Methylseleninic Acid Superactivates p53-Senescence Cancer Progression Barrier in Prostate Lesions of Pten-Knockout Mouse

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Abstract

Monomethylated selenium (MM-Se) forms that are precursors of methylselenol, such as methylseleninic acid (MSeA), differ in metabolism and anticancer activities in preclinical cell and animal models from seleno-methionine that had failed to exert preventive efficacy against prostate cancer in North American men. Given that human prostate cancer arises from precancerous lesions such as high-grade prostatic intraepithelial neoplasia (HG-PIN), which frequently have lost phosphatase and tensin homolog (PTEN) tumor suppressor permitting phosphatidylinositol-3-OH kinase (PI3K)–protein kinase B (AKT) oncogenic signaling, we tested the efficacy of MSeA to inhibit HG-PIN progression in Pten prostate-specific knockout (KO) mice and assessed the mechanistic involvement of p53-mediated cellular senescence and of the androgen receptor (AR). We observed that short-term (4 weeks) oral MSeA treatment significantly increased expression of P53 and P21Cip1 proteins and senescence-associated-β-galactosidase staining, and reduced Ki67 cell proliferation index in Pten KO prostate epithelium. Long-term (25 weeks) MSeA administration significantly suppressed HG-PIN phenotype, tumor weight, and prevented emergence of invasive carcinoma in Pten KO mice. Mechanistically, the long-term MSeA treatment not only sustained P53-mediated senescence, but also markedly reduced AKT phosphorylation and AR abundance in the Pten KO prostate. Importantly, these cellular and molecular changes were not observed in the prostate of wild-type littermates which were similarly treated with MSeA. Because p53 signaling is likely to be intact in HG-PIN compared with advanced prostate cancer, the selective superactivation of p53-mediated senescence by MSeA suggests a new paradigm of cancer chemoprevention by strengthening a cancer progression barrier through induction of irreversible senescence with additional suppression of AR and AKT oncogenic signaling. Cancer Prev Res; 1–8. ©2015 AACR.

Introduction

Selenium (Se) compounds have been studied for their chemopreventive potential in various animal models of carcinogenesis, notably mammary, colon, lung, and prostate cancer. Selenized yeast (Se-yeast) and its principal Se form Se-methionine (SeMet) have been tested in several human trials in North America for the prevention of prostate cancer (1–4). The outcomes of the Selenium and vitamin E Cancer Trial (SELECT) and other trials failed to demonstrate a preventive efficacy of these Se forms in the Se-adequate North American cohorts (2–4). Many, including us, have opined on the possible reasons for such failures (5–7, 8). One key factor was the selection of ineffective Se agents. In fact, the scarce animal efficacy data that existed prior to the initiation of these trials did not support prostate cancer preventive efficacy of SeMet and these negative data were not published in full-length until after SELECT was terminated (9, 10).

Preclinical and mechanistic research has demonstrated that SeMet has little in common with the mono-methylated methylselenol precursor Se forms (MM-Se), such as methylseleninic acid (MSeA), in terms of metabolism and anticancer activities (8, 11). We have posited that the failure of SeMet should not be taken to indicate that other Se forms are ineffective for prostate cancer chemoprevention (12). Indeed, we have shown that daily orally administered MSeA inhibited the growth of DU145 and PC-3 human prostate cancer xenografts in athymic nude mice, whereas an equal Se dose of SeMet was inactive, in spite of SeMet leading to much higher retention of Se in the xenograft tumors (13). We also reported the efficacy of MSeA to inhibit prostate carcinogenesis in the transgenic adenocarcinoma mouse prostate (TRAMP) model, which improved survival with no observable long-term adverse effect (12). More efficacy and biomarker assessments in clinically relevant prostate carcinogenesis models with MM-Se will be essential to evaluate their prostate cancer chemoprevention potential in the post-SELECT era to support future translation of these data to humans.

The PTEN (phosphatase and tensin homolog) protein antagonizes the phosphatidylinositol-3-OH kinase (PI3K)–protein kinase B (AKT) signaling pathway that stimulates cancer cell...
metabolism, proliferation, and survival (14). Human PTEN loss has been identified in 45% of high-grade prostatic intraepithelial neoplasia (HG-PIN) and 70% of advanced prostate cancer (ref. 14 and reference therein). Mouse genetic studies have demonstrated that loss of Pten in prostate epithelium rapidly causes HG-PIN that ultimately progresses to invasive adenocarcinoma and metastatic disease (15). As men diagnosed with HG-PIN are at increased risk of developing prostate cancer, this prostate-specific conditional Pten KO mouse model recapitulates essential characteristics of human prostate carcinogenesis and is considered clinically relevant for studies of prostate cancer chemoprevention. In the Pten KO model, the sustained activation of AKT not only initiates and perpetuates oncogenic signaling and progression pathways, but at the same time induces cellular senescence (known as Pten-deficiency Induced Cellular Senescence, PICS), which acts as a formidable barrier to restrain oncogenic progression to invasive and metastatic disease (16, 17). Mechanistic studies suggest that PICS primarily depends on P53 protein overexpression, which is induced through AKT/mTOR-mediated protein synthesis and p19ARF sequestration of MDM-2, resulting in inhibition of proteasome-mediated P53 degradation (16).

The critical role of androgen receptor (AR) signaling in prostate cancer, even at the advanced metastatic castration-resistant stage, is well established and therapeutically exploited.

Figure 1. Oral supplement of MSeA (3 mg Se/kg body weight, 5 days/week) for 4 weeks induced p53–p21-mediated senescence and inhibited prostate epithelial cell proliferation in vivo. MSeA or water treatment commenced at 12 weeks of age. A, prostate weight of Pten KO (PtenΔ/Δ) mice (n = 5) and WT littermates (Pten+/+) (n = 3). B, immunoblot analyses of Pten, p-Akt, p53, and p21Cip1 proteins in pooled samples of each group. Tubulin served as loading control. C, representative photomicrographs of immunohistochemical staining of androgen receptor (AR), Ki67, and SA-galactosidase staining of Pten KO prostate from water-treated control mice and MSeA-treated mice. Magnification 100×. D, quantitation results from individual mice for each group are presented as mean ± SEM, n = 5 for PtenΔ/Δ mice and n = 3 for Pten+/+ mice. For AR and SA-gal staining, the integrated optical density (IOD) was normalized to control mice as 100. For Ki67, percentage of positive nuclei in the prostate epithelium was quantified.
(18). Unfortunately, recent studies have shown that inhibition of AR signaling by castration or antagonist drugs inadvertently promotes the progression of stable HG-PIN to invasive carcinomas in Pten KO model (19), raising concerns for utilization of these androgen deprivation strategies for chemoprevention in high-risk men and prostate cancer patients with Pten deficiency or mutations.

In cell culture studies, we and others have shown MSeA suppression of AR abundance and signaling in prostate cancer cells (20, 21), and the phosphorylative activation of AKT Ser473 (pAKT; ref. 22, 23). MSeA was recently shown to induce suppression of AR abundance and signaling in prostate cancer (20, 21), and the phosphorylative activation of AKT (24, 25). Therefore, in this study, we tested the hypothesis that MSeA would inhibit Pten-deficient carcinogenesis and prostate cancer progression and assessed the mechanistic involvement of p53, PICS, AKT, and AR in conferring the hypothesized in vivo efficacy.

Materials and Methods

Generation of Pten KO mice

The conditional Pten flox/+ mouse was generated as previously described (26). PB-Cre4 transgenic mice were obtained from the NCI Mouse Repository. Female mice carrying Pten flox/+ were crossed with male mice harboring PB-Cre4 "Pten flox/lox" to generate mutant mice with prostate epithelium-specific deletion of Pten. Tail DNA was used for PCR-based genotyping as described (26). All animal protocols were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Intervention experiments with MSeA

In the short-term experiment, 12-week-old Pten KO mice (Cre+; Pten flox/flox, hereafter indicated as Pten−/− mice) were randomly assigned, 5 mice per group, to receive water or MSeA for 4 weeks by daily (5 days/week) oral application at the base of the tongue as before (12, 13). Wild-type (WT) littermates (Cre−; Pten flox/flox, hereafter as Pten+/+; n = 3) were treated identically with water or MSeA to provide comparison control tissues. AIN93G semipurified diet and water were provided ad libitum. Mouse body weight was monitored weekly. At necropsy, total prostate was dissected, photographed, and weighed. One portion of prostate tissues from each mouse was fixed in formalin for hematoxylin and eosin (H&E) and immunohistochemistry staining. The reminder was stored frozen at −80°C for senescence-associated β-galactosidase (SA-gal) staining and Western blot analyses.

The long-term experiment was carried out with same design, except that the mice were 10 weeks old at the start of the MSeA and water treatments (5 days/week), lasting for 25 weeks (8 mice per group). At necropsy, the genitourinary (GU) tract was collected and weighed. Then the different prostate lobes were dissected and weighed. The prostate lobes were saved and processed individually for histopathology and biochemical analyses.

Histopathology analysis

Tissue processing and staining were as performed previously (12, 26). H&E-stained lesions were verified by a pathologist (M. Bosland). The pathologic changes of all lobes of prostate were classified according to Shappell and colleagues (27).

Figure 2.

Effect of oral supplement of MSeA (3 mg Se/kg body weight, 5 days/week) for 25 weeks on Pten KO-driven growths of genitourinary organs and prostate tumors. MSeA or water treatment commenced at 10 weeks of age. A, genitourinary (GU) tract weight of Pten KO (Pten−/−) mice (n = 8) and WT littermates (Pten+/+) (n = 8); B, whole prostate weight of Pten−/− mice (n = 8) and Pten+/+ mice (n = 8); C, individual lobe weights of Pten−/− mice (n = 8) and Pten+/+ mice (n = 8). AP, anterior prostate; DLP, dorsolateral prostate; VP, ventral prostate. *P = 0.05 or two-sided t-test. P values were shown.
Senescence-associated β-galactosidase staining

Prostate tissues were embedded with optimal cutting temperature (OCT) compound and cut into 4-μm sections. The sections were stained for SA-gal and counterstained with eosin, as described previously (26). The integrated optical density (IOD) in the prostate epithelium/lesions was quantified by ImagePro-Plus 6.3 software.

Immunohistochemistry (IHC) and immunoblot analysis

IHC and immunoblot (Western) were performed as previously described (26). Briefly, antibodies against Ki67 (NeoMarker), AR (Millipore), cleaved caspase-3, and p-AKT Ser473 (Cell Signaling Technology) were diluted at 1:100 for IHC. Images were captured and analyzed by ImagePro-Plus 6.3 software for integrated optical density (IOD) semiquantitation. For immunoblot, the prostate tissues were homogenized in nondenaturing lysis buffer and subjected to SDS-PAGE and blotted with antibodies against P53, P21Cip1, p-AKT Ser473, AR, and tubulin. Pooling of prostate tissues from the short-term experiment was necessary due to limited amount of material available.

Statistical analyses

For parametric data, the mean and SEM were calculated for each experimental group. Differences among groups were analyzed by ANOVA for more than two groups. For comparison of only two groups, the Student t-test was used. Significant differences were accepted at P < 0.05.

Results

Short-term MSeA treatment of Pten KO mice led to superactivation of p53–p21 and cellular senescence in prostate epithelium

In the first experiment, we evaluated the effect of 4-week MSeA treatment to identify early biochemical and cellular changes that might correlate and predict its long-term preventive efficacy against HG-PIN growth and tumor progression in Pten KO mice. As shown in Fig. 1A, MSeA treatment led to a reduction (~20%) of prostate weight in Pten KO mice compared with the prostate weight in WT mice, which was not affected by MSeA. The protein levels of Pten and phospho-Akt Ser473 (p-Akt) were analyzed in pooled prostate samples of WT mice and Pten KO mice. As expected, Pten KO mice lacked Pten protein in the prostate and had greatly increased p-Akt expression (Fig. 1B, lanes 3, 4 vs. 1, 2). Consistent with previous results (16, 17, 26), Pten KO mouse prostate showed increased basal expression of P53 and P21Cip1 over the WT counterpart (Fig. 1B, lane 3 vs. lane 1). MSeA treatment of the Pten KO mice dramatically increased P53 and P21Cip1, but this did not occur in WT mice, whereas p-Akt expression was not affected in MSeA-treated KO or WT mice (Fig. 1B). In addition, there was no observable change in AR expression determined by IHC in Pten KO mice after 4 weeks of MSeA treatment (Fig. 1C and D).

Because increased P53 protein abundance causes PICS in the Pten KO mice (16, 17), we examined SA-gal expression in situ

Figure 3.

Long-term oral MSeA supplementation inhibited prostate cancer progression in Pten KO mice. Oral supplementation of MSeA (3 mg Se/kg body weight, 5 days/week) was initiated at 10 weeks of age for 25 weeks. A and B, representative photomicrographs of H&E-stained prostate lobes of (A) Pten KO (PtenKO) mice treated with water (Con) or MSeA and (B) WT (Pten+/+) mice treated with water (Con) or MSeA. C, quantitation of anterior (AP), ventral (VP), and dorsolateral (DLP) prostate pathologic changes. Mean ± SEM, PtenKO mice (n = 8), and Pten+/+ mice (n = 8). *, statistical difference from Pten KO control mice, P < 0.05.
Figure 4.
Effect of long-term MSeA supplementation on p-Akt and androgen receptor (AR), senescence, and cellular proliferative index in the anterior prostate of mice in Fig. 3. A and B, representative photomicrographs of IHC staining of p-Akt Ser473, AR, Ki67, and SA-galactosidase activity in anterior prostate of (A) Pten KO (Pten<sup>Δ/Δ</sup>) mice treated with water (Con) or MSeA and (B) WT (Pten<sup>+/+</sup>) mice treated with water (Con) or MSeA. C, quantitation of changes from A and B. Mean ± SEM, Pten<sup>Δ/Δ</sup> mice (n = 8) and Pten<sup>+/+</sup> mice (n = 8). The integrated optical density (IOD) was estimated by ImagePro-Plus software. *, statistical difference from Pten KO control mice, P < 0.05.
in frozen prostate sections (Fig. 1C). 

Prolonged MSeA treatment of Pten KO mice prevented prostate adenocarcinoma

The promising biochemical and cellular responses to the short-term MSeA intervention prompted us to evaluate its chemopreventive efficacy on Pten KO HG-PIN growth and progression in the second experiment with 23-week administration. Consistent with long-term safety of MSeA supplement in our previous study with the TRAMP model (12), no significant effect of MSeA was observed on the body weight gain of the mice of each genotype (Supplementary Fig. S1A). As shown in Fig. 2A, long-term MSeA daily treatment did not significantly affect the genitourinary tract (GU) weight of the WT mice, but decreased Pten KO-driven expansion of GU over the WT baseline by more than 70% (Fig. 2A). Similarly, the prostate weight was not affected in the WT mice by MSeA, but was decreased by more than 50% in the Pten KO mice over the WT baseline (Fig. 2B). At the gross anatomical level, blood-rich prostate tumors were visible in some water-treated control Pten KO mice (Supplementary Fig. S1B). Among the different lobes, the AP exhibited the most Pten KO-driven growth (Fig. 2C; Supplementary Fig. S1C), whereas in TRAMP mice the DLP undergo the most growth (12). The prostate weight suppressing effect of MSeA in the Pten KO mice was uniform across AP, DLP, and VP lobes (Fig. 2C; Supplementary Fig. S1C). In sharp contrast, the weight of prostate lobes in WT mice of the MSeA and control groups was not different (Fig. 2C).

Histologically, the prostate lesions from the control Pten KO mice showed HG-PIN phenotypes in all three lobes (Fig. 3A). Notably 38% (3 out of 8 mice) of Pten KO mice progressed from HG-PIN to invasive adenocarcinomas at the termination of the experiment at 35 weeks of age (Supplementary Fig. S1D). In contrast, MSeA-treated mice showed dramatic histopathologic modification, many approaching near normal appearance of the prostate of the WT mice and none of them with detectable invasive adenocarcinoma features (Fig. 3A and C). Consistent with selectivity of targeting oncogenic growth, MSeA treatment of WT mice did not affect their typical normal glandular structures (Fig. 3B and 3C). These findings indicate that long-term MSeA treatment significantly inhibited HG-PIN growth and progression to carcinoma in vivo.

Long-term MSeA treatment decreased p-Akt and AR abundance in Pten KO prostate epithelium

Because short-term MSeA superactivated P53/P21Cip1 and increased senescence in the Pten KO epithelium in vivo, we examined whether long-term MSeA was able to sustain the cellular senescence phenotype. SA-gal staining of AP lobes showed intense senescence in the MSeA-treated Pten KO mice (Fig. 4A) with little effect in the WT mice (Fig. 4B and C). Ki67 staining confirmed the paucity of proliferating cells in the MSeA-treated Pten KO prostate epithelium (Fig. 4A and C). No appreciable apoptosis, indicated by cleaved caspase-3, was induced by MSeA treatment in Pten KO mice (data not shown).

It is noteworthy that IHC staining intensity of p-Akt and AR proteins in the AP lobe of MSeA-treated Pten KO mice was noticeably decreased (Fig. 4A and C) without corresponding observable changes in the WT mice (Fig. 4B and C). Immunoblot confirmed the IHC results for p-AKT and AR protein abundance suppression by MSeA in the Pten KO prostate (Fig. 5A).

Discussion

To our best knowledge, this study is the first in which any form of Se has been tested in the Pten KO prostate cancer mouse model for chemopreventive efficacy. It is also the first time that in vivo senescence was measurably increased by MSeA treatment selectively in the HG-PIN epithelium of Pten KO mice without any detectable impact on the prostate of the WT mice. The observed concomitant increase of P53 and P21Cip1 in the prostate of MSeA-treated Pten KO mice was not evident in the prostate of WT mice (Fig. 1B), consistent with the selective superactivation of this crucial senescence signaling axis. In addition to boosting and sustaining P53-P21Cip1 senescence as a cell proliferation barrier, long-term treatment with MSeA led to considerably reduced tumor burden (Fig. 2) with decreased AR abundance and phosphorylation of Akt (Figs. 4 and 5), together contributing to effective suppression of the progression of HG-PIN to carcinoma (shown schematically in Fig. 5B). Given that current Akt/mTOR...
inhibitor drugs activate AR signaling, whereas androgen ablation and AR antagonist drugs cross-induce the Akt pathway through a reciprocal feedback regulatory loop (28), the inhibition of both the Akt and the AR signaling pathways by prolonged MSeA supplement suggests that combined use of MSeA with these drugs may mitigate their undesirable side effects and result in greater prostate cancer risk reduction.

Human prostate cancer arises from precancerous HG-PIN lesions with a prolonged clinical course, affording unique windows of opportunity for chemoprevention/intervention. Because p53 signaling is more likely to be intact in precancerous lesions than advanced prostate cancer, the superactivation of p53-senescence by MSeA offers a new paradigm for prostate cancer chemoprevention through strengthening a cancer progression barrier in the precursor lesions. Our data support that MSeA superactivated and sustained P53-mediated cellular senescence and subsequently inhibited both the Akt and the AR signaling pathways to suppress Pro-deficient HG-PIN progression to adenocarcinoma (Fig 5A). Pro-deficient HG-PIN tumor-suppressive mechanisms mediating these cellular and molecular actions of MSeA are currently being elucidated.

The efficacy for chemoprevention of Pro-deficient HG-PIN progression by MSeA documented in this work and the previously demonstrated efficacy and safety of MSeA in other prostate cancer mouse models (12,13) provide strong justification for further development of MM-Se toward human translational studies.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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