Ann Hematol (2009) 88:733–742
DOI 10.1007/s00277-008-0676-4

ORIGINAL ARTICLE

Exposure of human leukemia NB4 cells to increasing concentrations of selenite switches the signaling from pro-survival to pro-apoptosis

Liying Guan · Bingshe Han · Jian Li · Zhushi Li · Fang Huang · Yang Yang · Caimin Xu

Received: 17 March 2008 / Accepted: 16 December 2008 / Published online: 20 January 2009

Abstract Selenium at low concentrations has a chemopreventive role against cancer, while at high concentrations, selenite exerts a direct antitumor effect. However, the mechanisms behind these effects remain elusive. In this study, we found that different concentrations of selenite triggered different signal pathways in human leukemia NB4 cells. Low concentrations of selenite elicited mild endoplasmic reticulum (ER) stress and mediated cell survival by activating unfolded protein response signaling, whereas high concentrations of selenite induced severe ER stress and caused cell death by activation of the pro-apoptotic transcription factors GADD153. In addition, selenite at low concentrations activated other anti-apoptotic pathways, such as AKT and ERK, whereas high concentrations of selenite induced activation of p53 and oxidative stress, which mediated the antitumor activity of selenite by causing mitochondrial dysfunction and caspase activation. These findings uncover the molecular mechanisms of the chemopreventive and antitumor effects of different concentrations of selenite.

L. Guan · B. Han · Z. Li · F. Huang · Y. Yang · C. Xu
National Laboratory of Medical Molecular Biology,
Institute of Basic Medicine, Peking Union Medical College
and Chinese Academy of Medical Sciences,
Beijing 100005, People's Republic of China

J. Li
Department of Hematology, Peking Union Hospital, Peking Union
Medical College and Chinese Academy of Medical Sciences,
Beijing 100730, People's Republic of China

C. Xu (✉)
Department of Biochemistry and Molecular Biology,
Institute of Basic Medical Sciences, CAMS & PUMC,
5 Dongdan Santiao, Dongcheng District,
Beijing 100005, China
e-mail: caiminxu@yahoo.com.cn

Keywords Selenite · UPR · ER stress · Survival · Apoptosis

Introduction

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML-M3), characterized by a specific chromosome translocation t(15;17) (q22;qll–12) and the expression of PML-RARαfusion protein [1]. The permanent cell line NB4 is derived from the marrow of a patient with APL in relapse [2]. All-trans retinoic acid and arsenic trioxide have been successful in treating APL [3, 4], but both drugs have some limitations. Thus, development of new therapeutic agents for APL patients is needed.

Selenium is an essential nutrient with a chemopreventive role against cancer at nutritional levels and antitumor potential at supranutritional doses [5, 6]. It has been reported that selenium at low concentrations can increase cell proliferation and suppress apoptosis caused by some stimuli while at higher concentrations decrease cell proliferation and cause apoptosis [7, 8]. Our previous works demonstrated that 20 μM selenite could inhibit the growth and proliferation of cultured cell lines including NB4, HL60, and U937 and induce cell apoptosis, while selenite at 2 μM did not have such effects [8–10]. cDNA microarray analysis found that both treatments induced significant gene-expression pattern changes. Different concentrations of selenite could have different effects on cells [10]. However, the mechanisms of these effects are not fully understood.

The accumulation of unfolded or misfolded protein can result in endoplasmic reticulum (ER) stress [11, 12]. ER triggers the unfolded protein response (UPR) to protect cells against ER stress. Three ER-resident transmembrane proteins, PERK, IRE1, and ATF6 have been identified as the proximal sensors of ER stress [13, 14]. Other survival

responses are also activated to counteract the adverse effects of ER stress [15]. The activation of AKT, ERK, and transcriptional regulator NF-κB has well-characterized roles in anti-apoptotic pathways [16–18]. However, if these survival responses are not sufficient to rescue cell, the cell will trigger apoptotic signals [19]. CHOP, also known as growth-arrest and DNA-damage inducible gene 153 (GADD153), is a key pro-apoptotic transcription factor that is closely related to ER stress [20, 21]. A paradox of UPR is that the response leads to the simultaneous activation of both adaptive and apoptotic pathways. Thus the UPR, survival response, and apoptotic response are simultaneously activated following ER stress, and the balance between them determines whether the cell survives or undergoes apoptosis.

Materials and methods

Reagents and antibodies

Sodium selenite, dimethyl thiazolyl diphenyl tetrazolium salt (MTT), and Rh123 were purchased from Sigma-Aldrich. Pifithrin-α and MnTMPyP were purchased from Calbiochem. The antibodies to the following proteins were used in this study: Cleaved Caspases Antibody Sampler Kit, casapse-2, eIF2α, phospho-eIF2α (Ser51), AKT, phospho-AKT (Ser 473), ERK, phospho-ERK (Thr202/Tyr204), NF-κB, p53, and phospho-p53 (Ser15) were purchased from Cell Signaling Technology; phospho-PERK (Thr981), CHOP/GADD153, ATF6 (N-16), and caspase-4 (N-15) from Santa Cruz; XBP-1 from Biolegend; and β-actin from Sigma-Aldrich. The reactive oxygen species (ROS) detection kit was purchased from Beyotime Company (Jiangsu, China).

Cell cultures

NB4 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2. Cells were exposed to different concentrations of selenite (2, 5, 10, 20 μM) for 24 h.

Cell viability assay

Approximately 10^4 cells were seeded to achieve a total volume of 200 μl in 96-well plates and treated with 2–20 μM selenite for up to an additional 3 days (0, 1, 2, or 3). Cells were collected by centrifugation at 250×*g* for 10 min. One hundred microliters of medium and 20 μl MTT (5 mg/ml in phosphate-buffered saline (PBS)) were added to each well and incubated for 4 h at 37°C. Cells were collected by centrifugation at 250×*g* for 10 min and resuspended in 150 μl dimethyl sulfoxide. The optical density (OD) value at a wavelength of 492 nm was measured as a direct indication of the relative vitality (RV) of cells. RV= (ODExp/ODCon)×100% is an indication of the inhibitory effect of the growth and proliferation of NB4 cells.

Apoptosis detection by flow cytometry

Approximately 10^6 cells were collected, washed twice with ice-cold PBS, and fixed with 70% ethanol at 4°C overnight. The cells were then collected by centrifugation and resuspended in 0.5 ml PBS containing 50 μg/ml RNase A. The cells were incubated at 37°C for 30 min, and then put on ice to stop the reaction. Propidium Iodide (PI) solution was then added to achieve a final concentration of 50 μg/ml, and cells were stained for at least 30 min on ice in the dark. The resulting cell suspension was then subjected to flow cytometry with excitation at 488 nm and emission at 620 nm.

Detection of mitochondrial membrane potential

Mitochondrial membrane potential (Δψm) was measured using flow cytometry with Rhodamine 123 (Rh123) and PI double labeling. Briefly, about 10^6 cells were collected and washed twice with PBS and incubated in 1 ml of staining solution containing 10 μg/ml Rh123 in PBS for 30 min in the dark at 37°C. Fifty micrograms per milliliter PI was then added to the cells and incubated for another 30 min. Fluorescence intensities were determined by flow cytometry.

Western blot

Cells were washed twice with ice-cold PBS and lysed in radioimmunoprecipitation assay cell lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM ethylenediamine tetraacetic acid, 1 mM ethyleneglycoltetraacetic acid, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na3VO4, 1 μg/ml Leupeptin, 1 mM phenylmethanesulfonyl fluoride). The cell lysates were sonicated on ice by ultrasonication (model VC 750, Sonics) for 20 s at 25% power output and centrifuged at 40,000×*g* for 10 min at 4°C. The supernatant was collected, protein concentration was determined by the Bradford assay, and equal amounts of protein were loaded on 12% (or 15%, depending on the protein of interest) sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked with Tris-buffered saline Tween-20 (TBST) containing 5% non-fat milk and incubated with primary antibodies overnight at 4°C. After washing with TBST containing 0.1% Tween 20, the membranes were incubated with sufficient dilutions of rabbit anti-mouse or goat anti-rabbit peroxidase-conjugated secondary antibodies for 60 min at room temperature. After a second wash, the

blots were probed with an enhanced chemiluminescence (ECL) substrate (Amersham) and exposed to Hyperfilm ECL to visualize the immunoreactive bands.

ROS measurement

Intracellular ROS levels were determined by measuring the oxidative conversion of cell permeable 2′,7′ dichlorofluorescein diacetate (DCFH-DA) to fluorescent dichlorofluorescein (DCF) in a fluorospectro-photometer. Cells treated with different concentrations of selenite for 10 min were washed with serum-free medium and incubated with 10 μM DCFH-DA at 37°C for 20 min. The DCF fluorescence distribution of 4×10^5 cells was detected by fluorospectrophotometer analysis at an excitation wavelength of 488 nm and at an emission wavelength of 535 nm.

Small interference RNA

The GADD153-specific siRNA (sense 5′-CCAGGAAACG GAAACAGA-3′; antisense 5′-CUCUGUUUCCGUUUCC UGG-3′) [22], p53-specific siRNA (sense 5′-CUACUUC CUGAAAACAACG-3′; antisense 5′-CGUUGUUUUCAG GAAGUAG-3′) and nonsilencing scrambled siRNA were synthesized and purchased from GenePharma (Shanghai Co. Ltd China). For siRNA transfection, NB4 cells were plated in six-well culture plates at density of 3.5×10^5 and transfected with siRNA by using Lipofectamine™ 2000 reagent (Invitrogen) according to the manufacturer's protocol. Briefly, for each well, 5 μl Lipofectamine2000 was diluted in 250 μl Opti-MEMI medium (Invitrogen). This mixture was carefully added to a solution containing 200 nmol/l siRNA in 250 μl Opti-MEMI medium. The solution was incubated for 20 min at room temperature and then gently dripped into the NB4 cells in 2 ml antibiotic-free medium. Regular growth medium was added 6–12 h after transfection. Twenty-four hours after transfections, cells were treated with 20 μM sodium selenite.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) analysis with Tukey's multiple comparisons and Student's *t* test. A value of $p<0.05$ was considered statistical significance.

Results

Different effects of different concentrations of selenite on cells

NB4 cells were exposed to different concentrations of selenite (2–20 μM) for up to an additional 3 days. MTT assay was used to determine cell viability. The results showed that selenite resulted in a dose-dependent change in cell viability. Selenite treatment (2 μM) for 24 h had no effect on cell viability but markedly increased cell viability for 48 h. The reduction in cell viability was observed starting at 5 μM selenite treatment, with maximum reduction observed at 20 μM (Fig. 1a). Cells treated with increasing concentrations of selenite were collected at 24 h, and the frequency of apoptosis was determined by flow cytometry analysis of the cells with sub-G1 DNA content, which is a marker of apoptosis. Figure 1b shows that the control and 2 μM selenite-treated groups have a very low percentage of sub-G1 cells, while a substantial increase of sub-G1 cells was observed with higher concentrations of selenite (5–20 μM) treatment. These results show that selenite above 5 μM reduces NB4 cell viability and induces apoptosis, while 2 μM selenite increases cell viability and has no effect on apoptosis.

Selenite activates UPR and GADD153 at low and high concentrations, respectively

To determine the effects of different concentrations of selenite on ER stress, we investigated the activation of ER stress markers, including UPR markers PERK-eIF2α, XBP-1S, ATF6, and apoptosis-related molecule GADD153. NB4 cells were treated with different concentrations of selenite for 24 h and subjected to Western blot analysis. Phosphorylation of PERK and eIF2α were induced at 2 μM selenite treatment and then decreased with selenite treatment above 5 μM, while total eIF2α protein levels were unchanged. (Fig. 2a). Activated IRE1 leads to XBP-1 mRNA splicing, and the spliced-XBP-1 mRNA encodes the potent transcription factor XBP-1S. Using IRE1-mediated XBP-1 splicing as a surrogate marker, we assessed the effect of selenite on IRE1 activation. As shown in Fig. 2a, 54 kDa XBP-1S protein was induced at 2 μM selenite and then decreased above 5 μM selenite treatment. Activated product of ATF6, the 50 kDa cleavage fragment, increased at 2–5 μM selenite treatments and returned to its basal level or below at higher concentrations of selenite (Fig. 2a). The inactivation of ATF6 is less sensitive to the other UPR signals. All three UPR pathways were significantly activated by selenite at concentration as low as 2 μM and decreased at higher concentrations. The apoptosis-related molecule GADD153, which is induced during ER stress and participates in ER stress-mediated apoptosis, was present at extremely low levels after 2 μM selenite treatment and was induced significantly above 5 μM selenite treatments. To further establish a functional role of GADD153 in selenite-induced apoptosis of NB4 cells, GADD153-specific siRNA was used to inhibit GADD153 expression. A scrambled RNA duplex was used as a

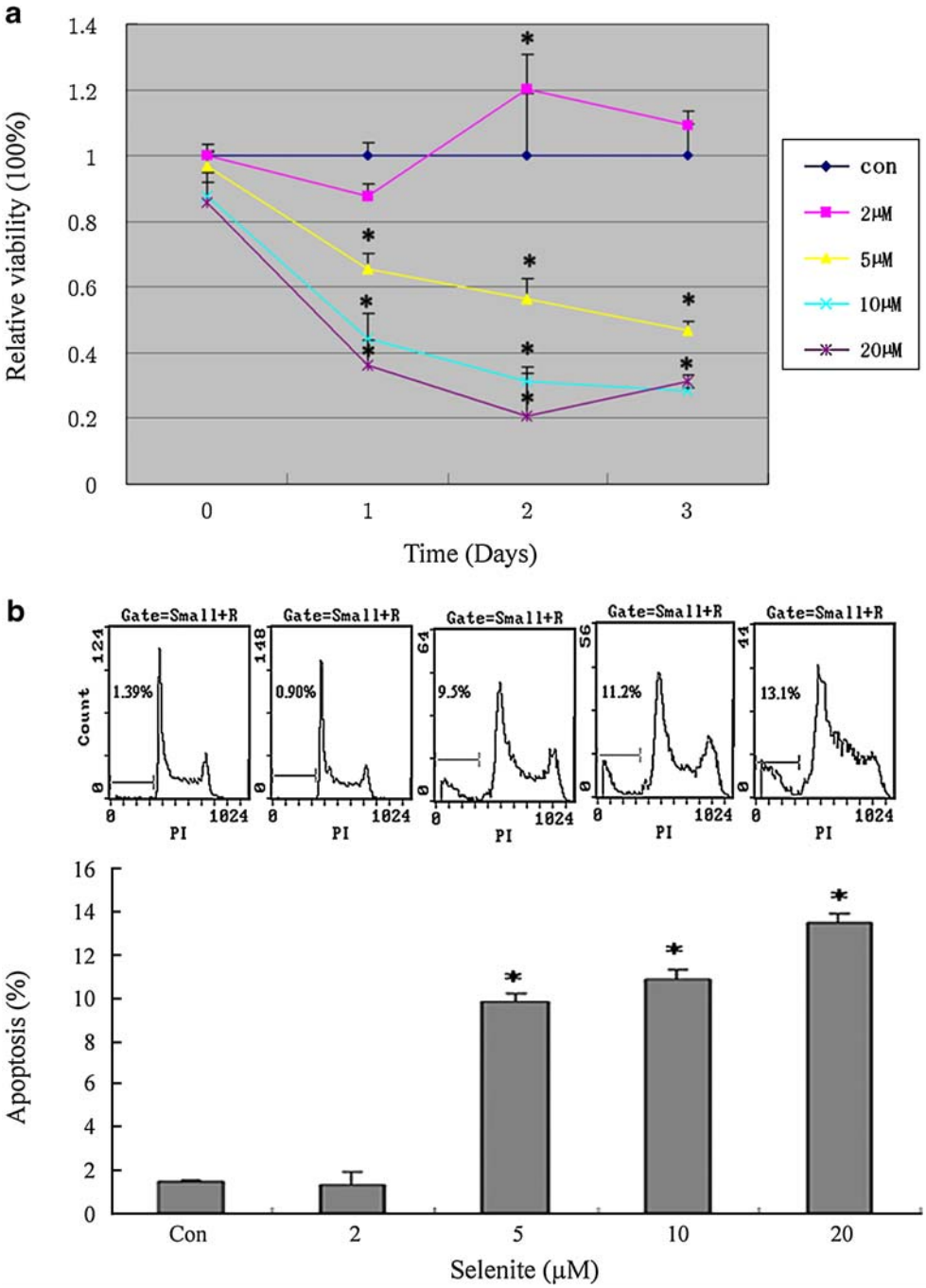

Fig. 1 Effects of different concentrations of selenite on cell viability and apoptosis. **a** Cell viability was determined by MTT assay. Relative vitality=(ODExp/ODCon)×100%, which is an indication of cell viability. Cells were treated with 0–20 μM selenite for 1, 2, or 3 days. **b** Cell apoptosis was determined by flow cytometry analysis of sub-G1 DNA cells, which is a marker of apoptosis. Cells were treated with 0–20 μM selenite for 24 h. *$p<$ 0.05 compared with the control group. Data are presented as the mean±SD of triplicates and analyzed using ANOVA analysis

negative control. As shown in Fig. 2b, GADD153-specific siRNA reduced GADD153 mRNA and protein expression and blocked selenite-induced cell apoptosis compared with cells transfected with scrambled siRNA, suggesting that GADD153 is involved in NB4 cell apoptosis induced by high concentrations of selenite.

Selenite activates AKT and ERK pathways at low concentrations

In addition to UPR, it remains unclear whether other cell survival mechanisms such as the AKT and ERK pathways are activated in parallel by ER stress. To evaluate the effects of selenite on the AKT and ERK pathways, NB4 cells were treated with different concentrations of selenite for 24 h and subjected to Western blot analysis. Results showed that low concentrations of selenite upregulated AKT and ERK phosphorylation, while high concentrations of selenite inhibited AKT and ERK phosphorylation. Phosphorylation of AKT was more sensitive to selenite treatment than ERK, and no apparent changes of AKT and ERK basal levels were observed (Fig. 3). These observations suggest low concentrations of selenite activate the AKT and ERK pathways and mediate cell survival.

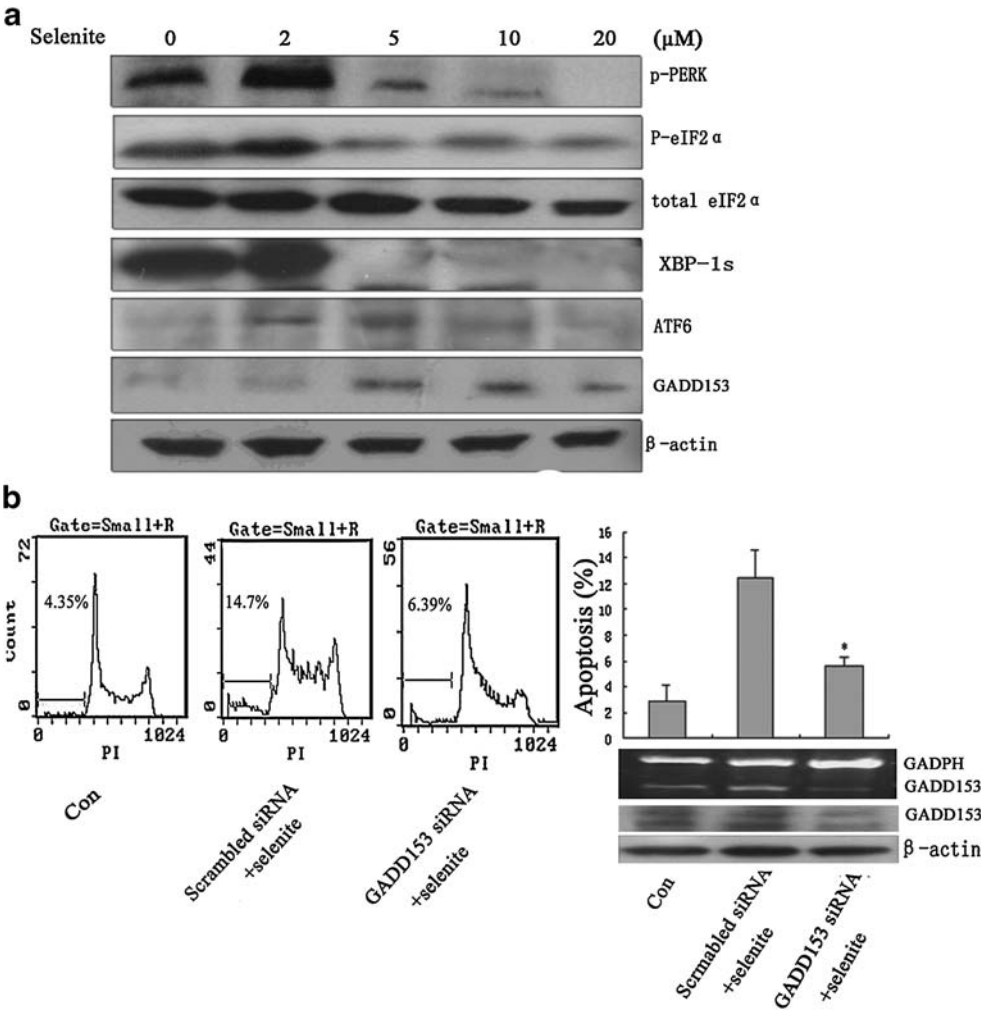

Fig. 2 Selenite induces ER stress, and GADD153 mediates cell apoptosis. **a** Total cell lysates were prepared and subjected to Western blot using antibodies directed against p-PERK, p-eIF2α, eIF2α, XBP-1S, ATF6, and GADD153, respectively. β-actin was used as a loading control. **b** Cells were transfected with GADD153 siRNA and scrambled siRNA as described in "Materials and methods" and treated with 20 μM selenite for 24 h. The mRNA and protein levels of GADD153 were determined by reverse transcription polymerase chain reaction and Western blot, respectively. Apoptosis was determined by flow cytometry. *$p<0.05$ compared with the scrambled siRNA. Data are presented as the mean±SD of triplicates and analyzed using Student's *t* test

Selenite blocks NF-κB signaling and activates p53 at high concentrations

The transcription factors NF-κB and p53 regulate the expression of genes critical for multiple biological processes, including inflammatory reactions, immune responses, cell proliferation, and apoptosis. The activation of NF-κB involves the nuclear translocation of the p65 subunit of NF-κB. In order to evaluate the effects of different concentrations of selenite on the activation of NF-κB, the amount of nuclear translocation of p65 was determined by Western blot analysis of the nuclear fraction. Result shows that, above 5 μM selenite treatment markedly inhibited the nuclear translocation of the p65 (Fig. 4a). Ser15 phosphorylation is essential for p53 to exert its pro-apoptotic effect. Western blot analysis was performed with a specific anti-phospho-Ser15-p53 antibody to determine whether p53 was activated. As shown in Fig. 4a, significant elevation of phosphorylation of p53 at ser15 was detected following treatments above 5 μM for 24 h, while no elevation of total p53 levels was observed. To determine whether p53 is involved in the selenite-induced NB4 cell apoptosis, P53-specific siRNA was used to reduce p53 expression, and a specific p53 inhibitor, pifithrin-α (PFT), was used to inhibit p53 activity. Both treatments partially abrogated 20 μM selenite-induced NB4 cell apoptosis (Fig. 4b, c), suggesting that p53 plays a role in selenite-induced NB4 cell apoptosis.

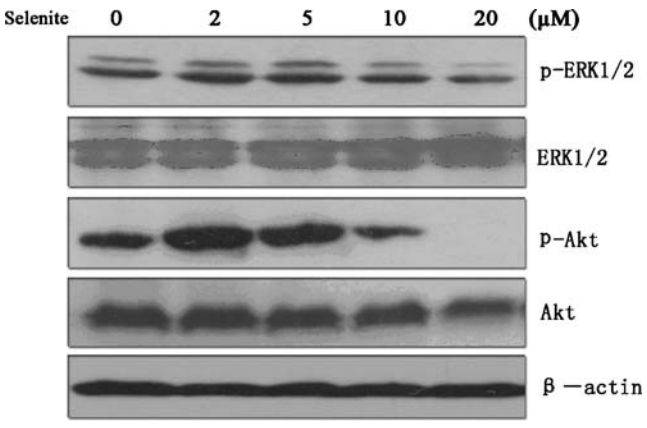

Fig. 3 Selenite activities AKT and ERK pathways at low concentrations. Total cell lysates were prepared and subjected to Western blot using antibodies directed to p-AKT, p-ERK, AKT, and ERK, respectively. β-actin was used as a loading control

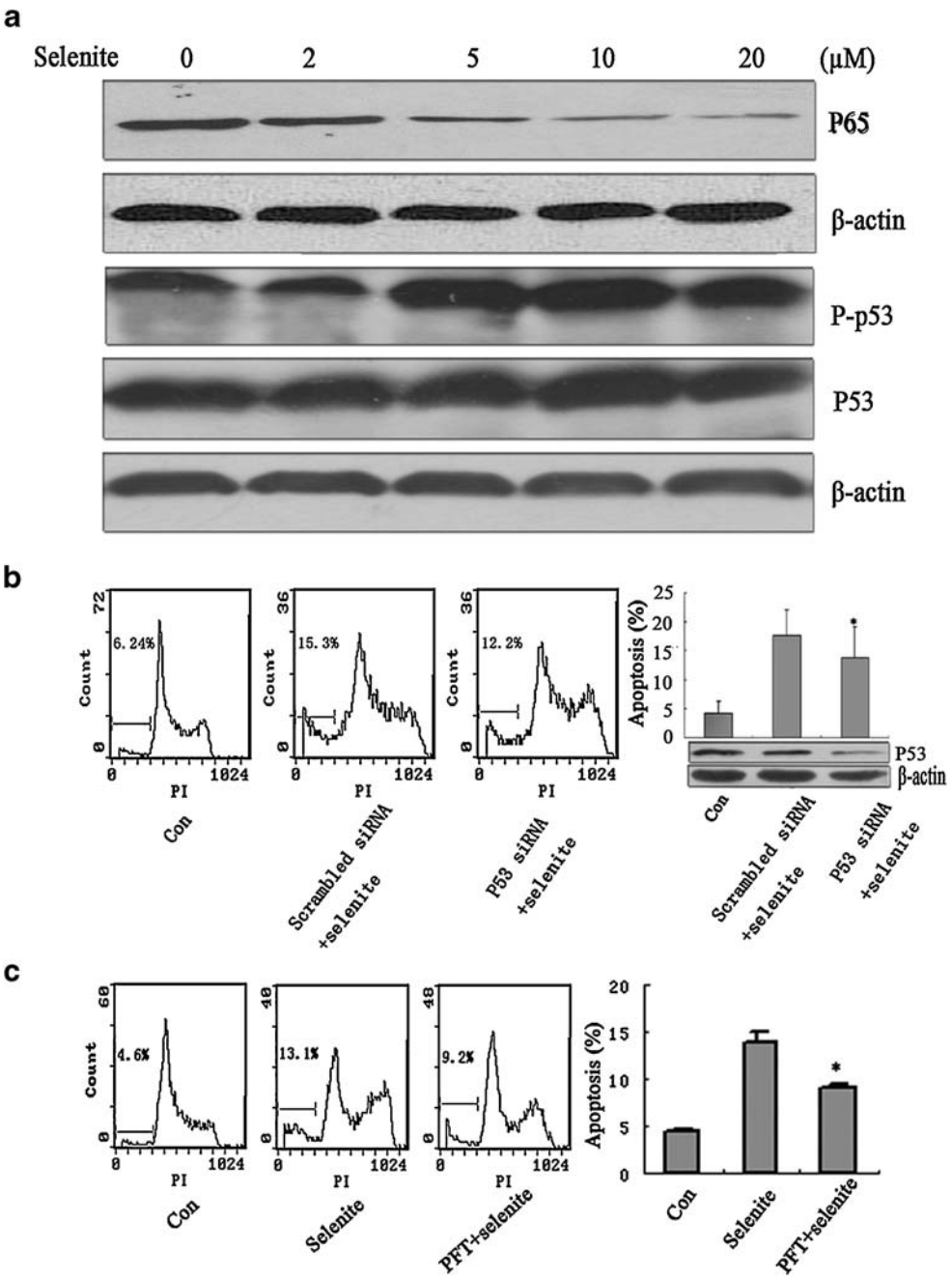

Fig. 4 Selenite blocks NF-κB but activates p53 at high concentrations. **a** Nuclear extraction and total cell lysates were prepared and subjected to Western blot using antibodies directed against p65, p-p53, and p53 respectively. β-actin was used as a loading control. **b** Cells were transfected with p53 siRNA and scrambled siRNA and treated with 20 μM selenite for 24 h. Apoptosis was determined by flow cytometry. *$p<0.05$ compared with the scrambled siRNA. **c** Cells were pretreated with or without PFT for 1.5 h and then treated with 20 μM selenite for 24 h. Apoptosis was analyzed by flow cytometry. *$p<0.05$ compared with the 20 μM selenite-treated cells. Data are presented as the mean± SD of triplicates and analyzed using Student's *t* test

Selenite activates the caspase cascades at high concentrations

Activation of caspases is a key process in cell apoptosis. We assessed the effects of different concentrations of selenite on caspase activation in NB4 cells by Western blot analysis. The results showed that the activated cleavage fragment of caspase-9 increased when cells were exposed to high concentrations of selenite (5–20 μM), whereas the activated cleavage fragment of caspase-8 was not detected (data not shown). Caspase-4 was also activated by treatment with high concentrations of selenite, as shown from the decrease of pro-caspase-4 and the increase of cleavage fragment. In parallel with the activation of caspase-9 and -4, there were also increases in the cleavage of effector caspases-3, -6, and -7 in response to high concentrations of selenite treatment (Fig. 5a). To test the functional significance of the activated caspases, we used general caspase inhibitors to interfere with the activity of total caspases. The general caspase inhibitor zVADfmk partially suppressed apoptosis induced by 20 μM selenite (Fig. 5b).

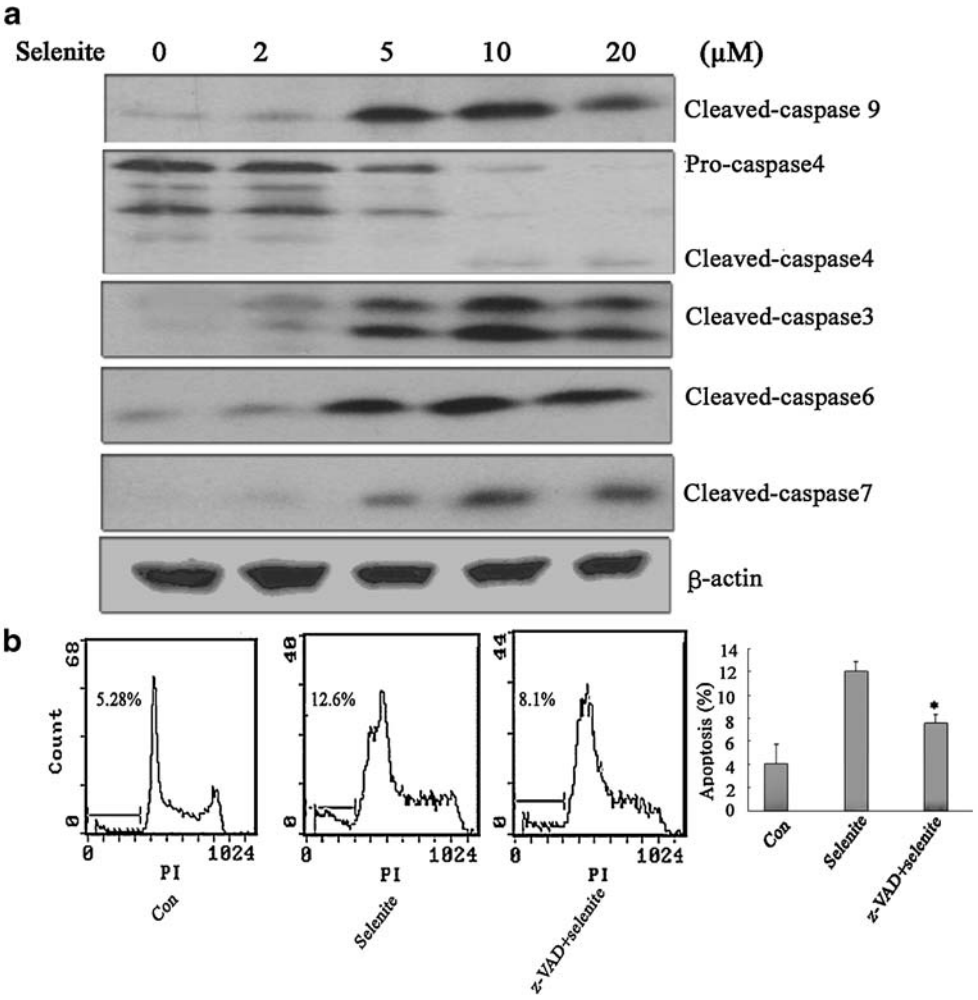

Fig. 5 Selenite activates caspase cascades at high concentrations. **a** Total cell lysates were prepared for 24 h and subjected to Western blot using antibodies directed against cleaved-caspase-9, -8, -3, -6, -7, and caspase-4, respectively. β-actin was used as a loading control. **b** NB4 cells were pretreated with or without z-VAD-fmk for 1.5 h and then treated with 20 μM selenite for 24 h. Cell apoptosis was analyzed by flow cytometry. *$p<0.05$ compared with the 20 μM selenite-treated cells. Data are presented as the mean± SD of triplicates and analyzed using Student's *t* test

Selenite induces mitochondrial membrane potential collapse at high concentrations

The loss of Δψm is involved in apoptosis of a variety of cell types. As shown in Fig. 6, high concentrations of selenite (5–20 μM) treatment for 24 h led to a pronounced decrease in Δψm, as represented by the increase of the proportion of PI and Rh123 double-negative cells. No significant alteration in Δψm was observed following 2 μM selenite treatment. Thus, we can conclude that high concentrations of selenite induce severe mitochondrial dysfunction.

High concentrations of selenite induce the production of Reactive Oxygen Species (ROS).

ROS can play an important role in depolarizing mitochondria and inducing apoptosis. A DCFH-DA-dependent measurement was used to assess ROS levels in NB4 cells treated with different concentrations of selenite for 10 min. A relatively low level of ROS was detected in control and 2 μM selenite treatment cells, whereas 20 μM selenite caused rapid increase in ROS production. Selenite raised the ROS level in a dose-dependent manner (Fig. 7a). A cell-permeable superoxide scavenger, MnTMPyp significantly suppressed selenite-induced cell death (Fig. 7b), suggesting an important role for superoxide radicals in selenite-induced cell apoptosis. Consistent with the blockage of cell apoptosis, MnTMPyp greatly prevented Δψm collapse and caspase activation induced by 20 μM selenite (Fig. 7c and d). These results suggest that high concentrations of selenite induce the production of ROS, which mediates NB4 cell apoptosis by causing mitochondrial dysfunction and caspase activation.

Discussion

Selenium could cause global thiol/disulfide redox modification of numerous proteins that may result in protein unfolding or misfolding in the ER, triggering ER stress [22, 23]. Thus, it is highly plausible that selenite could induce ER stress and oxidative stress. So we speculated that ER stress-induced pro-survival and pro-apoptotic pathways probably mediate the chemopreventive and antitumor effects of selenite. Treatment with selenite induced a number of signaling markers in a dose-dependent

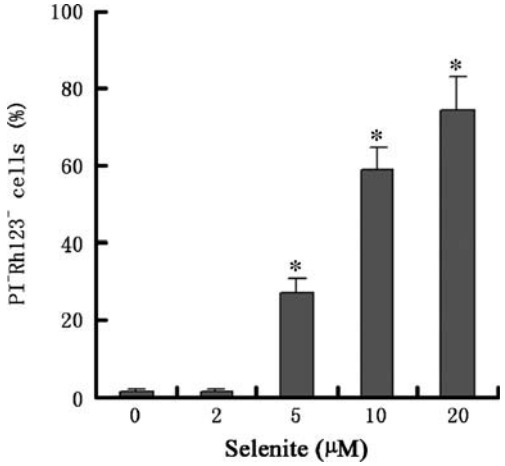

Fig. 6 Selenite induces Δψm collapse at high concentrations. Cells were treated with different concentrations of selenite for 24 h, and fluorescence intensities of Rh123 and PI were determined by flow cytometry. The results shown are the proportion of PI and Rh123 double-negative cells. *$p<0.05$ compared with the control group. Data are presented as the mean±SD of triplicates and analyzed using Student's *t* test

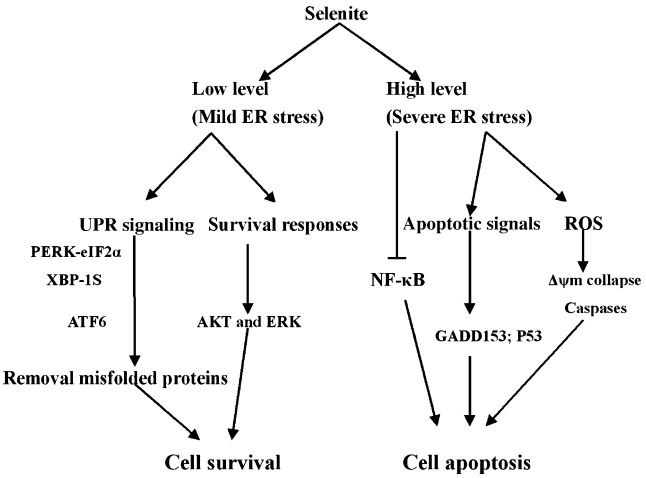

Fig. 8 The survival and apoptosis signaling cascades stimulated with low and high concentrations of selenite, respectively

manner. These markers can be categorized into two groups: (a) Survival molecules such as UPR pathway (including PERK-eIF2α, IRE1-XBP-1S, and ATF6), AKT, ERK, and NF-κB; (b) apoptotic molecules such as GADD153, p53,

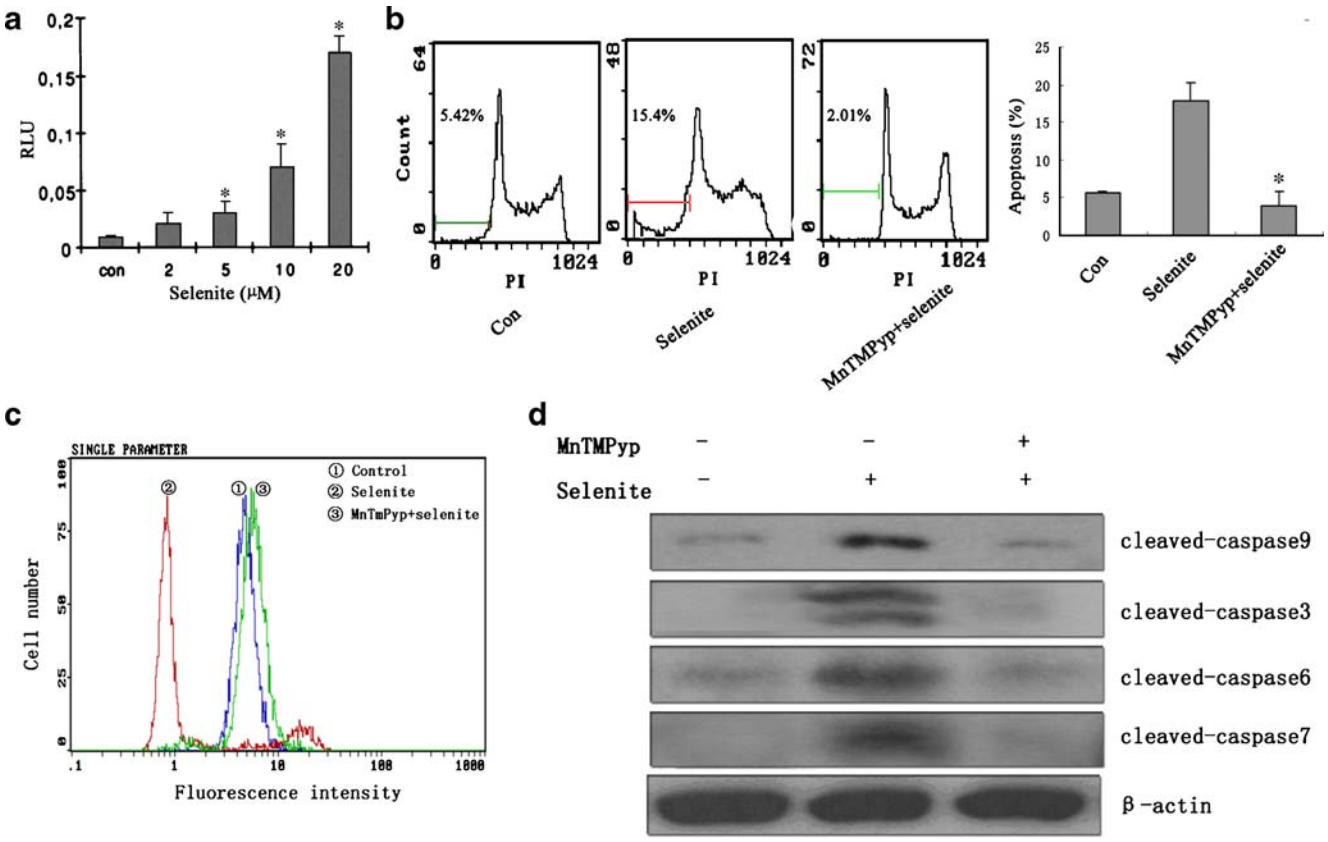

Fig. 7 High concentrations of selenite induce oxidative stress-mediated cell apoptosis. **a** ROS level was detected using a DCFH-DA-dependent assay. *$p<0.05$ compared with the control group. Cells were pretreated with or without MnTMPYP for 1.5 h and then treated with selenite for 24 h. **b** Cell apoptosis was analyzed by flow cytometry. *$p<0.05$ compared with the 20 μM selenite-treated cells. **c** Mitochondrial membrane potential was determined by flow cytometry and **d** the activation of caspases was analyzed by Western blot. Data are presented as the mean±SD of triplicates and analyzed using Student's *t* test

and caspases. Low concentrations of selenite preferentially activated the survival responses, whereas high concentrations of selenite led to assembly of the apoptotic molecules and ROS, which induced mitochondrial membrane permeabilization and caspases activation. The net balance between these signaling cascades probably governs cell survival or apoptosis. We have schematically presented these events in Fig. 8.

The present work showed that the three UPR transducer pathways, PERK-eIF2α, IRE1-XBP1S, and ATF6, were activated by selenite at a concentration as low as 2 μM and inhibited at higher concentrations. The phosphorylation of eIF2α shuts off general translation to reduce the biosynthetic load and thus prevents further accumulation of unfolded proteins [24]. The activation of XBP-1S and ATF6 involves up-regulation of ER chaperone proteins, which increase protein-folding activity and prevent protein aggregation [25]. In addition, XBP-1S also induces expression of a subset of genes encoding protein degradation enzymes (e.g., EDEM), which mediate the degradation of misfolded proteins by ER-associated degradation (ERAD) [25]. These UPR signaling pathways protect cells against ER stress-induced damage by transiently inhibiting translation (PERK pathway), enhancing protein-folding capacity (ER chaperone proteins) and degrading terminally misfolded or aggregated proteins (ERAD system) [19].

In parallel to the UPR, low concentrations of selenite activate AKT and ERK that directly prevent apoptotic signals to provide time for the UPR to function [15, 16, 26, 27]. Activation of endogenous AKT and ERK plays a critical role in controlling cell survival by resisting the ER stress-induced apoptotic signals [16, 26]. In conclusion, low concentrations of selenite protect cells against apoptosis via activation of UPR, AKT, and ERK in NB4 cells.

However, these cell-protection mechanisms have a limit. If the stress level is too severe to be repaired by cells, the apoptotic signals will be triggered [19]. Higher concentrations of selenite induce activation of ER-stress apoptotic markers GADD153, which switches the signaling from pro-survival to pro-apoptosis [20]. At the same time, higher concentrations of selenite activate p53 but block the activation of NF-κB. NF-κB has been suggested to be associated with increased survival in many tumor cells [15, 18]. P53 is a well-known tumor suppressor, and its anti-tumor activity is achieved primarily through the induction of apoptosis. A recent study has found that ER stress can trigger p53 activation in a PERK-dependent manner [28]. NB4 cells express a mutant form of p53 that is incapable of binding DNA [29]. Inhibition of p53 by RNA interference or PFT-prevented selenite-induced cytotoxicity indicated that this transactivation-deficient mutant of p53 in NB4 cells participates in the apoptotic process in a transcription-independent manner.

Caspase-9 and -4 rather than caspase-8 are activated by higher concentrations of selenite, indicating the possible involvement of both mitochondria and ER stress-dependent apoptotic pathways in selenite-induced NB4 cell apoptosis [30, 31]. Selenite has been reported to increase the generation of ROS during apoptosis [32, 33]. As anticipated, DCFH-DA-dependent measurements showed that intracellular ROS levels were significantly increased in cells treated with 20 μM selenite. The removal of ROS by an antioxidant drug, MnTMPyP, abrogated the increase in ROS and simultaneously completely inhibited cell apoptosis induced by selenite. MnTMPyP pretreatment impeded selenite-induced $\Delta\psi m$ collapse and activation of caspases. These results suggest that oxidative stress also mediates the antitumor activity of selenite, probably through induction of mitochondria dysfunction [34, 35].

Thus, it takes more selenite to elicit ER-stress apoptotic markers as well as oxidative stress compared with the UPR and survival markers (over 5 μM for the former versus 2 μM for the later). Low concentrations of selenite elicit mild ER stress and activate the UPR and survival pathways, which help cells to cope with and recover from ER stress. But this rescue effort is limited because these UPR and survival markers fail to keep up with the increasing challenge from selenite. Apoptosis-related markers are activated after selenite treatment above 5 μM. Taken together, low concentrations of selenite activate UPR and multiple survival mechanisms that counteract ER stress-induced cell apoptosis, while high concentrations of selenite block these survival pathways and at the same time activate multiple mechanisms that directly trigger cell apoptosis. These different molecular mechanisms control selenite adaptation and selenite-induced cell apoptosis.

Acknowledgments This work was supported by grants from National Natural Sciences Foundation of China (no. 30370348 and no. 30770491), Doctoral Point Foundation of National Educational Committee (no. 20010023029), and Natural Sciences Foundation of Beijing (no. 7032034 and no. 5082015).

References

1. Kakizuka A, Miller WH, Umesono K (1991) Chromosomal translocation t (15; 17) in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor PML. Cell 66:663–674 doi:10.1016/0092-8674(91)90112-C
2. Lanotte M, Martin-Thouvenin V, Najman S, Balerini P, Valensi F, Berger R (1991) NB4, a Maturation Inducible Cell Line With t (15;17) Marker Isolated From a Human Acute Promyelocytic Leukemia (M3). Blood 77:1080–1086
3. Chen Z, Chen GQ, Shen ZX, Chen SJ, Wang ZY (2001) Treatment of acute promyelocytic leukemia with arsenic compounds: in vitro and in vivo studies. Semin Hematol 38:26–36 doi:10.1053/shem.2001.20863

4. Shen ZX, Shi ZZ, Fang J (2004) All-trans retinoic acid/As2O3 combination yields a high quality remission and survival in newly diagnosed acute promyelocytic leukemia. Proc Natl Acad Sci USA 101:5328–5335 doi:10.1073/pnas.0400053101
5. Diwadkar-Navsariwala V, Diamond AM (2004) The link between Selenium and chemoprevention: a case for selenoproteins. J Nutr 134:2899–2902
6. Zeng H, Combs GF Jr (2007) Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion. J Nutr Biochem 19:1–7 doi:10.1016/j.jnutbio.2007.02.005
7. Gopee NV, Johnson VJ, Sharma RP (2004) Selenite-induced apoptosis in murine B-Lymphoma cells is associated with inhibition of protein kinase C-δ, nuclear factor κB, and inhibitor of apoptosis protein. Toxicol Sci 78:204–214 doi:10.1093/toxsci/kfh072
8. Zuo L, Li J, Shen T, Zhang ZN (2002) The comparison between the mechanisms of sodium selenite induced apoptosis and arsenic trioxide induced apoptosis in human acute promyelocytic leukemia cell line NB4 cells. Zhongguo Shi Yan Xue Ye Xue Za Zhi 10:195–200
9. Zuo L, Li J, Yang Y, Wang X, Shen T, Xu CM, Zhang ZN (2004) Selenite induces apoptosis in acute promyelocytic leukemia-derived NB4 cells by a caspase-3-dependent mechanism and a redox pathway different from that of arsenic trioxide. Ann Hematol 83:751–758 doi:10.1007/s00277-004-0920-5
10. Cao TM, Hua FY, Xu CM, Han BS, Dong H, Zuo L, Wang X, Yang Y, Pan HZ, Zhang ZN (2006) Distinct effects of different concentrations of selenite on apoptosis, cell cycle, and gene expression profile in acute promyeloytic leukemia-derived NB4 cells. Ann Hematol 85:434–442 doi:10.1007/s00277-005-0046-4
11. Faitova J, Krekac D, Hrstka R, Vojtesek B (2006) Endoplasmic reticulum stress and apoptosis. Cell Mol Biol Lett 11:488–505 doi:10.2478/s11658-006-0040-4
12. Rutkowski DT, Kaufman RJ (2004) A trip to the ER: coping with stress. Trends Cell Biol 14:20–28 doi:10.1016/j.tcb.2003.11.001
13. Szegezdi E, Logue SE, Gorman AM, Samali A (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7:880–885 doi:10.1038/sj.embor.7400779
14. Zhang KZ, Kaufman RJ (2004) Signaling the Unfolded Protein Response from the Endoplasmic Reticulum. J Biol Chem 279:25935–25938 doi:10.1074/jbc.R400008200
15. Misra UK, Deedwania R, Pizzo SV (2006) Activation and cross-talk between AKT, NF-κB, and unfolded protein response signaling in 1-LN prostate cancer cells consequent to ligation of cell surface-associated GRP78. J Biol Chem 281:13694–13707 doi:10.1074/jbc.M511694200
16. Hu P, Han Z, Couvillon AD, Exton JH (2004) Critical role of endogenous Akt/IAPs and MEK1/ERK pathways in counteracting endoplasmic reticulum stress-induced cell death. J Biol Chem 279:49420–49429 doi:10.1074/jbc.M407700200
17. Jiang HY, Wek RC (2005) GCN2 phosphorylation of eIF2α activates NF-κB in response to UV irradiation. Biochem J 385:371–380 doi:10.1042/BJ20041348
18. Deng J, Lu PD, Zhang YH, Scheuner D, Kaufman RJ, Sonenberg N, Harding HP, Ron D (2004) Translational repression mediates activation of Nuclear Factor Kappa B by phosphorylated translation initiation factor 2. Mol Cell Biol 24:10161–10168 doi:10.1128/MCB.24.23.10161-10168.2004
19. Kadowaki H, Nishitoh H, Ichijo H (2004) Survival and apoptosis signals in ER stress: the role of protein kinases. J Chem Neuroanat 28:93–100
20. Oyadomari S, Mori M (2004) Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 11:381–389 doi:10.1038/sj.cdd.4401373
21. Wang XZ, Lawson B, Brewer JW, Zinszner H, Sanjay A, Mi LJ, Boorstein R, Hendershot LM, Ron D (1996) Signals from the stressed endoplasmic reticulum induce C/EBP-homologous protein (CHOP/GADD153). Mol Cell Biol 16:4273–4280
22. Wu Y, Zhang HT, Dong Y, Park YM, Ip C (2005) Endoplasmic Reticulum stress signal mediators are targets of selenium action. Cancer Res 65:9073–9079 doi:10.1158/0008-5472.CAN-05-2016
23. Zu K, Bihani T, Lin A, Park YM, Mori K, Ip C (2006) Enhanced selenium effect on growth arrest by BiP/GRP78 knockdown in p53-null human prostate cancer cells. Oncogene 25:546–554
24. Jiang HY, Wek RC (2005) Phosphorylation of the α-Subunit of the eukaryotic initiation factor-2 (eIF2α) reduces protein synthesis and enhances apoptosis in response to proteasome inhibition. J Biol Chem 280:14189–14202 doi:10.1074/jbc.M413660200
25. Lee AH, Iwakoshi NN, Glimcher LH (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol 23:7448–7459 doi:10.1128/MCB.23.21.7448-7459.2003
26. Wang Z, Jiang C, Ganther H, Lu J (2001) Antimitogenic and proapoptotic activities of methylseleninic acid in vascular endothelial cells and associated effects on PI3K-AKT, ERK, JNK and p38 MAPK signaling. Cancer Res 61:7171–7178
27. Yoon SO, Kim MM, Park SJ, Kim D, Chung J, Chung AS (2002) Selenite suppresses hydrogen peroxide-induced cell apoptosis through inhibition of ASK/JNK and activation of PI3-K/AKT pathways. FASEB J 16:111–113
28. Zhang F, Hamanaka RB, Bobrovnikova-Marjon E, Gordan JD, Dai MS, Lu H (2006) Ribosomal stress couples the unfolded protein response to p53-dependent cell cycle arrest. J Biol Chem 281:30036–30045 doi:10.1074/jbc.M604674200
29. Song XD, Sheppard HM, Norman AW, Liu X (1998) Mitogen-activated Protein Kinase Is Involved in the Degradation of p53 Protein in the Bryostatin-1-induced Differentiation of the Acute Promyelocytic Leukemia NB4 Cell Line. J Biol Chem 18:3205–3212
30. Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309–1312 doi:10.1126/science.281.5381.1309
31. Hitomi J, Katayama T, Eguchi Y, Kudo T, Taniguchi M, Koyama Y, Manabe T, Yamagishi S, Bando Y, Imaizumi K, Tsujimoto Y, Tohyama M (2004) Involvement of caspase-4 in endoplasmic reticulum stress-induced apoptosis and Aβ-induced cell death. J Cell Biol 165:347–356 doi:10.1083/jcb.200310015
32. Shen HM, Yang CF, Ong CN (1999) Sodium selenite-induced oxidative stress and apoptosis in human hepatoma HepG2 cells. Int J Cancer 81:820–828 doi:10.1002/(SICI)1097-0215(19990531)81:5<820::AID-IJC25>3.0.CO;2-F
33. Li GX, Hu H, Jiang C, Schuster T, Lv J (2007) Differential involvement of reactive oxygen species in apoptosis induced by two classes of selenium compounds in human prostate cancer cells. Int J Cancer 120:2034–2043 doi:10.1002/ijc.22480
34. Wei W, Han BS, Guan LY, Fang H, Lei F, Yang Y, Xu CM (2007) Mitochondrial transmembrane potential loss caused by reactive oxygen species plays a major role in sodium selenite-induced apoptosis in NB4 cells. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 29:324–328
35. Chung YW, Kim TS, Lee SY, Lee SH, Choi Y, Kim N, Min BM, Jeong DW, Kim IY (2006) Selenite-induced apoptosis of osteoclasts mediated by the mitochondrial pathway. Toxicol Lett 160:143–150 doi:10.1016/j.toxlet.2005.06.019