

## Requirement for ERK Activity in Sodium Selenite-induced Apoptosis of Acute Promyelocytic Leukemia-derived NB4 Cells

Bingshe Han, Wei Wei, Fangyuan Hua, Tingming Cao, Hua Dong, Tao Yang, Yang Yang, Huazhen Pan and Caimin Xu\*

National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China

Received 2 April 2006, Accepted 17 October 2006

**Our previous study has shown that sodium selenite can cause apoptosis in acute promyelocytic leukemia-derived NB4 cells in a caspase-dependent manner, but the detailed mechanism is unknown. Here we demonstrate a requirement for extracellular signal-regulated protein kinase (ERK) in mediating sodium selenite -induced apoptosis in NB4 cell. Though no apparent elevation of ERK activity was observed during the apoptosis in NB4 cells caused by 20  $\mu$ M sodium selenite treatment, PD98059 and U0126, specific chemical inhibitors of the MEK/ERK signaling pathway, were shown to strongly prevent the apoptosis process, while ERK activator TPA enhanced the process. It is also known that p38 MAPK inhibitor SB203580 and JNK inhibitor SP600125 had slight effects on apoptosis. Further study indicated that ERK exerted its proapoptotic effect only at the early stage of apoptosis and played an antiapoptotic role at the later stages. Taken together, our findings suggest that ERK plays an active role in mediating sodium selenite-induced apoptosis in NB4 cells .**

**Keywords:** Acute promyelocytic leukemia-derived NB4 cells, Apoptosis, ERK Activity, MEK Inhibitor, Sodium Selenite

### Introduction

Acute promyelocytic leukemia (APL) is a unique subtype of acute myeloid leukemia (AML) both in its biology and response to chemotherapy, APL is characterized by a specific chromosome translocation t(15;17) and the expression of PML-RAR $\alpha$  fusion protein, which presumably plays a role in the etiology of APL (Kakizuka *et al.*, 1991). All-trans retinoic

acid (ATRA) and arsenic trioxide have been successfully put into clinical practice to achieve complete remission (CR) in APL patients (Grignani *et al.*, 1994; Chen *et al.*, 2001). However, development of new therapeutic agents for APL patients is needed, since both drugs mentioned above have some limitations.

The trace element selenium is an essential dietary nutrient that has been extensively studied as an potential anticancer agent (Patrick *et al.*, 2004). Recent reports show that sodium selenite, a common dietary form of selenium, can effectively induce several cancer cell lines to undergo apoptosis (Wang *et al.*, 2003; Gopee *et al.*, 2004; Kim *et al.*, 2004), raising a new idea for its clinical application. Our pioneering work has shown that sodium selenite, at concentrations of 5-20  $\mu$ M, can disrupt  $\Delta\psi_m$  and induce apoptosis in NB4 cells in a caspase 3-dependent manner, and this proapoptotic activity of sodium selenite was mainly attributed to its ability of inducing oxidative stress and downregulating anti-apoptotic protein Bcl-2 (Zuo *et al.*, 2004).

It is well established that mitogen-activated protein kinases (MAPKs) play important roles in mediating apoptosis caused by various stimuli. To determine the role that MAPKs may play in sodium selenite-induced apoptosis, three groups of mammalian MAPKs were studied: extracellular signal-regulated kinase (ERK, also referred to as p42/p44 MAPK), c-jun NH<sub>2</sub>-terminal kinase (JNK) or stress-activated kinase (SAPKs), and p38 MAPK. Of the three main members, p38 MAPK and JNK are generally associated with apoptosis induction, ERK are generally associated with mitogenesis and reversely related to apoptosis, but some reports also show that ERK may exert proapoptotic effect (Amra *et al.*, 2005), and that p38 MAPK and JNK can play antiapoptotic roles in some cases (Bachelor *et al.*, 2003; Lee *et al.*, 2005).

In this study, we demonstrated that sodium selenite-induced apoptosis in NB4 cells required the activity of ERK according to the following facts: blocking MEK/ERK pathway with specific inhibitors could effectively attenuate sodium selenite-induced apoptosis, while activating MEK/ERK function with

\*To whom correspondence should be addressed.  
Tel: 86-10-65296445; Fax: 86-10-65296445  
E-mail: caiminxu@yahoo.com.cn

specific activator potentiated the process. Further study showed us that this requirement for ERK activity for apoptosis induction occurred only at the early stage of apoptosis. While at the later stages, ERK showed antiapoptotic activity. These findings indicated that ERK played an essential and complex role in sodium selenite-induced apoptosis.

## Materials and Methods

**Materials.** The chemical inhibitors PD98059, U0126, SB203580 and SP600125 were purchased from Promega. Rabbit polyclonal antibodies against phospho-p44/42 MAPK (Thr202/Tyr204), phospho-p38 MAPK (Thr180/Tyr182), phospho-SAPK/JNK (Thr183/Tyr185), phospho-c-Raf (Ser338) (56A6), and phospho-p90RSK (Ser380); Rabbit monoclonal antibody against phospho-MEK1/2 (Ser217/221), mouse monoclonal antibody (7E11) against phospho-Bad (Ser112) were purchased from Cell Signaling Technology; non-phosphorylated Erk antibody was purchased from Beijing Zhongshan. All other reagents were purchased from Sigma.

**Cells and treatments.** Human acute promyelocytic leukemia-derived NB4 cells were routinely grown in RPMI 1640 supplemented with 10% (v/v) heat-inactivated fetal calf serum and 0.2% sodium bicarbonate plus antibiotics in a humidified 5% CO<sub>2</sub> atmosphere at 37°C. For all experiments, the cells were seeded at  $2 \times 10^5$  cells/ml. Stock solutions of TPA (50  $\mu$ M), PD98059 (50 mM), U0126 (10 mM), SB203580 (10 mM), SP600125 (10 mM), and LY294002 (50 mM) were prepared in dimethyl sulfoxide (DMSO). The doses used by us of 20 or 40  $\mu$ M for PD98059, 5 or 10  $\mu$ M for U0126, 10  $\mu$ M for SB203580 and 10  $\mu$ M for SP600125 were those that were proved effective in vitro in leukemic cells as documented both by ourselves and other researchers (Lunghi *et al.*, 2004; Wu *et al.*, 2004). These solutions were stored at -20°C. Stock solution of sodium selenite (10 mM) were prepared in distilled water

**Flow cytometric determination of sub-G1 cells.** After treatment with chemicals,  $1-3 \times 10^6$  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 stained with propidium iodide solution. The fluorescence intensities of 10,000 cells were measured by an EPICS XL-MCL flow cytometer (Coulter) with excitation at 488 nm and emission at 620 nm.

**Preparation of Cell Lysates and Immunoblot.** Protein samples were extracted for immunoblot assay after the treatment times indicated. The cells were washed twice with ice-cold PBS, and lysed in Cell Lysis Buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM  $\beta$ -glycerolphosphate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1  $\mu$ g/ml Leupeptin, 1 mM PMSF). After centrifugation, the cell lysates were normalized by the Bradford assay and equal amounts of protein were separated by 12% or 15% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes. The membranes were blocked with 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 horseradish 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.

## Results

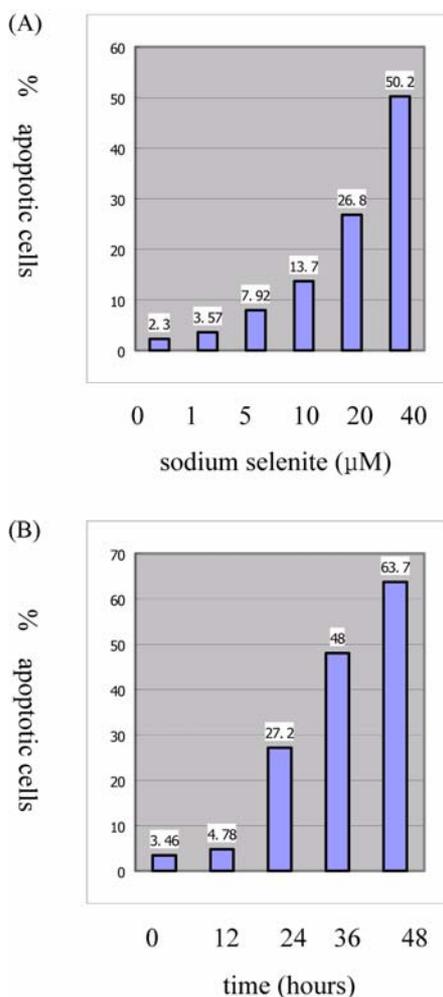
**Sodium selenite induced apoptosis in NB4 cells in a time- and dose-dependent Manner.** First, we investigated the effect of different concentrations of sodium selenite on NB4 cells. Cell cultures were treated with various doses (0, 1, 5, 10, 20, 40  $\mu$ M) of the agent for 24 h, and the frequency of apoptosis was determined by counting cells with a sub-G1 DNA content, which is a marker of apoptosis. It was observed that (Fig. 1A) sodium selenite caused apoptosis in NB4 cells in a dose-dependent manner at higher concentrations (5-40  $\mu$ M), with the treatment with 40  $\mu$ M sodium selenite for 24 h resulting in the apoptosis of greater than 50% of the cell population.

We then examined the kinetics of sodium selenite -induced apoptosis using a 20  $\mu$ M concentration. Cell cultures were treated with 20  $\mu$ M sodium selenite for a period of 12 to 48 h before the frequency of apoptosis was determined by counting cells with a sub-G1 DNA content. Data showed (Fig. 1B) that sodium selenite caused apoptosis in NB4 cells in a time-dependent manner, and that treatment with 20  $\mu$ M sodium selenite for 48 h resulted in the apoptosis of greater than 60% of the cell population.

### Effect of sodium selenite on MEK/ERK pathway activation.

To investigate the effect of sodium selenite on MEK/ERK pathway activation, we measured the phosphorylation of ERK by immunoblot analysis with whole cell lysates that were extracted from cells treated with sodium selenite (in a concentration range of 1-40  $\mu$ M) for 24 h. It's reported that activation of ERK requires phosphorylation at Thr202/Tyr204, which is used as a marker of ERK activation. (Fig. 2A) Results showed that, at lower concentrations (1-5  $\mu$ M), 24-h treatment with sodium selenite could upregulate ERK phosphorylation, while at higher concentrations (10-40  $\mu$ M) sodium selenite downregulated ERK phosphorylation. Phosphorylation of ERK1 was more sensitive to sodium selenite treatment than ERK2 and no apparent change of ERK basic level was observed. Experiments indicated that sodium selenite affected the activation of ERK in a dose-specific manner.

Further study was carried out at a 20  $\mu$ M concentration, which has been proved a physiologically tolerable concentration in our previous work. NB4 cells were exposed to sodium selenite for a period of 1 to 24 h, and the activation of MEK/ERK pathway was monitored by measuring ERK1/2 phosphorylation by immunoblot analysis. As shown in Fig.



**Fig. 1.** Sodium selenite induced apoptosis in NB4 cells in a time-dependent manner. NB4 cells were treated either with different doses of the sodium selenite for 24 h (A), or 20 μM sodium selenite for the indicated times (B). Cells were then fixed with 70% ethanol, stained with propidium iodide and assayed by a flow cytometer. (A) Sodium selenite induced apoptosis in NB4 cells in a dose-dependent manner. (B) Sodium selenite induced apoptosis in NB4 cells in a time-dependent manner.

2B, ERK1/2 phosphorylation was downregulated during apoptosis, although a slight increase of ERK1/2 phosphorylation could be observed at the earlier stages (1-3 h). We also observed that this downregulation of ERK1/2 phosphorylation continued into 48 h after treatment. These results showed that treatment with sodium selenite could downregulate ERK1/2 activation in a time-dependent manner.

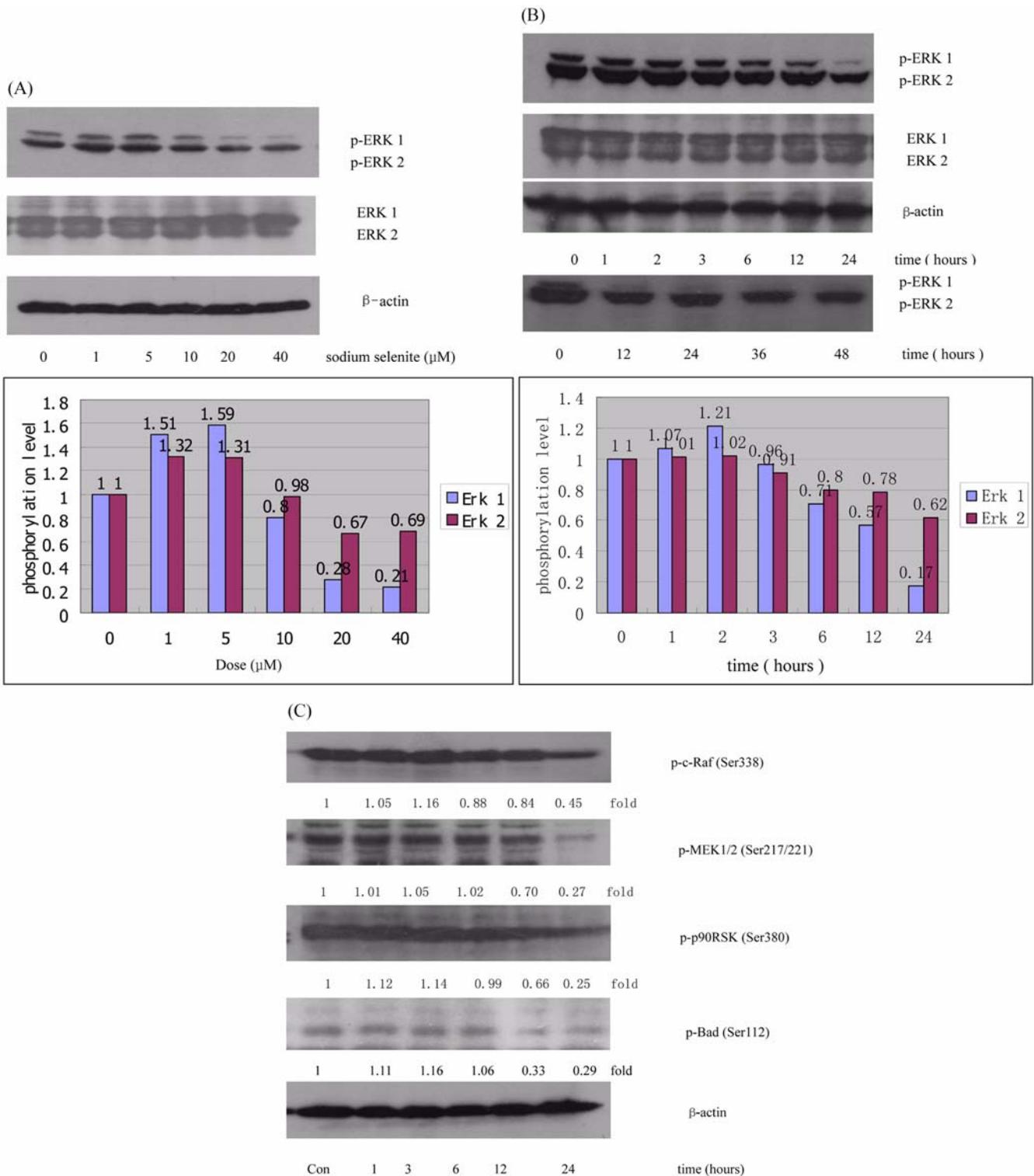
Meanwhile, activation of several other components of this pathway: Raf, MEK, Bad and p90RSK were measured by immunoblot analysis (Fig. 2C). Results showed that sodium selenite downregulated phosphorylation of Raf, MEK, Bad and p90RSK with similar kinetics. It is known that activation of Raf requires phosphorylation at Ser338, that Raf can activate MEK by phosphorylating at Ser217 and Ser 221, that

MEK can activate ERK by phosphorylating at Thr202 and Tyr204, and that p90RSK, which is an important substrate of ERK, can inactivate proapoptotic Bad protein by phosphorylating at Ser 112 (Scheid *et al.*, 1999; Ballif *et al.*, 2001; Klumpp *et al.*, 2002). All these results showed that ERK pathway was downregulated in apoptosis.

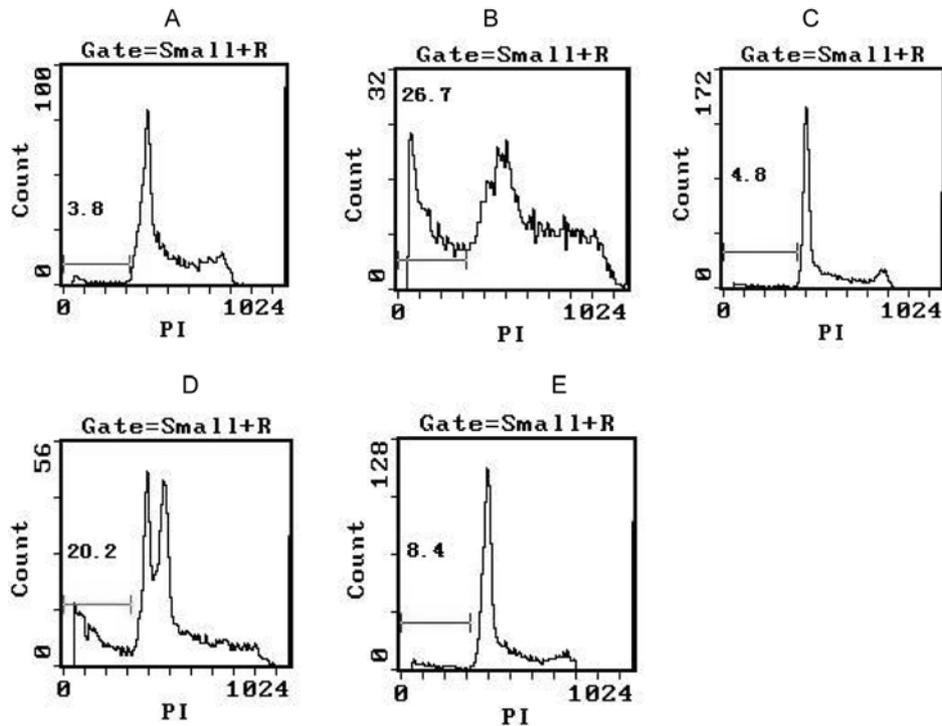
**MEK1/2 inhibitors abolished sodium selenite-induced apoptosis in NB4 cells.** To investigate the role that the MEK/ERK pathway may play in sodium selenite-induced apoptosis, PD98059 and U0126, two specific chemical inhibitors of MEK1/2 which are highly selective in their inhibition of the ERK phosphorylation/activation, were used. NB4 cells were pretreated with various doses of PD98059 or U0126 for 90 min, and then treated with 20 μM sodium selenite for 24 h, after which the apoptosis frequency was measured (Fig. 3). Compared with treatment with sodium selenite, 40 μM PD98059 alone could not induce apoptosis in NB4 cells. Pretreatment with PD98059 could protect NB4 cells from sodium selenite induced-apoptosis in a dose-dependent manner, 40 μM PD98059 almost abolished sodium selenite-induced apoptosis. Similar results were obtained in cells pretreated with U0126 through Annexin-V-PI stained flow cytometry analysis (data not shown). These results suggested that activity of MEK/ERK was required in the apoptotic process of NB4 cells caused by sodium selenite.

**The involvement of p38 MAPK and JNK in sodium selenite-induced apoptosis.** We further investigated the involvement of p38 MAPK and JNK in sodium selenite-induced apoptosis. The activation of p38 MAPK and JNK were monitored by measuring their phosphorylation status by immunoblot analysis. Results showed that sodium selenite treatment caused strikingly increased p38 MAPK activation in a time-dependent manner, while just a slight decrease of JNK activation was observed. Then specific chemical inhibitors SB203580 (for p38 MAPK) and SP600125 (for JNK) were used for further study. Pretreatment with 10 μM SB203580 only slightly delayed apoptosis induction, and pretreatment with 10 μM SP600125 slightly enhanced sodium selenite induced apoptosis.

**TPA pretreatment enhanced sodium selenite -induced Apoptosis.** If the activity of ERK plays an important role in mediating apoptosis induced by sodium selenite, then agents capable of stimulating ERK activity, when combined with sodium selenite treatment, are expected to potentiate apoptosis. To address this possibility, TPA (12-*O*-Tetradecanoylphorbol-13-acetate), which has been proved a strong activator of the ERK signaling pathway at concentrations that do not alter JNK activities (Wang *et al.*, 2000), was used. Cells were pre-incubated with 25 or 50 nM TPA for 1.5 h, followed by treatment with 20 μM sodium selenite, then the cells were evaluated for apoptosis 24 h later. (Fig. 5) When incubated alone, TPA was not toxic to NB4 cells. However, TPA-



**Fig. 2.** Effect of sodium selenite on MEK/ERK pathway activation. (A) NB4 cells were treated with different doses of sodium selenite for 24 h, whole cell lysates were then used for immunoblot with antibodies against phospho-p44/42 MAPK (Thr202/Tyr204) and non-phospho-p44/42 MAPK (Thr202/Tyr204). (B) NB4 cells were treated with 20 μM sodium selenite for the indicated times, whole cell lysates were then used for immunoblot with antibodies against phospho-p44/42 MAPK (Thr202/Tyr204) and non-phospho-p44/42 MAPK (Thr202/Tyr204). (C) NB4 cells were treated with 20 μM sodium selenite for the indicated times, then whole cell lysates were used for immunoblot with phospho-c-Raf (Ser338) (56A6) rabbit mAb, phospho-MEK1/2 (Ser217/221) antibody, phospho-p90RSK (Ser380) antibody, and phospho-Bad (Ser112) (7E11) monoclonal antibody.



**Fig. 3.** MEK1/2 Inhibitor abolished sodium selenite-induced apoptosis. NB4 cells were pretreated with PD98059 at indicated concentrations for 1.5 h, followed by a 24-h treatment with 20  $\mu$ M sodium selenite. Cells were then stained with propidium iodide and assayed with FACS to detect apoptotic cells. Cells with sub-G1 DNA content were identified as apoptotic, and their percentage against total cell population in this area was given. Results shown are representative of three independent experiments. A: Control; B: 20  $\mu$ M sodium selenite; C: 40  $\mu$ M PD98059; D: 20  $\mu$ M PD98059 + 20  $\mu$ M sodium selenite; E: 40  $\mu$ M PD98059 + 20  $\mu$ M sodium selenite.

pretreated cells were much more sensitive to sodium selenite-induced apoptosis.

It has been reported that TPA can activate both ERK and PKC, thus an experiment was carried out to make sure that TPA did enhance sodium selenite-induced apoptosis through ERK activation. MEK inhibitor PD98059 was added before TPA incubation, then NB4 cells were treated with sodium selenite for 24 h in the presence of both PD98059 and TPA. (Fig. 5F) Results showed that higher concentration of PD98059 could protect NB4 cells from apoptosis caused by the combination treatment of sodium selenite and TPA, and these results indicated that TPA did enhance sodium selenite-induced apoptosis through ERK activation.

#### **ERK played opposite roles at different stages of apoptosis.**

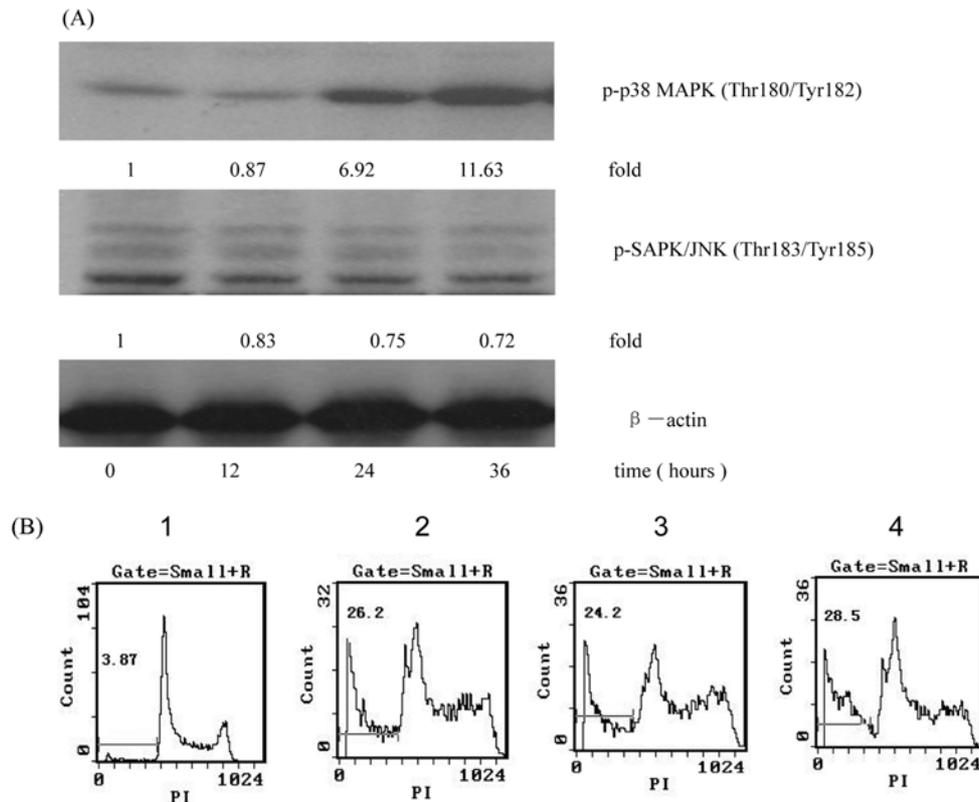
The results obtained above gave rise to a new question: if ERK activity is required for apoptosis, what is the effect of ERK downregulation on apoptosis induction, since ERK was downregulated during apoptosis. We thus carried out the following experiment: the chemical inhibitor PD98059 was added at different time points of sodium selenite treatment (1.5 h before, 6 h, 12 h, 16 h after sodium selenite exposure). After treatment with sodium selenite for 24 h, the culture were collected for FCM analysis.

To our surprise (Fig. 6), although pretreatment with PD98059

could protect NB4 cells from sodium selenite-induced apoptosis, inhibition of MEK/ERK pathway at other time points with the chemical inhibitor showed different effect on apoptosis. PD98059 added 6 h after sodium selenite exposure had little effect on apoptosis induction, PD98059 added 12 h after sodium selenite exposure potentiated the apoptotic action of sodium selenite and PD98059 added 16 h after sodium selenite exposure caused the most severe apoptosis. These results indicated that MEK/ERK pathway probably play different roles at different stages of the apoptosis. In other words, ERK activity was proapoptotic at the early stages (within 6 h) and antiapoptotic at the later stages (6-18 h).

#### **Discussion**

Selenium has been suggested as a potential anticarcinogenic agent. Recent researches show that Selenium compounds are able to induce tumor cell to apoptosis through distinct mechanisms according to cell type and compound pattern (Cheng *et al.*, 2002; Plunghi *et al.*, 2003; Wang *et al.*, 2003; Kim *et al.*, 2004). Some researchers reported that sodium selenite, at low concentrations (0.005-5  $\mu$ M), could increase cell proliferation and suppress apoptosis caused by some stimuli, while at higher concentrations (>5  $\mu$ M), could decrease



**Fig. 4.** The involvement of p38 MAPK and JNK in sodium selenite-induced apoptosis. (A) NB4 cells were treated with 20  $\mu$ M sodium selenite for the indicated times, then whole cell lysates were used for immunoblot with antibodies against phospho-p38 MAPK (Thr180/Tyr182) and phospho-SAPK/JNK (Thr183/Tyr185). (B) NB4 cells were pretreated with 10  $\mu$ M SB203580 and 10  $\mu$ M SP600125 for 1.5 h, followed by a 24-h treatment with 20  $\mu$ M sodium selenite. Cells were then stained with propidium iodide and assayed with FACS to detect apoptotic cells. Cells with sub-G1 DNA content were identified as apoptotic, and their percentage against total cell population in this area was given. Results shown are representative of three independent experiments. 1: Control; 2: 20  $\mu$ M sodium selenite; 3: 10  $\mu$ M SB203580 + 20  $\mu$ M sodium selenite; 4: 10  $\mu$ M SP600125 + 20  $\mu$ M sodium selenite.

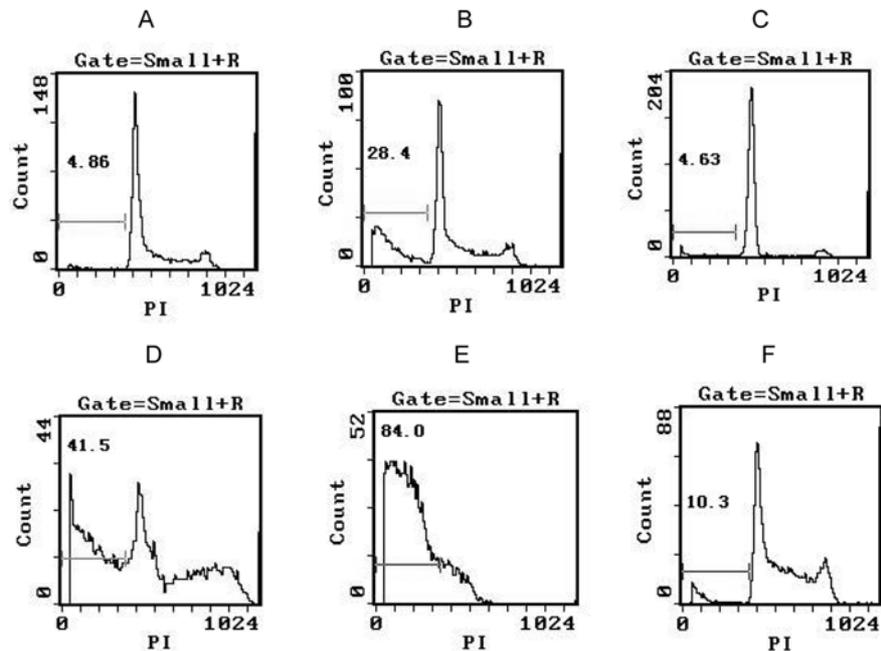
cell proliferation and cause apoptosis (Yoon *et al.*, 2001; Gopee *et al.*, 2004). Our previous study also showed that sodium selenite markedly inhibited the proliferation and induced apoptosis in NB4 cells in a dose- and time-dependent manner at higher concentrations (5-20  $\mu$ M) (Zuo *et al.*, 2004). In this study, we first showed that sodium selenite could cause apoptosis in acute promyelocytic leukemia-derived NB4 cells in dose- and time-dependent manner at higher concentrations (5-40  $\mu$ M), while no apoptosis was observed in cells treated with 1  $\mu$ M sodium selenite. However, the mechanism of this effect is not fully understood.

It is known that ERK pathway plays essential roles in mediating mitogenic and antiapoptotic signals (Platanias, 2003). Our results also showed that sodium selenite could upregulate ERK activation at lower concentrations (1-5  $\mu$ M) and downregulate ERK activation at higher concentrations (10-40  $\mu$ M). All the results mentioned above indicated that ERK activation is likely to be responsible, at least partly, for the different effects of different doses of sodium selenite on proliferation and apoptosis of NB4 cells.

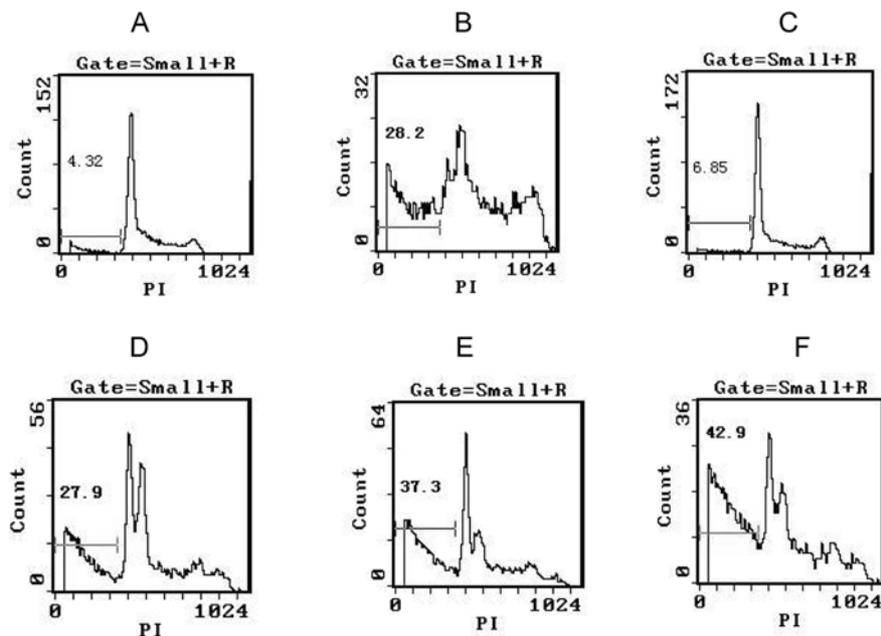
Then we demonstrated that treatment with 20  $\mu$ M sodium

selenite could downregulate the activation of Raf/MEK/ERK pathway during apoptosis, which was further confirmed by the downregulation of p90RSK phosphorylation. It is accepted that dephosphorylation of p90RSK will downregulate transcription factor CREB, which plays a critical role in cell survival through transcriptional upregulating of antiapoptotic Bcl-2 family members Bcl-2, Bcl-X<sub>L</sub>, and Mcl-1, and that dephosphorylation of Bad can activate its proapoptotic ability by dissociating Bad from 14-3-3 protein. (Bryan *et al.*, 2001).

According to the results outlined above, it is natural to think that downregulation of ERK signaling pathway contributes to the apoptosis induction through downregulation of its antiapoptotic effect such as inactivation of BAD proapoptotic protein. However to our surprise, inhibition of MEK/ERK pathway with specific chemical inhibitors abolished the apoptosis caused by sodium selenite, indicating a proapoptotic action of MEK/ERK pathway in apoptosis. Further experiment using ERK activator TPA also showed that activation of MEK/ERK could enhance sodium selenite-induced apoptosis, although elevation of MEK/ERK activation by TPA alone could not cause any apoptosis in NB4 cells. At the same time,



**Fig. 5.** TPA pretreatment enhanced sodium selenite-induced apoptosis. NB4 cells were pretreated with TPA at indicated concentrations for 1.5 h, followed by a 24-h treatment with 20  $\mu$ M sodium selenite. Cells were then stained with propidium iodide and assayed with FACS to detect apoptotic cells. Cells with sub-G1 DNA content were identified as apoptotic, and their percentage against total cell population in this area was given. Results shown are representatives of three independent experiments. A: Control; B: 20  $\mu$ M sodium selenite; C: 50 nM TPA; D: 25 nM TPA + 20  $\mu$ M sodium selenite; E: 50 nM TPA + 20  $\mu$ M sodium selenite; F: 60  $\mu$ M PD98059 + 50 nM TPA + 20  $\mu$ M sodium selenite.



**Fig. 6.** ERK played opposite roles at different stages of apoptosis. NB4 cells were treated with 20  $\mu$ M sodium selenite for 24 h; 40  $\mu$ M PD98059 was added or not at the indicated time points. Cells were then stained with propidium iodide and assayed with FACS to detect apoptotic cells. Cells with sub-G1 DNA content were identified as apoptotic, and their percentage against total cell population in this area was given. Results shown are representatives of three independent experiments. A: control; B: 20  $\mu$ M sodium selenite; C: PD98059 pretreatment for 1.5 h before sodium selenite exposure; D: PD98059 added 6 h after sodium selenite exposure; E: PD98059 added 12 h after sodium selenite exposure; F: PD98059 added 16 h after sodium selenite exposure

we showed that treatment with 20  $\mu$ M sodium selenite could markedly upregulate p38 MAPK activation and slightly downregulate JNK activation, but inhibition of both pathways showed only slight effect on apoptosis induction.

Although some researches reported that ERK could be activated and exert proapoptotic action in some types of cells under some specific stimuli (Wang *et al.*, 2000; Amra *et al.*, 2005), some other researchers also reported that inhibition of MEK/ERK pathway with PD98058 could sensitize NB4 cells to apoptosis induced by such stimulus as Arsenic trioxide (ATO) (Lunghi *et al.*, 2004), and what is more, ERK was downregulated in sodium selenite-induced apoptosis in NB4 cells. Thus a question arises: is ERK activity proapoptotic in NB4 cells, if not, what converted it into a proapoptotic factor in sodium selenite-induced apoptosis.

To obtain more information about the role that ERK may play in sodium selenite-induced apoptosis, PD98059 was added to cell cultures at different time points during sodium selenite exposure. All results showed that ERK exerted distinct effects at different stages of apoptosis. At the early stages, ERK activity was necessary for apoptosis induction, and at the later stages, ERK played an antiapoptotic role by regulating its downstream substrates. Considering that TPA-caused activation of MEK/ERK alone could not cause apoptosis, these results indicated that ERK pathway was also antiapoptotic in NB4 cells but temporarily proapoptotic at the early stage of sodium selenite-induced apoptosis.

How ERK is involved in sodium selenite induced-apoptosis remains to be elucidated. Some researchers reported ERK was activated and required for proapoptotic action of Cisplatin by controlling the accumulation of the agent in cell (Amra *et al.*, 2005). Taking these considerations and results together, it is reasonable to propose that ERK can regulate apoptosis through many distinct mechanisms which await further discovery and elucidation.

**Acknowledgments** This work was supported by Beijing Natural Science Foundation (No. 7032034), China for Doctor-Training Unite (No. 20010023029) and National Natural Science Foundation (No. 30370348)

## References

- Amran, D., Sancho, P., Fernandez, C., Esteban, D., Ramos, A. M., de Blas E, Gomez, M., Palacios, M. A. and Aller, P. (2005) Pharmacological inhibitors of extracellular signal-regulated protein kinases attenuate the apoptotic action of cisplatin in human myeloid leukemia cells via glutathione-independent reduction in intracellular drug accumulation. *Biochim. Biophys. Acta* **1743**, 269-279.
- Bachelor, M. A. and Bowden, G. T. (2003) Ultraviolet A-induced modulation of Bcl-XL by p38 MAPK in human keratinocytes. *J. Biol. Chem.* **279**, 42658-42668.
- Ballif, B. A. and Blenis, J. (2001) Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Differ.* **12**, 397-408.
- Chen, Z., Chen, G. Q., Shen, Z. X., Chen, S. J. and Wang, Z. Y. (2001) Treatment of acute promyelocytic leukemia with arsenic compounds: *in vitro* and *in vivo* studies. *Semin Hematol.* **38**, 26-36.
- Gopee, N. V., Johnson, V. J. and Sharma, R. P. (2004) Sodium selenite-Induced apoptosis in murine B-Lymphoma cells is associated with inhibition of protein kinase C- $\delta$ , nuclear factor  $\kappa$ B, and inhibitor of apoptosis protein. *Toxicol Sci.* **78**, 204-214.
- Grignani, F., Fagioli, M., Alcalay, M., Longo, L., Pandolfi, P. P., Donti, E., Biondi, A., Lo Coco, F., Grignani, F. and Pelicci, P. G. (1994) Acute promyelocytic leukemia: from genetics to treatment. *Blood* **83**, 10-25.
- Jiang, C., Wang, Z., Ganther, H. and Lu, J. (2002) Distinct effects of methylseleninic acid versus selenite on apoptosis, cell cycle, and protein kinase pathways in DU145 human prostate cancer cells. *Mol. Cancer Ther.* **1**, 1059-1066.
- Kakizuka, A., Miller, W. H. Jr., Umesono, K., Warrell, R. P. Jr., Frankel, S. R., Murty, V. V., Dmitrovsky, E. and Evans, R. M. (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.
- Kim, Y. S., Jhon, D. Y. and Lee, K. Y. (2004) Involvement of ROS and JNK1 in selenite-induced apoptosis in Chang liver cells. *Exp. Mol. Med.* **36**, 157-164.
- Klumpp, S. and Krieglstein, J. (2002) Serine/threonine protein phosphatases in apoptosis. *Curr. Opin. Pharmacol.* **2**, 458-462.
- Kyriakis, J. M. and Avruch, J. (2001) Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol. Rev.* **81**, 808-860.
- Lee, Y. J. and Shukla, S. D. (2005) Pro- and anti-apoptotic roles of c-Jun N-terminal kinase (JNK) in ethanol and acetaldehyde exposed rat hepatocytes. *Eur. J. Pharmacol.* **508**, 31-45.
- Lunghi, P., Tabilio, A., Lo-Coco, F., Pelicci, P. and Bonati, A. (2005) Arsenic trioxide (ATO) and MEK1 inhibition synergize to induce apoptosis in acute promyelocytic leukemia cells. *Leukemia.* **19**, 234-244.
- Lunghi, P., Tabilio, A., Dall'Aglio, P. P., Ridolo, E., Carlo-Stella, C., Pelicci, P. G. and Bonati A. (2003) Downmodulation of ERK activity inhibits the proliferation and induces the apoptosis of primary acute myelogenous leukemia blasts. *Leukemia.* **17**, 1783-1793.
- Patrick, L. (2004) Selenium Biochemistry and Cancer: A Review of the Literature. *Altern. Med. Rev.* **9**, 239-258.
- Platanias, L. C. (2003) Map kinase signaling pathway and hematologic malignancies. *Blood* **101**, 4667-4678.
- Scheid, M. P., Schubert, K. M. and Duronio, V. (1999) Regulation of Bad phosphorylation and association with Bcl-xL by the MAPK/Erk Kinase. *J. Biol. Chem.* **274**, 31108-31113.
- Wang, H., Yang, X., Zhang, Z. and Xu, H. (2003) Both calcium and ROS as common signals mediate Na<sub>2</sub>SeO<sub>3</sub> induced apoptosis in SW480 human colonic carcinoma cells. *J. Inorg. Biochem.* **97**, 221-230
- Wang, X., Martindale, J. L. and Holbrook, N. J. (2000) Requirement for ERK activation in cisplatin-induced apoptosis. *J. Biol. Chem.* **275**, 9435-9443.
- Wu, J., Wong, W. W., Khosravi, F., Minden, M. D. and Penn, L. Z. (2004) Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis.

- Cancer Res.* **64**, 6461-6468.
- Yoon, S. O., Kim, M. M., Park, S. J., Kim, D., Chung, J. and Chung, A. S. (2001) Selenite suppresses hydrogen peroxide-induced cell apoptosis through inhibition of ASK1/JNK and activation of PI3-K/Akt pathways. *FASEB J.* **16**, 111-113.
- Zuo, L., Li, J., Yang, Y., Wang, X., Shen, T., Xu, C. M. and Zhang, Z. N. (2004) Sodium 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.