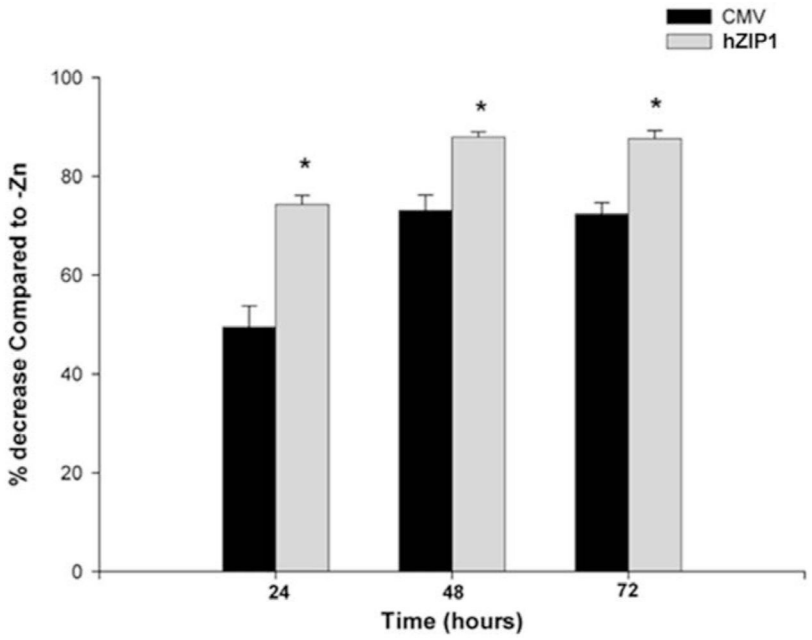

Fig. 9.

hZIP1 overexpression increases the growth inhibitory effect of zinc. CMV and CMV-hZIP1 transfected cells were plated into multi well plates and cultured overnight under standard conditions. The medium was changed to serum free medium containing 15 μM zinc. The cells were incubated for 24, 48 and 72 h, collected and the cell number determined. Bars show the % decrease in cell number of zinc treated cultures compared with no zinc treatment controls. *Statistically different; CMV vs. hZIP1, $P<0.05$.