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Sodium Selenite Inhibits Proliferation of Gastric Cancer Cells by Inducing SBP1 Expression

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Selenium is an essential trace element with an inhibitory effect on many types of human cancers, including gastric cancer. Selenium-binding protein 1 (SBP1) has been shown as a possible mediator of selenium's anti-cancer functions. Indeed, SBP1 was downregulated in gastric cancer, which is related with poor prognosis. However, the molecular mechanisms underlying the anti-tumor effects of SBP1 remain poorly understood. In this study, we aimed to assess the effects of selenium and/or SBP1 on the proliferation of gastric cancer cells. We used SGC7901 and N87 human gastric cancer cell lines and nude mice carrying subcutaneously implanted SGC7901 cells. Treatment with sodium selenite for 48 h caused the inhibition of cell proliferation and the increase in apoptosis of SGC7901 and N87 cells. Furthermore, sodium selenite increased the expression level of SBP1 and decreased the levels of nuclear factor erythroid 2-related factor 2 (Nrf2) and the Wnt pathway components and its downstream targets, including β-catenin, GSK-3β, c-myc and cyclinD1 in these cell lines. However, these effects of sodium selenite were attenuated in SGC7901 and N87 cells by knockdown of SBP1 expression. Thus, the sodium selenite-induced SBP1 expression is associated with the inhibition of cell proliferation and with the induced apoptosis. Importantly, sodium selenite treatment retarded the growth of the transplanted SGC7901 cells in nude mice, with the induction of SBP1 expression, which was associated with the decrease of Nrf2 expression and the inactivation of the Wnt/β-catenin signaling pathway. We suggest that sodium selenite may have a potential application in gastric cancer treatment.

Keywords: gastric cancer cells; Nrf2; sodium selenite; Wnt/β-catenin; xenografts
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Introduction

Gastric cancer is the second leading cause of cancer-related death in the world and occurs at very high rates in certain regions of China (Ferlay et al. 2010). Despite recent advances in the diagnosis and treatment of gastric cancer, the prognosis of individuals with an advanced stage of disease remains poor and treatment options mainly focus on surgery and chemoradiotherapy (Wagner et al. 2006). Selenium, an essential trace mineral, is considered a cancer preventive agent (Kong et al. 2011). Selenium-binding protein, which binds selenium by mechanisms that are currently unclear, is a selenium-containing protein found in mammals. The human selenium-binding protein 1 (*SBP1*, *SELENBP1* or *hSP56*) gene is located at chromosome position 1q21-22 and is the homologue of the mouse SP56 gene (Yang and Diamond 2013). SBP1 has been shown to bind selenium covalently and has been found to be the possible mediator of selenium's anticancer functions (Porat et al. 2000; Silvers et al. 2010; Huang et al. 2012). Selenium supplementation causes the increase in SBP1 expression, which affects cell viability and chemosensitivity of esophageal adenocarcinoma cells (Silvers et al. 2010). A large body of evidence has recently demonstrated the protective role of selenium against several major types of cancers including gastric cancer (Liu and Russell 2008; Lee et al. 2011). For example, several studies have reported that sodium selenite induces apoptosis in human osteosarcoma, prostate cancer and leukemia (Cao et al. 2006; Xiang et al. 2009; Chen et al. 2012). However, the precise mechanism by which sodium selenite exerts an inhibitory effect on gastric cancer is unclear.

The nuclear factor erythroid 2-related factor 2 (Nrf2), which belongs to the redox-sensitive bZIP family of transcription factors, is a master transcriptional activator of intracellular antioxidants and phase II detoxification enzymes, through the upregulation of antioxidant responsive element (ARE)-driven genes (Deng 2014). Recent

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data showed that Nrf2 expression is an independent prognostic indicator for gastric cancer, but the role of Nrf2 in gastric cancer remains incompletely understood (Hu et al. 2013). The Wnt signaling pathway has emerged as a critical regulator in various processes of embryogenesis and development, as well as pathogenesis of human diseases, including cancer (Logan and Nusse 2004). In the Wnt signaling pathway, the central event is the stabilization of cytosolic β-catenin that translocates into the nucleus to activate the expression of a variety of Wnt/β-catenin target genes, many of which have been implicated in cancer, such as c-myc and cyclin D1 (Mazieres et al. 2005).

In this study, we analyzed the effects of sodium selenite on the proliferation and apoptosis of gastric cancer SGC7901 and N87 cell lines. We also analyzed whether sodium selenite inhibited progression of gastric cancer cells transplanted in nude mice. Furthermore, the role of SBP1 in the Nrf2 expression and the Wnt/β-catenin signaling pathway was studied *in vitro* and *in vivo*.

Materials and Methods

Chemicals and cell culture

Sodium selenite was purchased from Sigma-Aldrich (St. Louis, MO, USA). The sodium selenite was dissolved in PBS and the concentrations of working solutions were 10, 20, 30, and 40 μM. Human gastric cancer cell lines (SGC7901 and N87) were obtained from the Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Cells were cultivated in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 1% (v/v) penicillin-streptomycin solution at 37°C in a humidified incubator with 5% CO_2.

RNA interference

The small interfering RNA (siRNAs) against human SBP1 (siSBP1; sense, 5′-CUU GGA GGC ACC AAG AAA UTT-3′, and antisense, 5′-AUU UCU UGG UGC CUC CAA GTT-3′) and scramble control siRNA (siControl; sense, 5′-UUC AUG UGC AUC GTT AAC UTT-3′, and antisense, 5′-ACG UUG UGC UUC CGC AGA ATT-3′) were synthesized by GenePharma Company (Shanghai, China). Transfection of siRNAs into SGC7901 and N87 cell lines was carried out with Lipofectamine 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. All cells were harvested 48 h after transfection.

Cell proliferation assay

Cells were seeded in 96-well plates at 4×10^3 cells/well overnight. Cells were treated with different concentrations of sodium selenite (0, 10, 20, 30, and 40 μM) for 0, 24, 48, 72, and 96 h. Then, 20 μl MTT (Sigma-Aldrich, 5 mg/ml in PBS) was added to each well and incubated for 4 h at 37°C. Then the medium was discarded and replaced with 100 μl/well of DMSO to dissolve the precipitate. Absorbance at 570 nm was detected using a microplate spectrophotometer (Molecular devices, VERSAmax tunable, CA, USA).

To directly measure cell proliferation, a bromodeoxyuridine (BrdU) incorporation assay was used in this study to determine the fraction of cells undergoing DNA replication, which correlates well with cell proliferation. Briefly, after the cells were treated with different concentrations of sodium selenite for 48 and 96 h, cell proliferation was determined by measuring BrdU incorporation using the BrdU Cell Proliferation ELISA kit (Roche Molecular Biochemicals, Mannheim, Germany) based on the manufacturer's instructions. The results were presented as BrdU-incorporation (%) compared to cells at 0 h.

Apoptosis assay

Apoptosis was measured by flow cytometry using the Annexin V-FITC apoptosis Detection Kit (BD Pharmingen, San Diego, CA, USA). Cells (1×10^6/well) were incubated with 30 μM of sodium selenite for 48 h. After washing, cells were resuspended in Annexin V binding buffer, and then 5 μl Annexin V-FITC and 10 μl propidium iodide (PI) were added into each well. Samples were mixed and analyzed using a fluorescence-activated cell sorter (FACS).

Western blot analysis

After SGC7901 and N87 gastric cancer cells were treated with 30 μM sodium selenite for 48 h, cells were lysed with RIPA buffer (Sigma-Aldrich). Protein was quantified using a BCA protein assay (Pierce, Rockford, IL, USA). Equal amounts of each sample protein (20 μg/lane) were separated using SDS-PAGE on a 12% polyacrylamide gel and transferred onto nitrocellulose membranes (Amersham, Little Chalfont, UK), which were blocked for 1 h at room temperature in Tris-buffered saline containing 0.1% Tween-20 and 5% skimmed milk. Next, the membranes were probed at 4°C overnight using primary antibodies: anti-Ki67 (Immunotech Inc., Westbrook, Maine, USA), anti-caspase-3 (Pharmingen, San Diego, CA, USA), anti-GSK-3β and anti-cleaved caspase-3 (Cell Signaling Technology, Danvers, MA, USA), anti-Nrf2, anti-c-myc, anti-β-actin and anti-Bax (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), anti-Bcl-2 (Cell Signaling Technology), anti-SBP1 (Sigma-Aldrich) and anti-cyclinD1 (Thermo Fisher Scientific, Rockford, IL, USA). After the membranes were washed, the appropriate horseradish peroxidase-conjugated secondary antibodies (Calbiochem, Shanghai, China) were added. The target proteins were visualized on X-ray film using an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The immunoreactive proteins were analyzed using Quantity One analysis software (Bio-Rad, Hercules, CA, USA).

Animal experiments

Animal experiments were approved by the Committee on Experimental Animals of Zhengzhou Central Hospital Affiliated to Zhengzhou University. Female BALB/c nu/nu mice (4-6 weeks old, 15-20 g) were purchased from the Laboratory Animal Center of Shanghai (Chinese Academy of Sciences, Shanghai, China). The xenografts were established by subcutaneous injection of SGC7901 cells (3×10^6 cells/200 μl/animal) on the left side of the back. The volume of the xenografts was measured with calipers every five days. Tumor volume was calculated as follows: tumor volume (mm^3) = (the longest diameter) × (the shortest diameter)^2/2. After the tumors were palpable, animals were randomly divided into two groups (n = 6 mice per group, on the 10th day). Subsequently, the mice were injected intraperitoneally with PBS or sodium selenite (3 mg/kg) every 3 days for 15 days (Shi et al. 2014). All the mice were euthanized at day 25 and the xenografts were resected for tumor weight measurement and western blot analysis.

Statistical analysis

All data were presented as the mean ± standard deviation and analyzed by SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Statistical analyses were tested using Student's *t*-test or ANOVA. $P < 0.05$ was considered statistically significant.

Results

Sodium selenite inhibits proliferation of gastric cancer cells

We initially measured the effect of sodium selenite on the viability of SGC7901 and N87 gastric cancer cells using MTT assay. Treatment with sodium selenite (10, 20, 30, or 40 μM) for 48, 72, and 96 h markedly inhibited the growth of gastric cancer cells, compared with the control group (Fig. 1A, D). Moreover, in accordance with the data of cell viability, the expression of Ki67 protein was decreased in comparison with the control group (Fig. 1B, E). Furthermore, the BrdU incorporation assay showed that incubation with sodium selenite at 10, 20, 30, or 40 μM for 48 or 96 h decreased the amount of BrdU incorporated into newly synthesized DNA (Fig. 1C, F).

Sodium selenite induces apoptosis in gastric cancer cells

We next investigated the effect of sodium selenite on apoptosis in SGC7901 and N87 cells by flow cytometry. After treatment with 30 μM sodium selenite for 48 h, the rate of apoptosis in SGC7901 and N87 cells is significantly increased in comparison with the control group (Fig. 2A, C). In addition, Western blot analyses showed that compared with the controls, the expression levels of cleaved caspase-3 and Bax were clearly increased, whereas the expression levels of Bcl-2 were decreased in both SGC7901 and N87 cells after 48 h of treatment with 30 μM sodium selenite (Fig. 2B, D).

Sodium selenite increases the expression level of SBP1

SBP1 is a selenium-associated protein shown to be downregulated in most human tumors (Diamond et al. 2015). Recently, SBP1 was identified as a tumor suppressor and a prognostic indicator of clinical outcome (Yang and Diamond 2013). To understand the role of SBP1 in the inhibitory effect of selenium on gastric cancer cells, we measured the change of SBP1 expression in SGC7901 and N87 cells treated with 30 μM sodium selenite for 48 h. The results showed that sodium selenite elevated the expression level of SBP1 in both SGC7901 and N87 cells (Fig. 3A, B).

Sodium selenite suppresses the Nrf2 and Wnt/β-catenin pathway in gastric cancer cells

We also measured the expression levels of Nrf2 and the Wnt pathway components and downstream targets, including *β*-catenin, GSK-3*β*, c-myc and cyclinD1 by Western blot analysis. In SGC7901 and N87 cells, sodium selenite treatment for 48 h substantially decreased the expression of these five proteins compared with control cells without sodium selenite treatment (Fig. 3A, B). These results suggest that sodium selenite may inhibit cell proliferation and induce apoptosis at least partially by downregulating Nrf2 expression and inhibiting the Wnt/*β*-catenin signaling pathway.

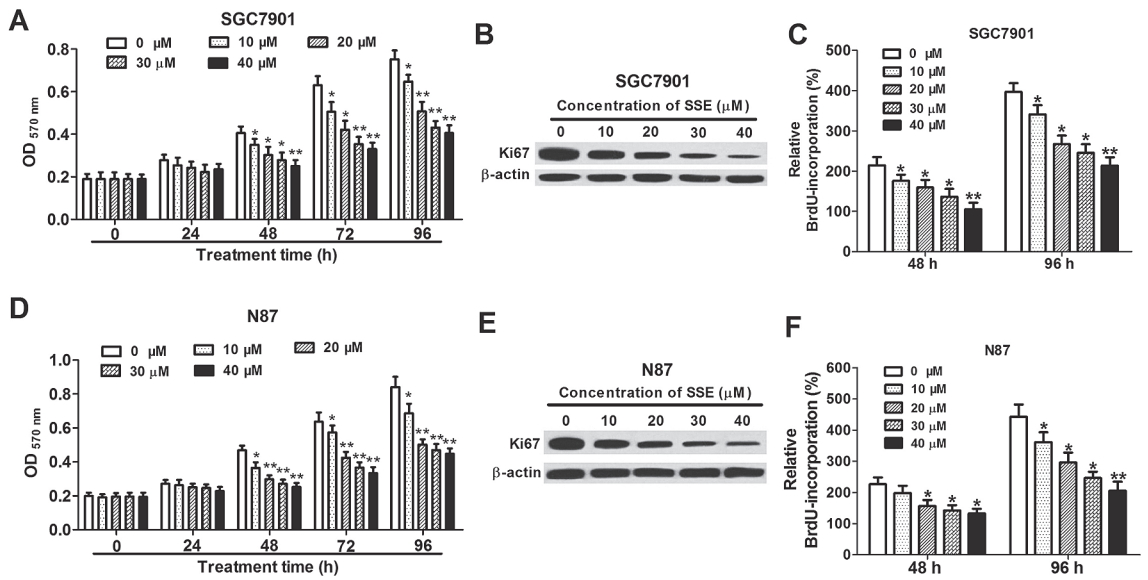

Fig. 1. Effect of sodium selenite on growth of gastric cancer cells.
Sodium selenite dose-dependently decreased the viable cell number of SGC7901 cells (A) and N87 cells (D). After the cells were exposed to different concentrations of sodium selenite (0, 10, 20, 30 and 40 μM) for 0, 24, 48, 72, and 96 h, the light absorption of each group was detected at 570 nm. After the cells were treated with different concentrations of sodium selenite (0, 10, 20, 30, and 40 μM) for 96 h, protein levels of Ki67 in SGC7901 cells (B) and N87 cells (E) were detected by Western blot analysis. The BrdU incorporation assay was used to detect the proliferation of SGC7901 cells (C) and N87 cells (F). The results are presented as BrdU-incorporation (%) compared to cells at 0 h. SSE represents sodium selenite. All experiments were performed in triplicate. Data are expressed as mean ± SD. *$P < 0.05$, *$P < 0.01$.

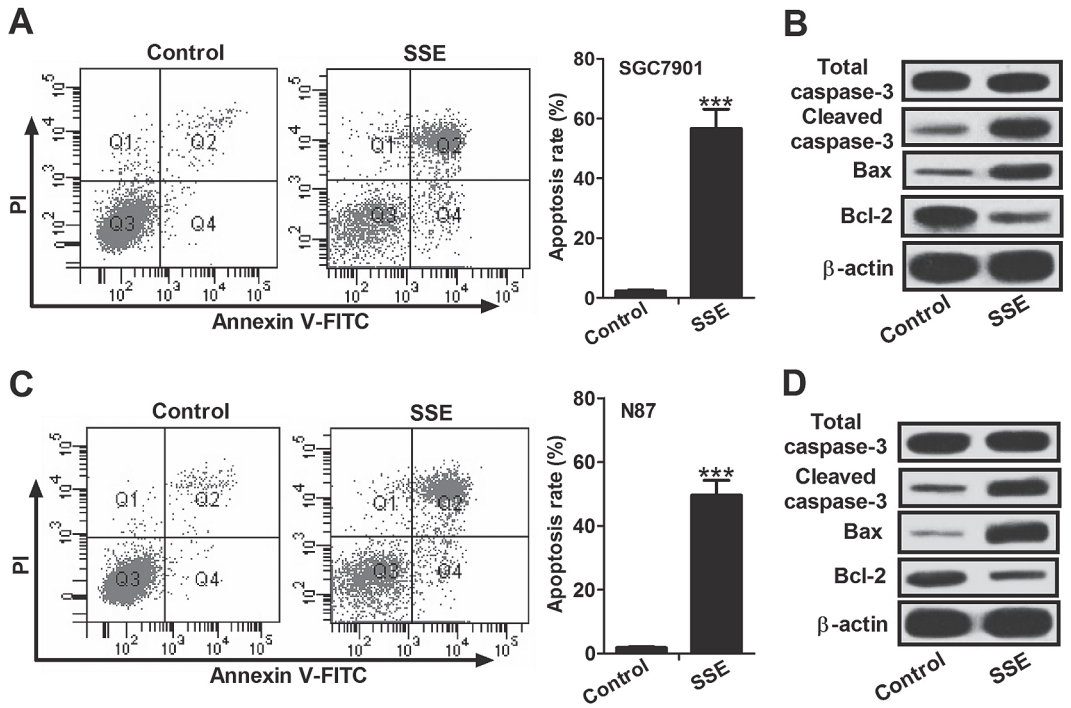

Fig. 2. Effect of sodium selenite on apoptosis of gastric cancer cells.
Sodium selenite induced apoptosis of SGC7901 cells (A) and N87 cells (C). After the cells were treated with 30 μM of sodium selenite for 48 h, the relative rate of apoptosis of each group was detected by flow cytometry. Protein levels of apoptosis-related proteins in SGC7901 (B) and N87 cells (D) were detected by Western blot analysis. SSE represents sodium selenite. The results were reproducible in three independent experiments. Data are expressed as mean ± SD. ***$P < 0.001$.

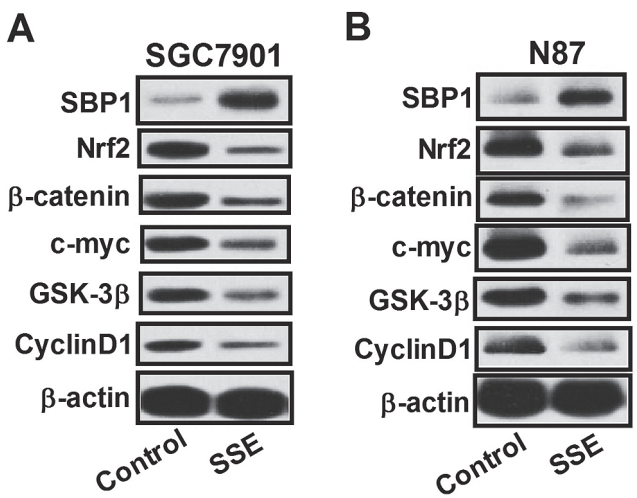

Fig. 3. Effect of sodium selenite on SBP1 and Nrf2 expression and the Wnt/β-catenin pathway in gastric cancer cells.
After cells were treated with 30 μM sodium selenite for 48 h, the relative expression levels of SBP1, Nrf2, β-catenin, c-myc, GSK-3β, and cyclinD1 were detected by Western blot analysis. Sodium selenite elevated SBP1 expression, but decreased the expression levels of Nrf2, β-catenin, c-myc, GSK-3β, and cyclinD1 in SGC7901 cells (A) and N87 cells (B). SSE represents sodium selenite. Data are from three independent assays.

Knockdown of SBP1 reverses the inhibitory effect of sodium selenite on gastric cancer cells

To further understand whether SBP1 was involved in the growth-inhibitory effect of sodium selenite on gastric cancer cells, we performed the SBP1 silencing experiments. When SGC7901 and N87 cells were treated with siRNA against SBP1 and 30 μM of sodium selenite for 48 h, the apoptosis rates were significantly decreased in comparison with control cells (Fig. 4A, B). We next investigated the relationship between the increase in SBP1 and the decrease of Nrf2 and Wnt/ß-catenin signaling components. After SBP1 levels had been reduced by siRNA and the SGC7901 and N87 cells had been treated with sodium selenite for 48 h, the level of SBP1 is decreased and the expression of Nrf2 and Wnt/ß-catenin signaling components was increased (Fig. 4C, D). The results indicate that sodium selenite suppresses the Nrf2 expression and the Wnt/β-catenin pathway in gastric cancer cells, and that SBP1 plays an important role in this process.

Sodium selenite ameliorates the progression of transplanted gastric cancer cells

Because SGC7901 cells were more susceptible to the anti-tumor effect of sodium selenite compared to N87 cells (see Fig. 2A, C), we used SGC7901 cells for the *in vivo* study. The volume and the weight of xenografts were measured after treatment with sodium selenite in nude mice (Fig. 5A, B). The results show that tumor growth was

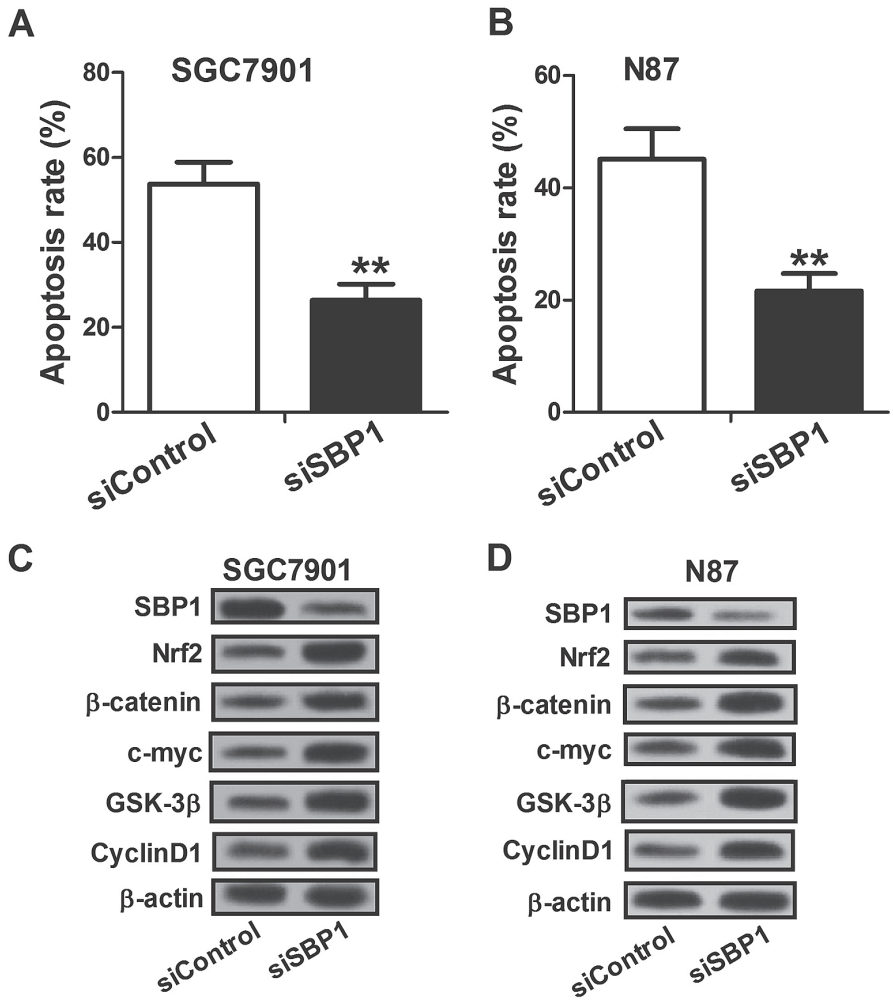

Fig. 4. SBP1 silencing reverses the inhibitory effect of sodium selenite on gastric cancer cells.
After reducing the protein level of SBP1 using siRNA, cells were treated with sodium selenite and the rates of apoptosis were determined by flow cytometry for SGC7901 (A) and N87 cells (B). After SBP1 knockdown and sodium selenite treatment, protein levels of SBP1, Nrf2 and Wnt/ß-catenin signaling components in SGC7901 (C) and N87 cells (D) were determined by Western blot analysis. Data are expressed as mean ± SD. $^{**}P < 0.01$.

inhibited in the sodium selenite-treated group compared with the control group from the 10th day to the 25th day. In addition, sodium selenite significantly reduced the average tumor weight. Importantly, Western blot analysis of the transplanted SGC7901 cells revealed that sodium selenite treatment decreased the expression levels of Ki67, Bcl-2, Nrf2, *β*-catenin, c-myc, GSK-3*β* and cyclinD1 (Fig. 5C, D), while it increased the expression levels of cleaved caspase-3, Bax and SBP1 (Fig. 5C).

Discussion

In the current study, we showed that sodium selenite inhibited proliferation of SGC7901 and N87 cells in a dose-dependent manner and induced apoptosis. The SGC7901 xenografts in nude mice were also ameliorated by sodium selenite treatment. Moreover, the Nrf2 expression and Wnt/ß-catenin signaling components were decreased both *in vitro* and *in vivo*.

Selenium compounds, including sodium selenite, selenomethionine, and monomethylated Se, have been considered to possess cancer prevention activity (Combs 2001; Kong et al. 2011; Yan and DeMars 2012). Results from a large number of clinical and epidemiological studies have shown that selenium compounds inhibit the growth of some cancers such as prostate, colon and lung tumors, and the anti-cancer effects involved underlying mechanisms such as anti-oxidation, apoptosis and immunological response (Yu et al. 1997; Bjelakovic et al. 2004; Kong et al. 2011). The induction of apoptosis is an important mechanism for the inhibition of cancer growth (Wong 2011). In the present study, after SGC7901 and N87 cells were treated with sodium selenite, cell proliferation was markedly decreased. Sodium selenite treatment also resulted in an increased rate of apoptosis of SGC7901 and N87 cells, which is also supported by the change in expression of apoptosis-related proteins. These findings indicate that sodium selenite eliminated cancer cells through inhibition of cell proliferation and induction of apoptosis.

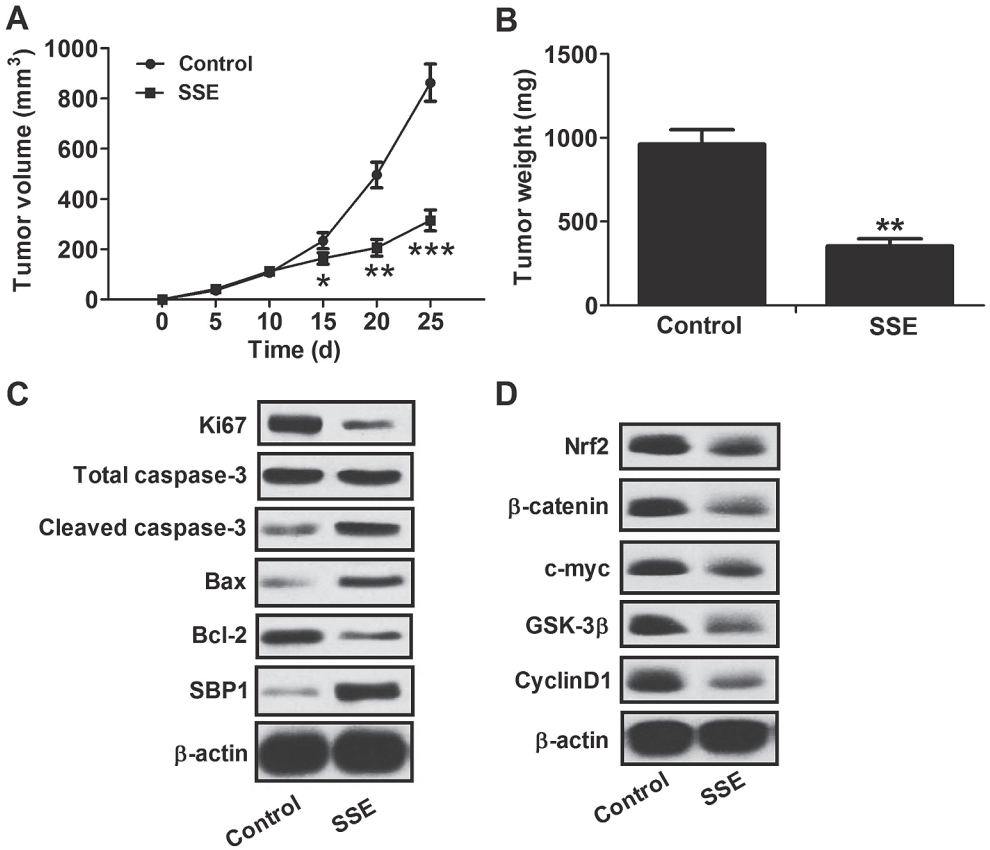

Fig. 5. Effect of sodium selenite on SGC7901 xenografts in nude mice.
(A) Sodium selenite inhibits tumor volume. After the tumors were palpable, animals were randomly divided into two groups (n = 6 mice per group, at day 10). Subsequently, the mice were injected intraperitoneally with PBS or sodium selenite (3 mg/kg) every 3 days for 15 days. (B) Sodium selenite treatment decreases tumor weight in comparison with the control group. (C) Sodium selenite decreases the protein levels of Ki67 and Bcl-2, but increases the expression of cleaved caspase-3, Bax and SBP1 in xenografts. (D) Sodium selenite inhibits the expression of Nrf2, β-catenin, c-myc, GSK-3β and cyclinD1 in xenografts. SSE represents sodium selenite. The data were derived from three independent experiments. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001.

SBP1, a selenium-containing protein, has been reported in a variety of human malignancies and the low-level expression of SBP1 is postulated to play a crucial role in cancer development, growth and progression (Diamond et al. 2015). Compared with normal tissue, downregulation of SBP1 expression has been observed in several cancer types, such as gastric carcinoma, prostate, colon and ovarian cancer (Xia et al. 2011; Giusti et al. 2012; Yang and Diamond 2013). Previous data showed that SBP1 suppression promoted gastric cancer cell proliferation and cell migration, while SBP1 overexpression was associated with anti-cancer activities (Zhang et al. 2013). In our study, administration of sodium selenite increased the SBP1 expression in both gastric cancer cells and xenografted tumors. In addition, knockdown of SBP1 reversed the inhibitory effect of sodium selenite on gastric cancer, indicating that SBP1 is required for the anti-tumor effect of sodium selenite.

Many human cancers are associated with the Nrf2-dependent defensive system. Lung cancer patients with upregulation of Nrf2 have lower survival rates (Merikallio et al. 2012). Inhibition of Nrf2 in cervical carcinoma cells reduced tumor growth and elevated sensitivity to chemotherapy (Ma et al. 2012). Our data showed that sodium selenite treatment considerably downregulated the expression of Nrf2 in both SGC7901 and N87 cell lines, as well as in xenografts. These results suggest that sodium selenite may inhibit the progression of gastric cancer through the Nrf2 pathway. In addition, the expression levels of β-catenin, GSK-3β, c-myc and cyclinD1 were downregulated both *in vitro* and *in vivo* by administration of sodium selenite, indicating the inhibition of the Wnt/β-catenin signaling pathway.

In conclusion, sodium selenite induces SBP1 expression in gastric cancer cells *in vitro* and *in vivo*. The induction of SBP1 expression is responsible for anti-cancer activities towards gastric cancer probably by inhibition of Nrf2 activity and the Wnt/β-catenin signaling pathway. Our findings suggest that sodium selenite may have a potential application in gastric cancer treatment.

Conflict of Interest

The authors declare no conflict of interest.

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