Selenium Compounds Activate Early Barriers of Tumorigenesis*§

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Selenium chemoprevention by apoptosis has been well studied, but it is not clear whether selenium can activate early barriers of tumorigenesis, namely senescence and DNA damage response. To test this hypothesis, we treated normal and cancerous cells with a gradient concentration of sodium selenite, methylseleninic acid and methylselenocysteine for 48 h, followed by a recovery of 1–7 days. Here we show that selenium compounds at doses of ≤LD₅₀ can induce cellular senescence, as evidenced by the expression of senescence-associated β-galactosidase and 5-bromo-2-deoxyuridine incorporation, in normal but not cancerous cells. In response to clastogens, the ataxia telangiectasia mutated (ATM) protein is rapidly activated, which in turn initiates a cascade of DNA damage response. We found that the ATM pathway is activated by the selenium compounds, and the kinase activity is required for the selenium-induced senescence response. Pretreatment of the MRC-5 non-cancerous cells with the antioxidant N-acetylcysteine or 2,2,6,6-tetramethylpiperidine-1-oxyl suppresses the selenium-induced ATM activation and senescence. Taken together, the results suggest a novel role of selenium in the activation of early tumorigenesis barriers specific in non-cancerous cells, whereby selenium induces an ATM-dependent senescence response that depends on reactive oxygen species.

Genome instability is a hallmark of carcinogenesis. Recent advances suggest that major barriers of human tumorigenesis at the early stage include DNA damage response and senescence (1–3), both of which involve ATM activation. Heritable mutations in ATM cause ataxia telangiectasia, a genomic instability syndrome characterized by cancer predisposition, neurodegeneration, and premature aging. In response to DNA damage, the ATM kinase is rapidly activated and mediates multiple downstream pathways, resulting in DNA damage checkpoint response and repair. ATM pathway activation in humans requires ATM phosphorylation at Ser-1981 (4). On the other hand, cellular senescence, a form of cell cycle withdrawal, can limit the proliferation of cells with persistent genomic instability. In the MRC-5 diploid fibroblasts expressing mos, the oncogene-induced senescence can be suppressed by inhibition of ATM, suggesting that ATM plays a major role linking pathways of senescence and DNA damage response during early tumorigenesis (2).

Selenium is an essential trace mineral widely distributed in inorganic forms in soil and in organic forms in certain foods. The Nutritional Prevention of Cancer Trial conducted in the United States concluded that daily selenium intake at a supra-nutritional level significantly decreases risks of cancer, and the prevention is most successful for prostate, lung, and colon cancers (5, 6). Furthermore, a role of selenium in preventing cancer in patients with prostatic intraepithelial neoplasia has been inferred (7, 8). However, the recent Selenium and Vitamin E Cancer Prevention Trial concluded that selenium supplementation alone or in combination with vitamin E does not prevent prostate cancer risks in the cohort of relatively healthy men (9). Differences in selenium formulation and the body selenium status prior to entering the trials may explain the seeming discrepancies between these two cohorts of clinical studies (5, 6, 9, 10). Nonetheless, a consensus drawn from the two trials is that selenium supplementation prevents cancer risks only in men entering the trial with suboptimal levels of body selenium. Thus, it is necessary to elucidate the mechanism of tumorigenesis suppression offered by selenium.

Metabolites of selenium compounds have been shown to induce reactive oxygen species (ROS), which in turn can induce oxidative modifications and breaks on DNA. Previous studies have focused on selenium-induced stress responses in various cultured cancer cells, from which it is suggested that much of the role of selenium in cancer prevention is attributable to ROS-induced apoptosis or cell cycle arrest in cancer cells (11–13). Consistent with this notion, it has been shown that selenium-induced apoptosis in cancer cells can be suppressed by antioxidants (14) and is p53-dependent (15). Furthermore, selenium can sensitize cancer cells to other apoptotic inducers, including TRAIL and doxorubicin (11, 16).

Available evidence from the literature has not provided a full understanding of selenium in tumorigenesis or the linkage of selenium metabolites to genomic maintenance (17). It is known that selenium-induced oxidative stress or DNA damage can

* This work was supported in part by an award from the General Research Board, the University of Maryland at College Park (to W.-H. C.), and United States Department of Agriculture Agricultural Research Service Project 1235-51530-052-00D (to N. W. S.).

‡ The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables 1–4 and Figs. 1–7.

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2 The abbreviations used are: ROS, reactive oxygen species; SA-β-galactosidase, senescence-associated β-galactosidase; BrdUrd, 5-bromo-2-deoxyuridine; MSeA, methylseleninic acid; MSeC, methylselenocysteine; NAC, N-acetylcysteine; PBS, phosphate-buffered saline; DAPI, 4′,6-diamidino-2-phenylindole; pATM, phosphorylated ATM; SASP, senescence-associated secretory phenotype; Tempo, 2,2,6,6-tetramethylpiperidine-1-oxyl.
lead to apoptosis in some cancer cells (18); however, the roles of selenium in cellular senescence have not been studied. To investigate additional barriers of tumorigenesis elicited by selenium, we treated the MRC-5 normal lung fibroblasts, the CRL-1790 normal colon fibroblasts, and the PC-3 prostate and HCT116 colon cancer cells with selenium compounds of organic and inorganic origins in a series of studies. We employed a number of senescence markers, including the measurement of senescence-associated β-galactosidase (SA-β-galactosidase) expression and levels of the pulse-labeled 5-bromo-2-deoxyuridine (BrdUrd), together with investigating the ATM-mediated DNA damage response pathway, to explore a role of selenium in the activation of early tumorigenesis barriers. Our results indicate that selenium induces an ATM-dependent senescence response via redox regulation in non-cancerous but not in cancerous cells, suggesting a novel mechanism of selenium in countering tumorigenesis.

**MATERIALS AND METHODS**

**Cells and Culture Conditions**—The MRC-5 normal lung fibroblasts (Coriell Institute, Camden, NJ), CRL-1790 normal colon fibroblasts, and PC-3 prostate cancer cells (ATCC, Manassas, VA) were maintained in Eagle’s minimum essential medium (Mediatech Inc., Herndon, VA) supplemented with 15% heat-inactivated fetal bovine serum, 1 ng/ml essential amino acid, 1 ng/ml nonessential amino acid, 1 ng/ml vitamins, and 100 units/ml penicillin and streptomycin at 37 °C in a 5% CO₂ incubator. The HCT116 colon cancer cells (ATCC, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s medium (Mediatech Inc.) supplemented with 10% heat-inactivated fetal bovine serum and 100 units/ml penicillin and streptomycin. Sodium selenite (Na₂SeO₃), methylselenolycysteine (MSeC), N-acetylcysteine (NAC), and 2,2,6,6-tetramethylpiperidine-1-oxyl (Tempo) were obtained from Sigma and were dissolved in phosphate-buffered saline (PBS). KU55933 and NU7026 were purchased from Tocris (Ellisville, MO) and were dissolved in DMSO. The cells were treated with the selenium compounds for 48 h and washed once with PBS, followed by a 1–7-day recovery.

**Senescence Assays**—We detected SA-βgalactosidase by using a senescence detection kit (MBL Co. Ltd., Woburn, MA) according to the manufacturer’s instructions. Briefly, cells were seeded onto 24-well plates with a density of 5,000 cells/well and treated with the selenium compounds for 48 h, followed by a 7-day recovery. The cells were washed once in PBS, fixed at room temperature for 15 min, washed three times in PBS, and stained in the staining solution containing X-gal at a concentration of 1 mg/ml for 8 h. Cells were then overlaid with 70% glycerol and observed under a light microscope.

We performed a BrdUrd incorporation assay to indicate status of DNA replication. Cells were cultured on coverslips and incubated with Na₂SeO₃ (1 μM), MSeA (1 μM) and MSeC (50 μM) for 48 h followed by a 7-day recovery. Cells were pulse-labeled with BrdUrd (10 μM) for 1 h, followed by washing three times in PBS and fixation in 4% paraformaldehyde (in PBS) for 30 min. The fixed cells were then permeabilized in HCl (0.1 N) containing pepsin (100 μg/ml) for 30 min at 37 °C. DNA was denatured by HCl (1.5 N) for 15 min and then by sodium borate (0.1 M) for 5 min. After washing three times in PBS, the cells were incubated in an anti-BrdUrd antibody conjugated with fluorescein isothiocyanate for 1 h according to the manufacturer’s instructions (BD Pharmingen). A drop of ProLong® gold antifade reagent with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen) was added to a slide, and then coverslips were mounted on the slides. We employed filter cube set 49 (excitation, 365 nm; filter, 395 nm; emission, 445 nm) and set 38 (excitation, 470 nm; filter, 495 nm; emission, 525 nm) for visualization of DAPI and fluorescein isothiocyanate, respectively, through a Zeiss AxioObserver 100 fluorescence microscope (Carl Zeiss, Oberkochen, Germany). All of the photomicrographs were taken using the same magnification scale (×200) and exposure time (400 ms) within 10 min to avoid autofading of the fluorescence signal.

**Immunofluorescence**—Cells were seeded onto coverslips and incubated with Na₂SeO₃ (1–2 μM), MSeA (1–2 μM), and MSeC (50–100 μM) for 2 days, followed by a 0–7-day recovery. The cells were washed three times in PBS, fixed in 4% paraformaldehyde (in PBS) for 30 min, permeabilized in 90% methanol for 10 min at −20 °C, and washed three times in PBS (5 min/wash). The cells were then incubated in 0.3% Triton-X for 15 min and washed three times in PBS. Cells were blocked in 3% bovine serum albumin in PBS for 30 min, followed by overnight incubation at 4 °C with antibodies against H2AX (1:2000; Abcam, Cambridge, MA), γH2AX (phospho-H2AX on Ser-139, 1:2000; Abcam), ATM (1:2000; Epitomics, Burlingame, CA), and anti-phospho-ATM on Ser-181 (pATM Ser-181, 1:2000; Rockland, Gilbertsville, PA). After washing, the cells were incubated with secondary antibodies conjugated with fluorescence dyes (Alexa 488 goat anti-rabbit IgG and Alexa 594 goat anti-mouse IgG, Invitrogen) for 1.5 h at room temperature. A drop of ProLong® Gold antifade reagent containing DAPI (Invitrogen) was added to a slide, and the coverslips were mounted onto the slide. The slides were then placed under a Zeiss AxioObserver 100 fluorescence microscope (Carl Zeiss) for image acquisition. The fluorescence signals were visualized by using filter cube set 49 for DAPI (excitation 365 nm, filter 395 nm, and emission 445 nm), filter cube set 38 for GFP (excitation 470 nm, filter 495 nm, and emission 525 nm), and filter cube set 43 for DsRed (excitation 560 nm, filter 570 nm, and emission 605 nm). All of the photos were taken using the same magnification scale (×630) and exposure time (100 ms for DAPI, 400 ms for GFP, and 800 ms for DsRed), followed by deconvolution according to the manufacturer’s instruction. The ATM phosphorylated at Ser-181 (pATM Ser-181) and γH2AX focus-positive cells are defined as those containing at least five foci (19). Five pictures were randomly taken from each slide. All experiments were run in duplication and performed a minimum of three times.

**Flow Cytometry Assay**—Cells were cultured in 10-cm² dishes and treated with 1–2 μM Na₂SeO₃, 1–2 μM MSeA, and 50–100 μM MSeC for 48 h, followed by a 1–7-day recovery. The cell monolayers were rinsed with PBS, incubated in trypsin/EDTA, resuspended in ice-cold PBS, and fixed and stored in 70% ethanol at −20 °C until analysis. Prior to the analysis, cells were stained in propidium iodide (20 μg/ml) containing RNase. The DNA was then analyzed by a FACScalibur cytometer with the CELLQuest program (BD Biosciences). ModFit LT (version 3.0, 2003).
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Verity Software House, Topsham, ME) was applied for cell cycle analysis on overlaid histograms.

Cell Survival Assay—Cells were seeded at a concentration of 10,000 MRC-5 cells, 5,000 PC-3 cells, and 5,000 HCT 116 cells per 3.5-mm well, incubated with the selenium compounds at the aforementioned dosages for 48 h, followed by a 7-day recovery. On day 7, the cells were washed once with PBS and then trypsinized. Cells were transferred to the INCYTO C-Chip disposable hemacytometers (SKC Inc., Eighty Four, PA), and the cell number was counted under a light microscope.

Statistical Analysis—The data were analyzed by using the SAS version 9.0 software (SAS Institute Inc., Cary, NC). A two-tailed Student's t test was applied to determine statistical significance between the treatments and the control. The linear regression was also computed to confirm the senescence response to gradient concentrations of selenium in Fig. 1 (p < 0.0001).

RESULTS

Senescence Is Induced by Selenium Compounds in MRC-5 and CRL-1790 Non-cancerous Cells but Not in PC-3 or HCT 116 Cancerous Cells—To determine whether or not selenium can counteract tumorigenesis through cancer barriers other than the well studied apoptosis, we first assessed senescence phenotypes after cellular exposure to the selenium compounds. MRC-5, CRL-1790, PC-3, and HCT 116 cells were treated with Na$_2$SeO$_3$ (0.1–10 μM), MSeA (0.1–10 μM), and MSeC (5–500 μM) for 2 days, followed by a 7-day recovery in normal medium in the absence of the selenium treatment. The cells were then subjected to the detection of SA-β-galactosidase, a hallmark of cellular senescence. The selenium treatment resulted in the expression of SA-β-galactosidase in the non-cancerous MRC-5 and CRL-1790 cells in a dose-dependent manner (by regression analysis, p < 0.0001) from a concentration as low as 0.1 μM (Na$_2$SeO$_3$ or MSeA) and 5 μM (MSeC) (Fig. 1, A–C, and supplemental Figs. 1 and 2). Strikingly, there was no detectable SA-β-galactosidase in the cancerous PC-3 and HCT 116 cells (Fig. 1, A–C, and supplemental Figs. 3 and 4). Thus, treatment of MRC-5 cells with the selenium compounds resulted in DNA replication suppression, a feature of cellular senescence. Taken together, selenium compounds induce cellular senescence in the non-cancerous but not in the cancerous cells.

We next performed survival assays to determine the cellular sensitivity to the selenium compounds and estimate their respective LD$_{50}$ values. Results from the cell proliferation analysis showed that PC-3 and HCT 116 cancerous cells are more resistant than MRC-5 cells to treatment with Na$_2$SeO$_3$ (Fig. 2A), MSeA (Fig. 2B), and MSeC (Fig. 2C) at day 7. When the two cancerous cell lines were treated with doses of selenium equivalent to their respective LD$_{50}$ range, there was no SA-β-galactosidase detected (Fig. 1, A–C, and supplemental Figs. 3 and 4). In contrast, treatment of MRC-5 cells...
with selenium at doses comparable with their LD_{50} resulted in significant SAβ-galactosidase expression. Moreover, there was no increase in sub-G1 cell population 3 days after cellular exposure to the selenium compounds (supplemental Table 1), suggesting that an apoptotic cell death pathway is not activated. The results further support the observation that the selenium-induced senescence occurs specifically in non-cancerous cells; cancerous cells are deficient in selenium-induced senescence.

Selenium Treatment Results in ATM Ser-1981 Phosphorylation and γH2AX Formation in Non-cancerous Cells—ATM activation and DNA damage response occur in senescent cells at an early stage of tumorigenesis (1–3, 18). Because pATM Ser–1981 is known as a marker of ATM pathway activation in response to DNA breaks and other types of damage in humans (4), we tested whether selenium can induce pATM Ser–1981 formation in MRC-5 cells by immunofluorescence studies. Treatment of MRC-5 cells with Na_{2}SeO_{3} (0–2 μM; Fig. 3A), MSeA (0–2 μM; Fig. 3B), and MSeC (0–100 μM; Fig. 3C) resulted in a significant increase in pATM Ser–1981 focus-positive cells in a dose- and time-dependent manner. The ATM protein was localized in the nucleus at a comparable level in either the presence or absence of the selenium treatment (Fig. 3G). Co-treating MRC-5 cells with the selenium compounds and the ATM kinase inhibitor KU55933 (10 μM) and/or the DNA-PKcs kinase inhibitor NU 7026 (50 μM), *, p < 0.01 compared with the cells treated with only selenium. K and L, PC-3 cells were treated with the selenium compounds, followed by the immunofluorescent analysis. *, p < 0.01 compared with cells treated with selenium only.

**FIGURE 3. The phosphorylation of ATM at Ser-1981 (pATM1981) and H2AX at Ser-139 (γH2AX) is induced in selenium-treated cells.** MRC-5 cells were cultured on coverslips and treated with 1–2 μM Na_{2}SeO_{3}, 1–2 μM MSeA, and 50–100 μM MSeC, followed by a 3- or 7-day recovery. Ratios of cells expressing pATM Ser–1981 (A–C) or γH2AX foci (D–F) to cells expression total ATM or total H2AX are presented with their respective S.D. values. *, p < 0.01 compared with the no selenium controls. **, p < 0.05. G, representative pictures showing immunofluorescent signals of DAPI (blue), total ATM or H2AX (red), and pATM Ser–1981 or γH2AX (green) in MRC-5 cells with or without selenium treatment. H–J, MRC-5 cells were treated with the selenium compounds, together with the ATM kinase inhibitor KU55933 (10 μM) and/or the DNA-PKcs kinase inhibitor NU 7026 (50 μM), *, p < 0.01 compared with the cells treated with only selenium.
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FIGURE 4. The kinase activity of ATM and ROS are involved in selenium-induced senescence in MRC-5 cells. MRC-5 cells were treated with the selenium compounds alone or together with KU55933 (10 μM), NAC (10 μM), or Tempo (1 mM). SA-β-galactosidase was measured as described in the legend to Fig. 1. *, p < 0.01 compared with cells treated with selenium only.

.. protein kinase (DNA-PKcs) (18, 21, 22). Thus, we determined whether the selenium compounds can induce γH2AX formation. Analysis of the immunofluorescent results indicated that treatment of MRC-5 cells with Na₂SeO₃ (1–2 μM; Fig. 3D), MSeA (1–2 μM; Fig. 3E), and MSeC (50–100 μM; Fig. 3F) resulted in significant increases in the population of cells expressing γH2AX foci, the extent of which is comparable between days 3 and 7 post-treatment. To determine whether ATM kinase activity is involved in the increased γH2AX focus formation, we co-treated the MRC-5 cells with the selenium compounds and KU55933 (20). Immunofluorescent analyses of γH2AX foci showed that KU55933 marginally suppresses γH2AX focus formation in the selenium-treated MRC-5 cells (Fig. 3, H–J, and supplemental Fig. 7), suggesting that ATM is not the major kinase attributable to the selenium-induced γH2AX focus formation. We next co-treated the MRC-5 cells with the selenium compounds and NU 7026, a kinase inhibitor of DNA-PKcs. Compared with KU55933, treatment of the cells with NU 7026 resulted in a more robust suppression in the selenium-induced γH2AX focus formation (Fig. 3, H–J). Interestingly, the effect of KU55933 and NU 7026 on the inhibition of selenium-induced γH2AX focus formation appears to be additive. Thus, ATM is not the major kinase involved in the selenium-induced γH2AX focus formation in MRC-5 cells.

Cancer cells are characterized by genomic instability and increased oxidative stress (23–26). Thus, we assessed pATM Ser-1981 and γH2AX expression in PC-3 cells, which is negative in selenium-induced senescence (Fig. 1, A–C, and supplemental Fig. 3). Compared with the non-cancerous MRC-5 cells, there were significantly greater PC-3 cell populations exhibiting intrinsic pATM Ser-1981 (58.4 versus 4.7%; Fig. 3, A versus K) and γH2AX (85.0 versus 33.3%; Fig. 3, D versus L) foci in the absence of selenium treatment. Although 7 days after recovery from treatment with Na₂SeO₃ (1 μM), MSeA (1 μM), and MSeC (50 μM), PC-3 cells showed an increase in cells containing pATM Ser-1981 foci, the extent of induction is much smaller (43% versus 14.6-fold) as compared with the non-cancerous MRC-5 cells. Noticeably, the PC-3 cells exhibit high levels of intrinsic γH2AX foci, and treatment of the cells with selenium did not further increase γH2AX expression (Fig. 3L). Thus, the PC-3 prostate cancer cells are predisposed to genomic instability, which may prevent the cells from responding to the selenium treatment for the activation of senescence and the ATM tumor-suppressing pathways.

A previous report indicates that senescent MRC-5 cells arrest in the G₁ phase of the cell cycle 7–10 days after cellular exposure to H₂O₂ at a concentration of 500 mM (27). Thus, we measured MRC-5 cell cycle profiles 1, 3, and 7 days post-treatment of the selenium compounds. We found that 1 and 3 days after recovery from treatment with MSeA and MSeC resulted in significant increases in MRC-5 cells in the S and G₂/M population, followed by cell cycle arrest in the G₁ phase at day 7 in cells treated with Na₂SeO₃, MSeA, or MSeC (supplemental Tables 2–4). The G₁ cell cycle arrest in MRC-5 cells after selenium treatment is consistent with the observation of the selenium-induced senescence phenotype.

Selenium-induced Senescence Requires ATM Kinase Activity—To determine whether ATM kinase activity is required for the selenium-induced senescence, we preincubated the MRC-5 cells with KU55933. Results from the SA-β-galactosidase analysis demonstrated that inhibition of ATM kinase activity prevented senescence induction in MRC-5 cells exposed to Na₂SeO₃, MSeA, or MSeC (supplemental Tables 2–4). The G₁ cell cycle arrest in MRC-5 cells after selenium treatment is consistent with the observation of the selenium-induced senescence phenotype.

Regulation of the Selenium-induced DNA Damage Response and Senescence by Oxidative Stress—It has been shown previously that MSeA and Na₂SeO₃ can induce ROS formation in a number of prostate cancer cells (28). Thus, we determined the involvement of ROS in the selenium-induced DNA damage response and senescence. Treatment of MRC-5 cells with antioxidants, NAC (a H₂O₂ scavenger) and Tempo (a superoxide dismutase mimic), significantly suppressed senescence in MRC-5 cells treated with the selenium compounds (Fig. 4).
Interestingly, Tempo is more potent than NAC in the attenuation of senescence induced by Na2SeO3 and MSeA. ROS contribute to the selenium-induced DNA damage response because the pATM Ser-1981 focus formation (Fig. 5, A–C) and γH2AX formation (Fig. 5, D–F) are attenuated by NAC treatment. Thus, selenium induces senescence and pATM Ser-1981 focus formation in MRC-5 cells in a manner dependent on reactive oxygen species.

**DISCUSSION**

The evidence for selenium being a chemoprevention agent includes that from geographic, animal, and epidemiological studies (5, 9, 10, 29). However, recent human clinical trials reported mixed results on the role of selenium in chemoprevention, which may potentially be explained by the differences in body selenium status of the subjects entering the trials as well as the selenium dose and formulation between the studies (5, 9, 31–34). Whatever the reason, the molecular mechanism by which selenium mitigates tumorigenesis is largely unknown. An increasing body of recent evidence has suggested that selenium and its metabolites induce apoptotic pathways in cancer cells (15, 18, 36). Nonetheless, selenium could in principle exert its chemoprevention function at the onset of tumorigenesis by other mechanisms. In line with this notion, recent reports indicate that DNA damage response and senescence are barriers of carcinogenesis that function at the interface between the pre-cancerous and the cancerous stages (1–3). The ATM kinase plays a pivotal role in the DNA damage-induced senescence (37, 38). Our findings suggest a new role of selenium in tumorigenesis; selenium induces an ATM-dependent senescence response in a manner dependent on ROS in non-cancerous but not in cancerous cells.

How do sublethal (≤LD50) doses of selenium compounds mount the ATM-dependent senescence response? One possibility is that selenium compounds induce ROS generation that impacts on genome stability. Ample evidence indicates that metabolites of selenium compounds at high doses can induce ROS formation and apoptosis in cancer cells (13, 15, 16, 28). Our findings identify an ATM-dependent senescence response induced by doses of selenium well below LD50 in non-cancerous cells, whereby this pathway is suppressed by antioxidant administration. Early experiments showed that doxorubicin-induced ROS formation or H2O2 treatment can activate the ATM pathway (39, 40) and that astrocytes and hematopoietic stem cells isolated from Atm−/− mice exhibit increased oxidative stress, early onset of senescence, and/or suppressed self-renewal capacity (41, 42). Although ATM is a prominent responder to DNA double strand breaks, this kinase can be activated by various forms of chromosome alterations (4, 20, 43). Importantly, our results suggest that the kinase activity of ATM mediates the senescence phenotype in the selenium-induced MRC-5 cells. Because inhibition of ATM kinase attenuates but does not prevent selenium-induced γH2AX formation.
formation (Figs. 3 and 5), other kinases are capable of phosphorylating H2AX. Altogether, we propose that selenium-induced oxidative stress activates the ATM pathway for the subsequent senescent response. Future studies are needed to elucidate the mechanism of ATM kinase activation by selenium-induced oxidative stress.

Of note, ROS is unlikely to be the only direct activator of the senescence and ATM pathways, as we do not observe a G1 cell cycle arrest as robust as reported previously in senescent MRC-5 cells treated with H2O2 (27). Rather, treatment of the cells with MSeA and MSeC at a dose of ~LD50 resulted in a minor S and G2/M arrest prior to senescence induction. It is conceivable that the ROS-induced DNA oxidation and the subsequent formation of DNA breaks in S phase may activate the ATM pathway for a checkpoint response at the early time point. ATM is known to function in the DNA damage checkpoint at G1/S, and G2/M and is activated by stalled or collapsed replication forks (20, 40, 43). We propose that, following ATM activation (day 1), the cells accumulate in the G1 phase (day 3) and eventually senesce (day 7).

There are several forms of senescence. Replicative senescence is mainly caused by telomere attrition, whereas premature senescence can be triggered by damaged DNA and oxidative stress. ATM is involved in both forms of senescence (37, 38, 44, 45). The p53 protein is mutated in the majority of human malignant tumors and is required for induction of senescence by DNA replication stress (topoisomerase inhibitors) in an array of cancer cell lines (46). Interestingly, we found that the selenium-induced senescence is missing in both the p53-proficient HCT 116 and the p53-deficient PC-3 cancer cells, suggesting that the lack of selenium-induced senescence in the two cancerous cell lines is not attributable to p53 status. Another candidate target of selenium action on senescence is p21, which is a gatekeeper of the G1/S transition and is implicated in a ROS-dependent senescence response in normal human fibroblasts (47, 48). In HCT 116 cells exposed to doxorubicin treatment, p21 expression is induced in both a p53-dependent and -independent manner (49). Further studies are therefore necessary to fully understand whether and how p53 and p21 regulate the ATM- and ROS-dependent senescence response after selenium exposure.

DNA replication stress and checkpoints are associated with oncogene-induced senescence (1–3). Genotoxic stress can induce persistent DNA damage, thus triggering a senescence-associated secretory phenotype (SASP) and suppressing p53 in normal cells (30). SASP can change the tissue microenvironment in a p53-independent fashion, and cells with SASP can secrete various factors, such as interleukin-6 (35). ATM is required for interleukin-6 secretion that facilitates cell communications and bypasses senescence in damaged cells (35). Our MRC-5 cells developed a senescence phenotype 7 days post-treatment of selenium, suggesting that persistent DNA damage and oxidative stress exist. Of note, our data indicate that the selenium-induced senescence is not associated with a robust S-phase checkpoint, as opposed to conditions such as significant DNA double strand breaks that induce S-phase checkpoint. We propose that SASP may develop and lead to senescence by changing its cellular microenvironment after the selenium exposure.

In conclusion, we have provided the first evidence that selenium can mitigate tumorigenesis by mechanisms other than the well studied apoptotic pathway. The observation that selenium specifically induces senescence response in non-cancerous cells suggests a cost-effective scenario by which tumorigenesis can be stifled at the very beginning in individuals who consume selenium with a cancer prevention perspective. It is of future interest to elucidate the mechanism by which selenium activates an ATM and ROS-dependent senescence response, especially at the interphase between the precancerous and cancerous stages by using models for initiation, promotion, and progression in carcinogenesis.

Acknowledgments—We thank Dr. Gerald (Jerry) F. Combs, Jr. and Dr. Yossi Shiloh for reagents and valuable suggestions and Dr. David K. Y. Lei for reagents and for sharing equipment.

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