

Published in final edited form as:

Int J Cancer. 2010 July 1; 127(1): 32–42. doi:10.1002/ijc.25029.

Tumor inhibition by sodium selenite is associated with activation of c-Jun NH2-terminal kinase 1 and suppression of β -catenin signaling

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Abstract

Epidemiological and clinical studies suggest that an increased intake of dietary selenium significantly reduces overall cancer risk, but the anti-cancer mechanism of selenium is not clear. In the present study, we fed intestinal cancer mouse model *Muc2/p21* double mutant mice with a selenium-enriched (sodium selenite) diet for 12 weeks or 24 weeks, and found that sodium selenite significantly inhibited intestinal tumor formation in these animals ($p < 0.01$), which was associated with phosphorylation of JNK1 and suppression of β -catenin and COX2. *In vitro* studies showed that sodium selenite promoted cell apoptosis and inhibited cell proliferation in human colon cancer cell lines HCT116 and SW620. These effects were dose- and time course- dependent, and were also linked to an increase of JNK1 phosphorylation and suppression of β -catenin signaling. Reduced JNK1 expression by small RNA interference abrogated sufficient activation of JNK1 by sodium selenite, leading to reduced inhibition of the β -catenin signaling, resulting in reduced efficacy of inhibiting cell proliferation. Taken together, our data demonstrate that sodium selenite inhibits intestinal carcinogenesis *in vivo* and *in vitro* through activating JNK1 and suppressing β -catenin signaling, a novel anti-cancer mechanism of selenium.

Keywords

selenium; JNK1; β -catenin; intestinal cancer

Introduction

Selenium is an important trace element that is involved in different physiological functions of human body. Epidemiological and clinical studies reported that inadequate status of selenium increased risk of cancer. Basic research and clinical trials strongly support the protective role of selenium against various types of cancer, the effect was most pronounced in prostate and colorectal cancers 1–7. Unfortunately, a recent report of the Selenium and Vitamin E Cancer Prevention Trial (SELECT) demonstrated that selenium or vitamin E,

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alone or in combination did not prevent prostate cancer and secondary cancers (including colorectal cancer) 8. However, the failure of one form of selenium does not disprove of other forms of selenium. In addition, the efficacy of selenium against cancer will also depend on the form of selenium and the dosage, organ site specificity of carcinogenesis, and on the baseline of selenium status and genotypes (e.g. polymorphisms) of glutathione peroxidase 1, a selenium enzyme, of the subjects. The anticancer effects of selenium has been postulated to link to inhibition of cell proliferation and induction of apoptosis through different signaling pathways, particularly the anti-oxidative and anti-inflammatory effects mediated through the activity of selenoenzymes 7. However, the targets and underlying mechanism of anticancer action by selenium are largely unknown.

Wnt/ β -catenin signal pathway plays a critical role in embryonic development, tissue homeostasis and carcinogenesis 9-11. Oncogenic activation of Wnt/ β -catenin signaling pathway by mutation of adenomatous polyposis coli (APC) or/and CTNNB1 interacts with TCF/LEF transcription factors that activate transcription of genes, such as c-myc and cyclin D1, and increases prevalence of many kinds of human carcinogenesis, especially colorectal cancer 12-13. Therefore, targeting β -catenin signaling will be an effective approach for colorectal cancer prevention. Indeed, non-steroidal anti-inflammatory drugs (e.g. aspirin, sulindac, celecoxib, rofecoxib), vitamins (e.g. vitamin A, D and their derivatives), have shown their anticancer effects through inhibiting Wnt/ β -catenin signal pathway 14-16. In addition, sulindac inhibits intestinal tumor formation by activating c-Jun NH2-terminal kinase 1 (JNK1) 17, a member of mitogen-activated protein kinase (MAPK) family, which plays a critical role in the regulation of cell proliferation, differentiation and apoptosis 18-20. Recently we demonstrated that activated JNK1 interacts with and downregulates β -catenin signaling 21. Therefore, JNK1 and Wnt/ β -catenin signaling could be promising targets for chemoprevention, chemotherapy and further anticancer drug discovery.

Using the *Muc2/p21* mouse model of intestinal cancer 22, 23 and colon cancer cell lines, we found that a selenium-enriched (sodium selenite) diet significantly inhibited intestinal tumorigenesis, and that sodium selenite promoted colon cancer cell apoptosis and inhibited cell proliferation, by activating JNK1 and suppressing β -catenin signaling.

Materials and methods

Animal study and mouse intestinal epithelial cell isolation

As described previously 23, *Muc2*^{-/-} mice (C57/BL6 background) 22 were mated with *p21*^{-/-} mice (C57/BL6 background) 24 to generate *Muc2*^{-/-}, *p21*^{+/-} double mutant mice 23, in which more intestinal tumors (including colorectal tumors) were expected. After weaning, the *Muc2/p21* mice were randomly grouped and fed an AIN-76 control diet (fat 5%, from corn oil), a Western-style high-risk diet (fat 20%), as reported recently 25, 26, or the Western-style diet supplemented with 0.00004% (4.0 ppm) sodium selenite (a 20 fold increase in dietary selenium level compared to that in the Western-style diet) for 12 weeks or 24 weeks, respectively. All diets were made by the Research Diets, Inc. (New Brunswick, NJ). At the endpoint, the animals were sacrificed, and tumorigenesis was evaluated as described 23. Intestinal epithelial cells from normal mucosa were isolated by incubating with 15 mM EDTA at 37°C for 20 minutes, as we described recently 18. The cell pellets were lysed with RIPA lysis buffer immediately or stored at -80°C for immunoblotting analysis. All animal studies were conducted in the University of Illinois at Chicago with an approval by the Animal Care and Use Committee at the University of Illinois at Chicago.

Cell lines and cell culture

Human colon cancer cell lines SW620 (wild-type β -catenin) and HCT116 (mutant β -catenin) were purchased from ATCC (American Type Culture Collection, Manassas, VA) and were maintained in MEM or McCoy's 5A medium, respectively. The medium was supplemented with 10% (v/v) fetal bovine serum (FBS), 1 \times antibiotic/ antimycotic (1 \times 10⁵ U/L streptomycin, 1 \times 10⁵ U/L penicillin, and 0.27 μ mol/L amphotericin B). All cell lines were cultured in humidified incubator at 37 °C with 5% CO₂. Sodium selenite (Sigma, St. Louis, MO) was dissolved in phosphate buffer solution (PBS) and stored at -20°C at 1mol/L stock solution.

Cell proliferation assay

1 \times 10⁴ cells were seeded in each well of a 96-well plate and incubated overnight. The medium was removed. 1 \times 10⁻⁴L of the medium with a final concentration of sodium selenite from physiological dosage (0, 25, 50, 100, 250, 500 and 1000 nmol/L) to pharmaceutical dosage (2.5, 5.0, 7.5, 10, 12.5 and 15 μ mol/L) was added to each well, PBS was used as a control. Three independent experiments were performed. After 24, 48 and 72 h exposure to sodium selenite, cell proliferation was determined by MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) according to the manufacturer's protocol (CellTiter 96 Non-Radioactive Cell Proliferation Assay Kit, Promega Corporation, Madison, WI).

Apoptosis analysis

6 \times 10⁵ cells were seeded in 6-well plates and incubated overnight until 40–50% confluency was reached. Cells were treated with different dosages of sodium selenite. PBS was used as a control. The cells were harvested at 24, 48, and 72 h, washed with cold PBS, then fixed with 80% ethanol for 8 h at 4 °C, followed by staining with propidium iodide buffer (0.075 mmol/L propidium iodide, 0.1% sodium citrate, and 0.1% TritonX-100) for 3 h at 4 °C. 10,000 cells were analyzed for apoptosis using a Becton Dickinson FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA). The percentage of apoptotic cells was quantified using Cell Quest software.

Immunoblotting analysis

Sodium selenite treated cells were harvested at various time points. The cell pellets were lysed with RIPA (radio immunoprecipitation assay) lysis buffer (50 mmol/L Tris-HCl, pH 8.0, with 150 mmol/L sodium chloride, 1.0% Igepal CA-630 (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate). 30 μ g of protein was loaded and separated in 12% SDS-PAGE gels and transferred to Immun-Blot PVDF membranes (Bio-Rad Laboratories). The following primary antibodies were used to probe the alterations of protein: JNK1, phosphorylated-JNK (p-JNK), β -catenin, cyclin D1 (Santa Cruz Biotechnology, Santa Cruz, CA), Caspase 3, cleaved caspase 3 (Cell Signaling Technology, Danvers, MA), CDK4 (BD PharMingen, San Diego, CA), cmyc (Sigma, St. Louis, MO), COX2 (Cayman Chemical, Ann Arbor, MI). All secondary antibodies against rabbit, goat or mouse IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Signal was detected by enhanced chemoluminescence techniques (Amersham Life Science, Piscataway, NJ). β -actin (Sigma, St. Louis, MO) was used as loading control. Signal quantification was obtained using Quantity One software (Bio-Rad Laboratories, Hercules, CA) and normalized to β -actin. The ratio is presented.

Transfection and luciferase reporter assays

To examine the effect of sodium selenite on β -catenin/TCF transcriptional signaling, cells were plated in 24-well plates at a density of 2 \times 10⁵ cells/well and incubated overnight. The

following day, cells were transiently transfected with β -catenin-TCF luciferase reporter construct TOPFlash which contains multiple optimal TCF/LEF (lymphocyte enhancing factor) binding sites that induce transcription of a luciferase reporter gene when activated by β -catenin, or FOPFlash which contains mutant and inactivated TCF/LEF binding sites, respectively (Upstate Biotechnology, Lake Placid, NY). To correct the differences caused by transfection efficiency, cells were co-transfected with a Renilla reporter plasmid using LipofectAMINE 2000 according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). 6 h after transfection, the medium was removed and the cells were treated with fresh medium supplemented with 5 μ mol/L of sodium selenite for 24, 48 and 72h, or treated with 0, 1, 2.5, 5 μ mol/L of sodium selenite for 48h. Cells were washed with cold PBS and lysed with passive lysis buffer (PLB) (Promega, Madison, WI). Luciferase activity was measured using the Dual Luciferase Report Assay System (Promega Corporation, Madison, WI). Lysate firefly luciferase values were normalized to Renilla luciferase activity. These experiments were triplicated.

Immunocytochemical staining

Human osteosarcoma cells U2OS grown on a chamber slide (BD Biosciences, San Jose, CA) were treated with 5 μ mol/L of sodium selenite or no sodium selenite MEM medium as a control. Forty-eight hours after treatment, cells were washed with cold PBS, fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min and rendered permeable by further treatment with 0.2% Triton X-100 for 5 min, blocked with 10% normal goat serum, and probed with goat anti- β -catenin polyclonal antibody (sc-1496; Santa Cruz Biotechnology, Santa Cruz, CA). Detection was with biotinylated anti-goat IgG, followed by incubation with avidin-biotin complex (Vector Laboratories, Burlingame, CA) and substrate 3', 5'-diaminobenzidine, followed with hematoxylin counterstaining. These experiments were triplicated.

Small Interfering RNA Experiments

Targeted small interfering RNA for human JNK1 (si-JNK1)(Sigma, St. Louis, MO) (sequence of si-JNK1 was 5'-GGGAUUUGUUAUCCAAAAU-3') was transfected into the human colon cancer cell line HCT116. 24 h after transfection, the cells were treated with 5 μ mol/L of sodium selenite for 48 h. The small interfering RNA for human green fluorescence protein (si-GFP) (Sigma, St. Louis, MO) was used as control. Cell proliferation was determined by MTS, as described above.

Results

Selenium-enriched diet inhibited the added effects of western style diet on tumorigenesis of *Muc2/p21* mice

To elucidate chemopreventive effects and the mechanisms of selenium *in vivo*, we fed the *Muc2/p21* mice with either a western-style high-risk diet (high fat and low calcium and vitamin D) or the western-style diet supplemented with sodium selenite for 12 weeks or 24 weeks after weaning. After 12 weeks of feeding, the western style high-risk diet (high fat and low calcium and vitamin D) was additive to the genetic mutation of the *Muc2* and *p21* genes to augment intestinal tumor formation at an average of 2.1 tumors per mouse (2.1 \pm 0.7, mean \pm SD, 9 mice) and tumor incidence of 100% (11/11) in *Muc2*^{-/-}*p21*^{+/-} mice, compared to about 1 tumor per mouse (12 mice) and tumor incidence of 78% (7/9) in the *Muc2*^{-/-}*p21*^{+/-} mice fed with AIN-76A control diet. However, intestinal tumorigenesis driven by the western-style diet was reversed by sodium selenite supplementation, reducing tumor numbers to 1.6 per mouse and tumor incidence to 83% (10/12) (Fig.1A).

After 24 weeks of feeding, similar to our previous report 23, the *Muc2*^{-/-},*p21*^{+/-} mice on AIN-76A control diet developed 1.9 intestinal tumors (1.9±0.2) per mouse, but the tumor number was increased to 4.3 (4.3±0.6) per mouse with feeding the Western-style high-risk diet (Fig. 1A) (p=0.003). However, selenium-supplemented Western-style diet dramatically inhibited intestinal tumorigenesis, in terms of a significant reduction of tumor frequency from 4.3 to 1.9 per mouse (1.9±0.4) (p=0.008) (Fig. 1A).

To elucidate the underlying mechanisms of tumor inhibition activity of sodium selenite, we isolated mouse small intestinal epithelial cells from the mice that were on diet for 24 weeks, analyzed protein alterations by Western blotting, and found that tumor inhibition by sodium selenite was strongly associated with the activation of JNK1 (phosphorylated JNK1, p-JNK1) and suppression of β -catenin in mouse intestinal epithelial cells (Fig. 1B). Interestingly, inhibition of COX2 by sodium selenite was also seen in mouse intestinal epithelial cells (Fig. 1B).

Sodium selenite inhibited cell proliferation and promoted apoptosis in colon cancer cells

To elucidate the anti-cancer activities of sodium selenite and the underlying mechanism *in vitro*, we treated human colon cancer cell lines HCT116 and SW620 with physiological dosages (0, 25, 50, 100, 250, 500 and 1000 nmol/L) and pharmaceutical dosages (2.5, 5, 7.5, 10, 12.5 and 15 μ mol/L) of sodium selenite for 48h, respectively, and found that the physiological range of sodium selenite did not affect colon cancer cell proliferation (data not shown), however, the pharmaceutical dosage of sodium selenite dramatically inhibited cell growth, and cell growth inhibition was in a dose-dependent manner in both cell lines (Fig. 2A, C). We therefore decided to use the pharmaceutical dosage of sodium selenite for the rest of our study. To investigate duration effect, cells were treated with 5 μ mol/L of sodium selenite for 12, 24, 48, 72, 96 and 120 h, respectively. As shown in Fig. 2B and 2D, sodium selenite inhibited cell proliferation in a time-dependent manner and its anticancer ability can be extended to more than five days, indicating that a low dosage and a long term intake of sodium selenite supplemental may be used as an approach for the chemoprevention and chemotherapy of colon cancer.

We also found that sodium selenite caused cell cycle arrest – S/G2 phase increased about 1.2 fold in both HCT116 and SW620 cell lines after 24 h and about 1.8 fold in both cell lines after 48 h treatment of 5 μ mol/L of sodium selenite, respectively, which is consistent with previous reports 27-30.

To determine whether sodium selenite has the ability to induce apoptosis in HCT116 and SW620 cells, we used P.I. staining cooperated with flow cytometry scanning to analyze apoptosis in both cell lines with treatment of sodium selenite at the final concentration of 2.5, 5 and 7.5 μ mol/L for 48 h. As shown in Fig. 3A and 3D, a lower dosage of sodium selenite (2.5 μ mol/L) could induce apoptosis, and a higher dosage from 5 μ mol/L significantly induced apoptosis in both colon cancer cell lines, indicating that the induction of apoptosis by sodium selenite was dosage-dependent. We then treated the HCT116 and SW620 cells with 5 μ mol/L of sodium selenite for 24, 48 and 72 h, respectively, and found that sodium selenite-induced apoptosis in both cell lines was in a time-dependent manner (Fig. 3B and 3E). The longer both cell lines were treated with sodium selenite, the more cells underwent apoptosis. To further confirm the induction of apoptosis, western-blot was performed and an increased cleavage of Caspase 3 was seen in both colon cancer cell lines after 48 and 72 h treatment of 5 μ mol/L of sodium selenite (Fig. 3C and 3F).

Sodium selenite inhibited β -catenin signaling in colon cancer cells

β -catenin signaling pathway plays critical roles in carcinogenesis and is a promising target for colorectal cancer prevention and chemotherapy. Thus, we determined whether sodium selenite-mediated inhibition of cell proliferation and induction of apoptosis was associated with β -catenin signaling. As shown in figure 4, β -catenin was indeed inhibited by sodium selenite in both HCT116 and SW620 cell lines, and the inhibition of β -catenin occurred in a dose- and time- dependent manner. Downstream targets of β -catenin, such as cyclin D1, CDK4 and c-myc, were also suppressed by sodium selenite (Fig. 4A-4D).

To further determine whether the suppression of β -catenin expression by sodium selenite corresponded to the reduction of β -catenin/TCF transcriptional activity, the two colon cancer cell lines were transiently co-transfected with the TCF/LEF luciferase reporter construct (TOPFlash) or mutant TCF/LEF luciferase reporter construct (FOPFlash) and Renilla reporter plasmid, respectively. Sodium selenite significantly reduced β -catenin/TCF/LEF luciferase activity (TOPFlash) in both cell lines, compared to FopFlash control (Fig. 5). The reduction of β -catenin transcriptional activities occurred furthermore in a dosage-dependent manner (Fig. 5A, 5C). Additional, sodium selenite-mediated reduction of β -catenin/TCF/LEF transcriptional activity occurred in a time-dependent manner (Fig. 5B, 5D). The reduction of β -catenin/TCF transcriptional activity could be affected by the reduction of β -catenin expression, which was consistent with the reduced expression of β -catenin, c-myc, cyclin D1 and CDK4 as shown in figure 4.

β -catenin activation, i.e., translocation into the nuclei, is an important event in promoting cell growth and carcinogenesis. To determine whether selenium inhibits of β -catenin nuclear translocation, in a pilot experiment, we treated human osteosarcoma cell line U2OS with 5 μ mol/L of sodium selenite for 48 hours. Immunocytochemical staining showed reduction of nuclear β -catenin expression as well as cytoplasmic expression by sodium selenite, compared to the control (Fig. 6), indicating selenium could cause β -catenin translocation from nuclei into cytoplasm in which β -catenin was degraded via other mechanisms, such as activation JNK1, as we recently reported that activated JNK1 interacts with and inhibits β -catenin expression 21.

JNK1 played an important role in sodium selenite-mediated inhibition of cell proliferation and suppression of β -catenin signaling

Previous studies have shown that JNK1 plays a critical role in intestinal cell maturation, including apoptosis, differentiation and proliferation 18. Our recent data also showed that the JNK1 interacts with β -catenin and downregulates β -catenin signaling *in vivo* and *in vitro* 21. Therefore, we elucidated whether the JNK1 was involved in selenium-mediated promotion of apoptosis and suppression of cell proliferation and β -catenin signaling in colon cancer cells. In both colon cancer cell lines HCT116 and SW620, phosphorylation of JNK1 (an active form of JNK1) was increased by 3.2 or 1.5 fold, respectively, corresponding with a 20% to 60% of inhibition of β -catenin and its targets (i.e. c-myc, cyclin D1 and CDK4) (Fig. 4). These effects of sodium selenite were in dose- and time-dependent manners, indicating that JNK1 was functioning in the effects of sodium selenite. Taken above, one could explain that selenium activates JNK1, triggering apoptosis (Fig.3), then the activated JNK1 inhibited β -catenin, resulting in cell growth inhibition (Fig.2).

To determine whether JNK1 was required for sodium selenite-mediated inhibition of cell proliferation and suppression of β -catenin signaling, we used targeted human JNK1 small interfering RNA (siRNA) to knockdown JNK1 expression and treated HCT116 cells with 5 μ mol/L sodium selenite for 48 h. Our previous study demonstrated that this siRNA (si-JNK1) was efficient to knockdown JNK expression and abrogated butyrate-induced

apoptosis 18. We found that cell proliferation was significantly inhibited (Fig. 7A) and the β -catenin protein levels were dramatically suppressed (Fig. 7B) in GFP-siRNA control transfected cells. However, knockdown of JNK1 by siRNA attenuated selenium-mediated inhibition of cell proliferation (Fig. 7A), because the activation of JNK1 was not induced as much as in the control cells (Fig. 7B). Consequently, the suppression of β -catenin, c-myc, CDK4 and cyclin-D1 expression in JNK1 siRNA transfected cells by sodium selenite was much less than those in the cells without si-JNK1 transfection (Fig. 7B).

Discussion

Several randomized controlled trials have demonstrated significant cancer prevention of selenium in colorectal cancer 3, 31-33. The milestone was cancer prevention by selenium supplementation conducted by Clark and his colleagues 3, although the SELECT trial was failed 8, in part due to the formulation of selenium (L-selenomethionine in SELECT trial v.s. selenized yeast in Clark's trial) and others addressed earlier. In some other studies and our current *in vitro* and *in vivo* studies, selenium showed effective inhibition of colorectal carcinogenesis. Moreover, we also found that sodium selenite alone or in combination with sulindac were effective in preventing intestinal tumor formation in the Apc/p21 mice (Bi and Yang, unpublished data). Therefore, selenium is still a promising chemopreventive agent. However, some issues should be considered for human use of selenium for cancer prevention, such as selection of population with determination of selenium status and glutathione peroxidase 1 genotypes, different dosage and formulation, etc.

It has been reported that anti-cancer activity of selenium was associated with multiple mechanisms, such as anti-oxidation 34, 35, anti-inflammation and/or suppression of β -catenin by 1,4-phenylene bis(methylene) selenocyanate 7, 36, 37, and increasing phosphorylation of mitogen-activated protein kinase (MAPK) in prostate cancer cells by methylseleninic acid 38, 39. But we found that tumor inhibition by sodium selenite in colorectal cancer was linked to the phosphorylation of JNK1 and consequent inhibition of β -catenin and its transcriptional targets c-myc, cyclin D1 and CDK4, leading to apoptosis induction and cell proliferation inhibition.

It has been known that JNK1 plays important roles in the regulation of cell proliferation, differentiation and apoptosis in response to cellular stress (e.g. cytokines and ultraviolet irradiation) and chemopreventive agents (e.g. sulindac). Our data strongly suggest that JNK1 plays a critical role in selenium-mediated chemoprevention of colorectal cancer – the activation of JNK1 might be one of the key factors through which selenium inhibited cell proliferation and induced apoptosis in colorectal cancer cells and prevented intestinal cancer formation in *Muc2/p21* mouse model of colorectal cancer. However, lack of JNK1 abrogated inhibition of cell proliferation by selenium, which proves the necessity of JNK1 in selenium-mediated tumor inhibition. Therefore, this report is the first to reveal a novel mechanism of selenium in cancer chemoprevention.

Increasing evidence shows that there is crosstalk between MAPK and Wnt signaling through other pathways, such as Akt, p38 and p53. But most recently we found that the MAPK JNK1 interacts with Wnt/ β -catenin signaling and negatively regulates β -catenin signaling through GSK3 β and the proteasome pathway 21. We found that activation of JNK1 dramatically inhibited β -catenin expression and transcriptional activities, but loss of JNK1 upregulated β -catenin signaling in HEK293T cells and human colon cancer cells. Most impressively, mice with JNK1 deficiency spontaneously developed intestinal tumors, which were linked to upregulation of β -catenin, resulting in increase of cell proliferation and decrease of cell differentiation and apoptosis in intestinal mucosa and tumor tissues 18. Here we show that phosphorylation of JNK1 stimulated by sodium selenite correlates with

downregulation of β -catenin in translational and transcriptional level, further supporting our observation that JNK1 suppresses β -catenin.

Activation and nuclear accumulation of β -catenin is known as a key event of transformation and carcinogenesis in colon cancer, therefore, targeting β -catenin has been a promising strategy for cancer prevention and therapy. Herein again, we found that sodium selenite inhibited β -catenin expression and transcription, rescued nuclear expression. It will be very interesting to investigate whether it was a direct target of selenium or a subsequence of activation of JNK1 by sodium selenite or both. Studying this will improve our understanding of the chemopreventive mechanisms by selenium.

We recently reported that intestinal tumorigenesis in the *Muc2*^{-/-} was associated with chronic intestinal inflammation, in terms of elevation of several cytokines and COX2 levels in *Muc2*^{-/-} mice 40. These results led us to determine whether sodium selenite functions in inhibiting inflammation since sodium selenite significantly prevented intestinal tumor formation (Fig. 1A). Interestingly, we found that COX2 expression was almost completely eliminated in sodium selenite-fed *Muc2*^{-/-} mouse intestinal epithelial cells (Fig. 1B). However, whether sodium selenite affected other cytokines and/or MAPK JNK1 regulates COX2 as seen in the β -catenin signaling cascade is still under investigation.

Studies from our group and others have suggested several potential regulations among JNK1, β -catenin and COX2, such as regulation of COX2 by JNK1 in MEFs 41, 42, regulation of COX2 by β -catenin in *Apc*^{+/-} mice, regulation of β -catenin by COX2 in *Apc*/*Muc2* mice 40, and regulation of β -catenin by PGE2 *in vitro* 43, 44. However, none of these regulators is well defined and it is important to be elucidated, which will provide important evidence of the regulations among JNK1, β -catenin and COX2, providing a rational for developing chemopreventive strategies of colorectal cancer.

In summary, our data demonstrate that sodium selenite is effective to promote apoptosis and inhibit proliferation, and to eventually inhibit intestinal carcinogenesis by targeting JNK1 and β -catenin signaling pathway, which provides more evidence of the anti-cancer bioactivity of selenium and improved our understanding of the mechanism of selenium-mediated chemoprevention and chemotherapy in colorectal cancer.

Acknowledgments

We would like to thank Drs. Anna Velcich and Leonard Augenlicht (Albert Einstein Cancer Center, Bronx, NY) for providing *Muc2*^{-/-} mice and support of experimental design and data interpretation, thank Dr. Philip Leder (Howard Hughes Medical Institute, Harvard University Medical School, Boston, MA) for providing *p21*^{-/-} mice, and thanks Dr. Deming Gou (University of Illinois at Chicago, Chicago, IL) for providing reagents. Thanks to Dr. Nicole M Pohl (University of Illinois at Chicago, Chicago, IL) for critical reading and editing this manuscript and thanks to Lindsay Gallagher (University of Illinois, Chicago, IL) for technical assistance.

Grant support: This work was supported in part by the National Cancer Institute grant (CA112081 to W.Y.) and the American Institute for Cancer Research grant (05A121 to W.Y.). Fang W is a recipient of the State Scholarship Fund of China (2007-3020).

Abbreviations

JNK1	c-Jun NH2-terminal kinase 1
APC	adenomatous polyposis coli
MAPK	mitogen-activated protein kinase
TCF	T cell factor

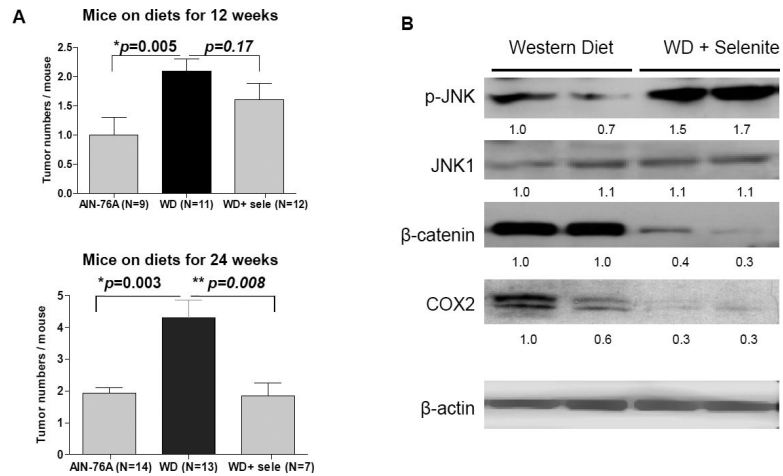
LEF	lymphoid enhancer factor
COX2	cyclooxygenase 2
siRNA	small interfering RNA

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**Figure 1.**

The Western-style diet (WD) accelerated mouse intestinal tumorigenesis, in comparison with the AIN-76A diet, but the Western-style diet supplemented with sodium selenite inhibited intestinal tumor formation in the *Muc2*^{-/-},*p21*^{+/-} mice for 12 or 24 weeks (A), which occurred through activating JNK1 (JNK1 phosphorylation, p-JNK) and suppressing β -catenin and COX2 in intestinal epithelial cells from normal mucosa (B). β -actin was used as internal control. The image signal was quantified and normalized to β -actin. The ratio was presented. WD, western-style diet; WD+Sele, western-style diet supplemented with sodium selenite. N, number of animals studied in each group.

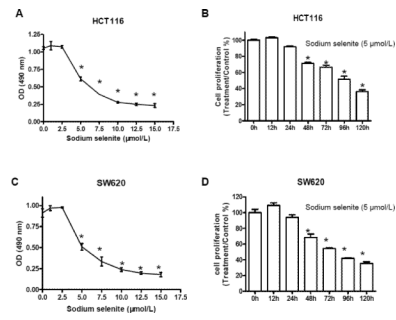


Figure 2. Sodium selenite inhibited cell proliferation in human colon cancer lines HCT116 and SW620. A and C, cell proliferation inhibition by sodium selenite occurred in a dosage dependent manner (48h); B and D, inhibition of cell proliferation by sodium selenite was time-dependent (5 μmol/L). * $p < 0.01$, compared to the control group (0 μmol/L, or 0 h, respectively). These experiments were triplicated independently.

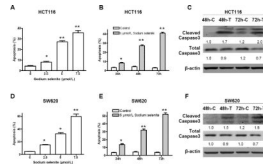


Figure 3.

Sodium selenite promoted apoptosis in colon cancer lines HCT116 and SW620. A and D, apoptosis induction by sodium selenite was in a dosage dependent manner (48h); B and E, apoptosis induction by sodium selenite was time-dependent (5 μ mol/L); C and F, increase of cleaved Caspase 3, a biomarker of apoptosis, was observed in both colon cancer cell lines after 48 h and 72 h treatment of 5 μ mol/L of sodium selenite, while the total caspases 3 was slightly reduced. “C” referred as “control”, and “T” referred as “sodium selenite treatment”. β -actin was used as loading control. The image signal was quantified and normalized to β -actin. The ratio was presented. These experiments were triplicated independently. (* $p < 0.05$, ** $p < 0.01$, compared to control, respectively).

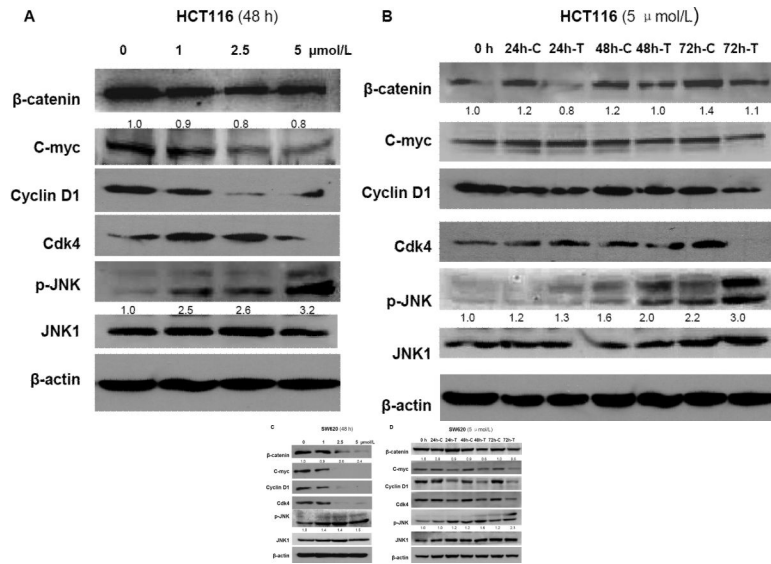


Figure 4. Sodium selenite activated JNK1 (increase of phosphorylated JNK1, p-JNK1) and inhibited β-catenin signaling in dose- and time-dependent manner in colon cancer cells HCT116 (A and B) and SW620 (C and D), respectively. “C” referred as “control”, and “T” referred as “sodium selenite treatment”. β-actin was used as loading control. The image signal of p-JNK1 and β-catenin was quantified and normalized to β-actin. The ratio was presented. These experiments were triplicated independently.

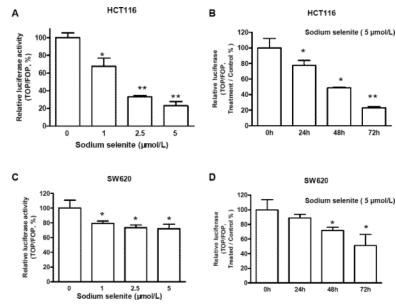


Figure 5.

Sodium selenite inhibited β -catenin/TCF4 transcriptional activities in dose- and time-dependent manner in colon cancer cells HCT116 (A and B) and SW620 (C and D). As described in “Materials and Methods”, colon cancer cells were co-transfected with TOPFlash (TOP) or FOPFlash (FOP) and with Renilla reporter plasmid using LipofectAMINE 2000. 6 h after transfection, the medium was removed and the cells were treated with fresh medium supplemented with 5 μ mol/L of sodium selenite for 24 , 48 h and 72h, or treated with 0, 1, 2.5 ,5 μ mol/L of sodium selenite for 48h. PBS was used as control. The presented data were the results from three independent luciferase activity assays (*p<0.05, **p<0.01, compared to control, respectively.)

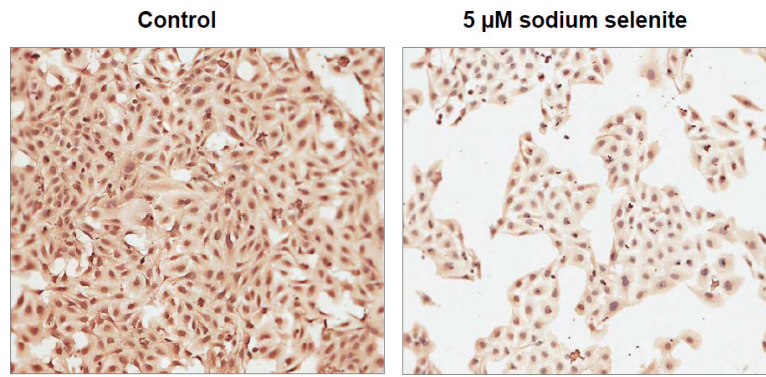


Figure 6. Sodium selenite inhibited nuclear β -catenin expression as well as cytoplasmic expression in human osteosarcoma cells after 48 h treatment of 5 μ mol/L of sodium selenite.

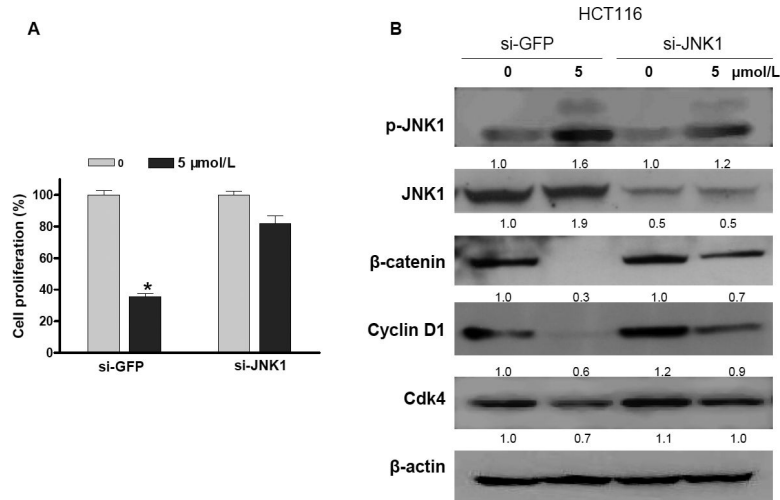


Figure 7.

Cell growth inhibition by sodium selenite was JNK1 dependent in colon cancer cells HCT116. Knockdown of JNK1 by siRNA abrogated sodium selenite-mediated inhibition of cell growth (A) and suppression of the expression of β -catenin signaling pathway (B) (48 h). si-GFP was a small interfering RNA targeting human green fluorescence protein (GFP) and was used as mock control; si-JNK1 was a small interfering RNA targeting human JNK1. The knockdown efficiency was confirmed by western blotting. These experiments were triplicated independently. The image signal was quantified and normalized to β -actin. The ratio was presented right below the image. (* $p < 0.01$, compared to the "0")