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Alteration of Cellular Phenotype and Responses to Oxidative Stress by Manganese Superoxide Dismutase and a Superoxide Dismutase Mimic in RWPE-2 Human Prostate Adenocarcinoma Cells

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ABSTRACT

To study biologic effects of increased manganese superoxide dismutase (MnSOD) on cell behavior, we overexpressed MnSOD in a human prostate cancer cell line RWPE-2 by cDNA transfection. Stable transfectants of MnSOD showed a two- to threefold increase in MnSOD protein and enzymatic activity and a decrease in growth rate with prolonged cell population doubling times. Western blot analysis showed a 1.5- to twofold increase in the cyclin-dependent kinase inhibitor $p21^{Waf1}$ in MnSOD transfectants. Overexpression of MnSOD resulted in a seven- to eightfold increase in reduced glutathione (GSH), 18- to 26-fold increase in oxidized glutathione (GSSG), and a two- to threefold decrease in the ratio of GSH to GSSG. MnSOD-overexpressing cells showed an increase in sensitivity to the cytotoxicity of buthionine sulfoximine, a glutathione-depleting agent, and vitamin C, but a decrease in sensitivity to sodium selenite. Treatment with a superoxide dismutase (SOD) mimic MnTMPyP resulted in similar effects of MnSOD overexpression on cell responses to vitamin C and selenium. These data demonstrate that overexpression of MnSOD or treatment with SOD mimics can result in antioxidant or prooxidant effects in cells, depending on the presence of other antioxidants and prooxidants. MnSOD also has redox regulatory effects on cell growth and gene expression. These findings suggest that MnSOD and SOD mimics have the potential for cancer prevention or treatment. *Antioxid. Redox Signal.* 6, 513–522.

INTRODUCTION

ACCUMULATING EVIDENCE indicates that redox-mediated regulation of signal transduction and gene expression is a fundamental mechanism in cell biology (12, 15). Reactive oxygen species (ROS) play an important role in the regulation of cell redox state. At high levels, ROS cause oxidative stress, resulting in a variety of pathologic states, including carcinogenesis and cell apoptosis. At low levels, ROS are involved in the regulation of gene expression by direct participation in cell signal transduction and/or modulation of the intracellular redox state. Redox regulation of ROS involves changes in intracellular free glutathione or thiol groups in proteins, thus altering the activities of the transcriptional factors activator protein-1 and nuclear factor-κB (1, 41). ROS have been demonstrated to be mediators of cell growth or differentiation induced by growth factors (3, 39, 40).

Intracellular levels of ROS, mainly superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl radical, can be produced from normal cellular metabolism, chemotherapeutic agents, and radiotherapy. They are regulated by antioxidant systems, particularly antioxidant enzymes, such as superoxide

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dismutases (SODs), catalase (CAT), and glutathione peroxidases (GPx). Manganese SOD (MnSOD) is one of the primary antioxidant enzymes and is located in mitochondria, which are the main source of ROS production in aerobic organisms. The main function of MnSOD is protection against oxidative stress by conversion of superoxide into H_2O_2, which is subsequently converted to O_2 and H_2O by GPx in mitochondria. In the cytosol, levels of ROS are regulated by copper-zinc SOD (CuZnSOD), CAT, and GPx. Antioxidant enzymes coordinately regulate levels of intracellular ROS and therefore also regulate intracellular redox state. The balance between ROS and antioxidants is important for maintenance of intracellular redox state. Additionally, the levels and the balance of antioxidant enzymes are crucial to regulate levels of ROS and total intracellular and organelle redox state.

A number of studies have demonstrated that levels of antioxidant enzymes were altered in a variety of tumor cells compared with their normal counterparts (27, 38). In general, MnSOD and CAT are almost always low in tumor cells; CuZnSOD is usually low; and GPx is variable. Decreased levels of MnSOD mRNA were shown to be responsible for reduced levels of MnSOD. Conversely, elevated levels of MnSOD by cDNA transfection suppressed tumor cell growth (9, 22, 50). Exogenous MnSOD inhibited tumor cell growth by elevating mitochondrial H_2O_2 levels, which could be reversed by cotransfection with GPx (23) or mitochondrial-targeted CAT (33). Thus, overexpression of MnSOD can either reduce oxidative stress or increase oxidative stress, depending on the level of MnSOD and the balance of other antioxidant enzymes.

Prostate cancer is the most commonly diagnosed non-skin cancer in men in the United States. Accumulating evidence has implicated the involvement of oxidative stress in prostate cancer development and progression. A recent randomized-controlled trial showed that selenium supplementation reduced prostate cancer incidence (10). An epidemiological study revealed that higher selenium levels in toenails were associated with a reduced risk of prostate cancer incidence and progression (46). An inverse relationship between serum levels of selenium and the incidence of prostate cancer has also been demonstrated by other studies (8, 43). Alteration of antioxidant enzymes has been demonstrated in recent studies of prostate cancer (4, 7, 28). In general, immunoreactive protein levels of MnSOD, CuZnSOD, and CAT were lower in prostatic intraepithelial neoplasia and prostate adenocarcinoma than benign epithelium. Interestingly, one study demonstrated that metastatic prostate cancer had higher levels of MnSOD and oxidative damage products than primary tumor (28). These results suggest that there is a shift in cellular redox state in both primary and metastatic prostate cancer, although redox state may be different in primary versus metastatic cancer. These changes partly contribute to prostate cancer carcinogenesis and the biologic properties of prostate cancer cells. Alteration of cellular redox state in prostate cancer cells may be the result of imbalance of antioxidant enzymes.

In the present study, effects of MnSOD on cellular phenotype and sensitivity to oxidative stress in a *ras*-transformed human prostate cancer cell line RWPE-2 were examined. Similar to the results of previous studies (9, 22, 50), overexpression of MnSOD altered the phenotype of RWPE-2 cells and resulted in an oxidative shift in cell redox state and growth inhibition. In addition, cells with MnSOD overexpression or treated with a SOD mimic manganese (III) tetrakis(1-methyl-4-pyridyl)porphyrin pentachloride (MnTMPyP) showed increased sensitivity to the cytotoxicity of vitamin C and buthionine sulfoximine (BSO), but decreased sensitivity to the cytotoxicity of selenium. These results suggest that overexpression of MnSOD or SOD mimic treatment may lead to an alteration of cell redox by modulation of intracellular ROS and result in changes in cellular sensitivity to other low-molecular-weight antioxidants and prooxidants, which may be of importance in cancer prevention and treatment.

MATERIALS AND METHODS

Chemicals and antibodies

Sodium selenite, BSO, vitamin C, and anti-β-actin antibody were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). MnTMPyP was purchased from Alexis Biochemicals (San Diego, CA, U.S.A.). Anti-MnSOD and anti-CuZnSOD antibodies were a gift from Dr. Larry Oberley (University of Iowa, Iowa City, IA, U.S.A.). $p21^{Waf1}$(C-19) antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). Anti-CAT antibody was purchased from Athens Research & Technology (Athens, GA, U.S.A.).

Cell culture

RWPE-2 cells were derived from a human papilloma virus-18 immortalized cell line (RWPE-1) following transformation by Ki-*ras* oncogene (5). Cells were routinely maintained in 100-mm tissue culture dishes (Corning) in keratinocyte-SF medium supplemented with 1% antibiotic–antimycotic (GibcoBRL, Rockville, MD, U.S.A.) at 37°C in a humidified atmosphere of 95% air and 5% CO_2. For biochemical analyses, cells were collected by rinsing in phosphate-buffered saline (PBS) three times, scraping with a rubber policeman in 10 ml of PBS, and then centrifuging at 2,000 rpm for 5 min. After removal of PBS, cell pellets were stored at −40°C until use.

Cell transfection

The plasmid vectors pcDNA3 and pcDNA3-MnSOD were gifts from Dr. Larry Oberley of the University of Iowa. Cells were seeded at a density of 1×10^5 cells per well in six-well plates overnight. Cells were incubated in 1 ml of serum- and antibiotic-free medium containing 6 μl of LipofectAMINE Plus (GibcoBRL) and 1 μg of pcDNA3 or pcDNA3-MnSOD plasmid vectors overnight and then grown in antibiotic-free medium for 24 h. Transfectants were selected in the regular medium with 500 μg/ml Geneticin (G418) (GibcoBRL) for 2 weeks. Overexpression of MnSOD in individual cell clones was screened by western blot analysis. Transfectants were routinely maintained in medium with 500 μg/ml G418. G418 was removed from the cultures for 4 days before the experiments were performed.

Western blot analysis

Cell pellets were lysed with M-PER™ mammalian protein extraction reagent (Pierce, Rockford, IL, U.S.A.), and protein concentrations were determined by the Bradford method

(Bio-Rad, Hercules, CA, U.S.A.). Cell lysates were electrophoresed in 12% sodium dodecyl sulfate–polyacrylamide gels and then transferred onto nitrocellulose membranes. After blocking in 5% nonfat dry milk in 10 m*M* Tris buffer, pH 7.8, with 0.1% Tween 20 (TTBS), the membranes were incubated with primary antibodies at 1:1,000 dilutions in TTBS overnight at 4°C and then secondary antibodies conjugated with horseradish peroxidase added at 1:10,000 dilution in TTBS for 1 h at room temperature. Protein bands were visualized on *x*-ray film using the enhanced chemiluminescence system (Pierce).

Colony formation and plating efficiency

Cells were seeded at a density of 200 cells per dish in 60-mm tissue culture dishes with 5 ml of culture medium and allowed to grow for 2 weeks without medium change. After staining with a solution of 0.1% crystal violet and 2% citric acid, cell colonies with >50 cells were counted under a dissecting microscope. Plating efficiency was calculated using a formula of (colonies formed/cells seeded) × 100.

SOD activity assay

SOD activity was measured as described previously (49). In brief, protein samples (0–800 μg of total protein) in 100 μl of 50 μ*M* potassium phosphate buffer, pH 7.8, were incubated in a mixture [50 m*M* potassium phosphate buffer, pH 7.8, 1 m*M* DETAPAC (diethylenetriaminepentaacetic acid), 0.13 mg of bovine serum albumin, 1 unit/ml CAT, 56 μ*M* nitro blue tetrazolium (NBT), 0.1 m*M* xanthine, and 50 μ*M* bathocuproine disulfonic acid] for 15–30 min. After mixing with xanthine oxidase that was adjusted to give an NBT reduction rate of 0.015–0.025/min at 560 nm, the reaction solution was immediately measured by a spectrophotometer (Beckman) at 560 nm for 6 min at 20-s intervals. MnSOD activity was measured by the addition of 5 m*M* NaCN in the mixture to inhibit CuZnSOD activity. CuZnSOD activity was determined by subtracting MnSOD from the total SOD activity. One unit of SOD activity is defined as the amount of protein required to give half-maximal inhibition of NBT reduction.

Glutathione assay

Cells were collected by trypsinization and washed with PBS after selenite treatment. The cells were suspended in phosphate buffer (PB, 50 m*M* potassium phosphate buffer, pH 7.8), and a small aliquot was used for protein quantitation. For glutathione assay, protein precipitation was carried out by mixing one volume of cell suspension with one volume of 10% 5-sulfosalicylic acid and centrifuged at 15,000 rpm for 5 min at 4°C. The supernatants were collected and total glutathione was measured in a DU640 spectrophotometer (Beckman) as previously described (48). Oxidized glutathione (GSSG) was indirectly estimated by measuring reduced glutathione (GSH) after the supernatants were incubated in 2-vinylpyridine for 90 min at 4°C.

Cell growth analysis

Cells were seeded at 2×10^4 cells per well in 12-well plates, and the medium was changed every 4 days. After trypsinization, cell numbers were counted every 4 days using a hemocytometer. Cell population doubling time was calculated by the formula $0.693t/\ln (N_t/N_0)$, where t is time in hours, N_t is the cell number at time t, and N_0 is the initial cell number.

Cytotoxicity assay

Cells were seeded at 5,000 cells per well in 96-well plates overnight before treatment with different agents and then allowed to grow for an additional 72 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to estimate cell viability as described previously (49).

GPx activity gel assay

Total protein of each sample was separated in a native 12.5% polyacrylamide gel. The gel was first rinsed in distilled water three times and then incubated in 1 m*M* glutathione and 0.008% cumene hydroperoxide for 10 min. The gel was then stained with 2% ferric chloride and 2% potassium ferricyanide. When achromatic bands appeared, the gel was washed extensively with distilled water and then photographed.

Statistical analysis

ANOVA analysis with Tukey post hoc multiple comparison was used to determine the significance of statistical difference of the data at the level of $p < 0.05$ using SPSS computer statistics software (SPSS Inc., Chicago, IL, U.S.A.).

RESULTS

Antioxidant enzyme expression in RWPE-2 cells

Multiple clones transfected with MnSOD cDNA or vector control only were selected by exposure to 500 μg/ml G418 and subsequently screened using anti-MnSOD antibody to determine levels of MnSOD immunoreactive protein. One neo (vector) control and three MnSOD-overexpressing clones, MnSOD1, MnSOD10, and MnSOD13, were chosen for this study. Western blot analysis showed that the neo control clone had a slightly higher level of MnSOD immunoreactive protein than the parental cell line whereas all three MnSOD-transfected clones had a 2.2–2.7-fold increase in MnSOD immunoreactive protein (Fig. 1A). Enzymatic assay confirmed that MnSOD enzymatic activity was increased by 2.5–3.0-fold in the MnSOD transfectants compared with parental cells (Table 1), which was consistent with increased levels of MnSOD immunoreactive protein. CuZnSOD immunoreactive protein showed a slight decrease in neo and MnSOD10 cells, a moderate decrease in MnSOD13 cells, and no change in MnSOD1 cells (Fig. 1A). However, the enzymatic assay did not show significant changes in CuZnSOD activity among these cell lines (Table 1). Levels of CAT immunoreactive protein were slightly increased in all MnSOD-overexpressing cells compared with parental cells (Fig. 1A). Two (MnSOD1 and MnSOD10) of the MnSOD transfectants had 1.6- and 1.7-fold increases in GPx enzymatic activity compared with parental cells, whereas MnSOD13 had no change in GPx activity. Neo control cells showed no significant changes in CAT immunoreactive protein and GPx enzymatic activity.

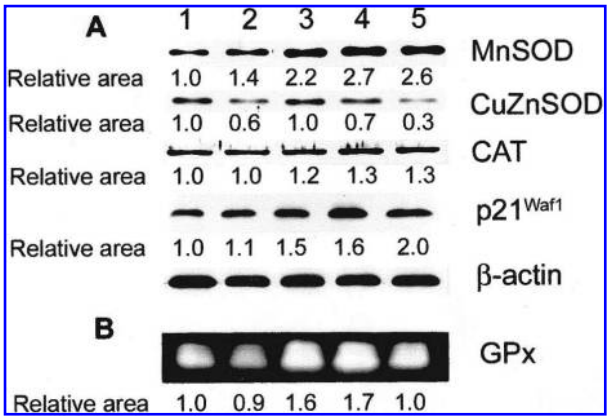

FIG. 1. **(A)** Western blot analysis of MnSOD, CuZnSOD, CAT, $p21^{Waf1}$, and β-actin in RWPE-2 cells. Twenty micrograms of total cellular protein was loaded per lane for MnSOD, CuZnSOD, CAT, and β-actin and 40 μg of protein per lane for $p21^{Waf1}$. **(B)** Activity gel analysis of GPx enzymatic activity. One hundred micrograms of total cellular protein was loaded per lane. Relative areas shown below individual protein bands are the intensity of individual bands measured by densitometry, and numbers present fold changes of band intensity relative to the corresponding parental cells. Data presented are one representative experiment of three independent experiments that showed similar results. Lane 1, parental cells; lane 2, neo (vector) control; lane 3, MnSOD1; lane 4, MnSOD10; lane 5, MnSOD13.

Effects of MnSOD overexpression on cell growth

RWPE-2 cells showed phenotypic changes after overexpression of MnSOD. Colony assay showed that overexpression of MnSOD decreased the size of colonies (Fig. 2A), but not the plating efficiency (Fig. 2B). All of the MnSOD transfectants had slower growth rates than parental and neo cells (Fig. 2C). Cell population doubling times were 31 h in parental cells and 36 h in neo control cells. Cell population doubling times were significantly increased in MnSOD-overexpressing cells: 42 h for MnSOD1 cells, 41 h for MnSOD10 cells, and 45 h for MnSOD13 cells. However, flow cytometric analysis showed no significant changes in cell population distribution in G_0/G_1, S, and G_2/M cell cycle phases (data not shown). Western blot analysis showed that levels of $p21^{Waf1}$ immunoreactive protein were increased by 1.5–2.0-fold in MnSOD-overexpressing clones compared with the parental cell line (Fig. 1A).

TABLE 1. SOD ACTIVITY IN RWPE-2 CELLS (UNITS/MG OF TOTAL PROTEIN)*

Cell line	*Total SOD*	*MnSOD*	*CuZnSOD*
Parental	42 ± 6	20 ± 3	22 ± 7
Neo	38 ± 4	25 ± 7	13 ± 8
MnSOD1	77 ± 6†	49 ± 8†	28 ± 10
MnSOD10	92 ± 4†	67 ± 6†	25 ± 7
MnSOD13	86 ± 9†	59 ± 7†	27 ± 11

*Values represent means ± SD ($n = 3$).
†$p < 0.05$ compared with parental cells.

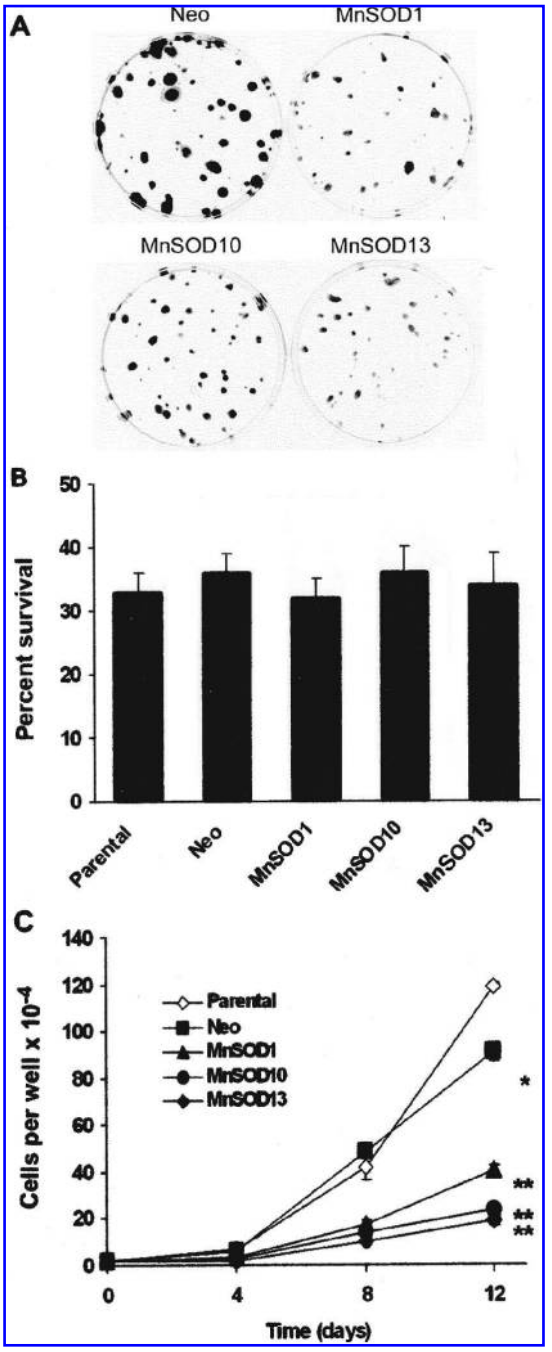

FIG. 2. **Effect of MnSOD overexpression on growth of RWPE-2 cells.** **(A)** Colony formation assay of cell growth. Cells were plated at a density of 200 cells per dish in 60-mm dishes and allowed to grow for 2 weeks without medium change. After staining in a solution with 0.1% crystal violet and 2% citric acid, cell colonies were photographed using a bright-field microscope. **(B)** Plating efficiency. The experiment was performed as described in A. Cell colonies were counted under a dissecting microscope. Plating efficiency was calculated as described in Materials and Methods. Data represent means ± SD ($n = 5$). **(C)** Cell growth curves. Cells were seeded at 2×10^4 cells per well in 12-well plates. Cell numbers were determined every 4 days using a hemocytometer. Medium was replaced every 4 days. Data represent means ± SD ($n = 3$). *$p < 0.05$ compared with parental cells on day 12; **$p < 0.01$ compared with parental and neo cells on day 12.

TABLE 2. LEVELS OF GSH AND GSSG IN RWPE-2 CELLS*

Cell line	*GSH (μg/10⁶ cells)*	*GSSG (μg/10⁶ cells)*	*GSH:GSSG*
Parental	5.72 ± 0.28	0.006 ± 0.001	904
Neo	8.32 ± 1.96	0.012 ± 0.003†	693
MnSOD1	41.91 ± 0.89‡	0.152 ± 0.031‡	275
MnSOD10	49.56 ± 0.51‡	0.153 ± 0.005‡	324
MnSOD13	43.99 ± 1.02‡	0.106 ± 0.005‡	412

*Values represent means ± SD ($n = 3$).
†$p < 0.05$ compared with parental cells.
‡$p < 0.01$ compared with parental and neo cells.

Alteration of intracellular levels of GSH and GSSG by MnSOD overexpression

To demonstrate alteration of intracellular redox state by MnSOD overexpression, levels of intracellular GSH and GSSG were measured using a chemical assay. As shown in Table 2, MnSOD-overexpressing clones had a seven- to ninefold increase in GSH, an 18–26-fold increase in GSSG, and a two- to threefold decrease in the ratio of GSH to GSSG (GSH:GSSG) compared with parental cells. For comparison, neo control cells showed minimal alterations in intracellular GSH (a 1.5-fold increase), GSSG (a twofold increase), and GSH:GSSG (a 1.3-fold decrease).

Effects of MnSOD overexpression on cellular sensitivity to the prooxidant BSO

Figure 3 shows that MnSOD transfectants dramatically increased their sensitivity to the cytotoxic effect of BSO, a GSH-depleting agent. Cellular sensitivity to BSO appeared to be correlated with an alteration of GPx activity in MnSOD transfectants. MnSOD13 cells had no change in GPx activity and were the most sensitive to BSO. MnSOD10 cells had a 1.7-fold increase in GPx activity and were less sensitive to BSO, whereas MnSOD1 cells had a 1.5-fold increase in GPx activity and their sensitivity to BSO was intermediate between that of MnSOD10 and MnSOD13 cells. Although neo control cells also had increased sensitivity to BSO, they were much less sensitive than MnSOD transfectants. After cells were treated with BSO for 24 h, levels of GSH were extremely low and levels of GSSG were undetectable (data not shown). Cell apoptosis was examined by fluorescence microscopy flowing Hoechst staining of the cells. There was only minimal increased apoptosis in MnSOD-overexpressing cells when treated with BSO (data not shown), suggesting that cell death is due to necrosis.

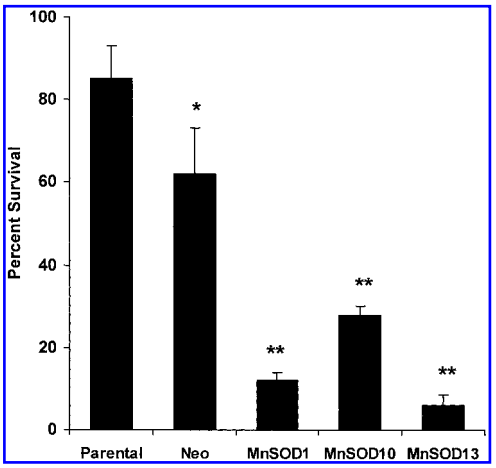

FIG. 3. MTT assay of cytotoxicity of BSO in RWPE-2 cells. Cells were plated at 5,000 cells per well in 96-well plates overnight and treated with 1 m*M* BSO for 72 h. Percent survival is cell viability relative to the corresponding cells without BSO treatment. Data represent means ± SD ($n = 5$). *$p < 0.05$ compared with the parental cells; **$p < 0.01$ compared with parental and neo cells.

Effects of MnSOD overexpression and a MnSOD mimic on cellular sensitivity to sodium selenite

Selenium is a key element for selenoproteins, most of which function as antioxidant enzymes, an example being GPx. On the other hand, at high levels selenium can function as a prooxidant by producing superoxide radicals (11). As shown in Fig. 4A, treatment with sodium selenite for 72 h resulted in cell death in RWPE-2 cells in a dose-dependent manner. All three MnSOD transfectants significantly decreased their sensitivity to the cytotoxic effect of sodium selenite as compared with the parental and the neo cells, whereas the neo cells had similar sensitivity to the parental cells. Similar to the effect of MnSOD overexpression, an MnSOD mimic MnTMPyP also protected the parental cells against the cytotoxic effect of sodium selenite in a dose-dependent manner (Fig 4B). There was no significant increase in apoptosis in cells treated with MnTMPyP as detected by Hoechst staining (data not shown). These results demonstrated that MnSOD could protect cells against the cytotoxic effects of selenium, suggesting that sodium selenite causes cell killing by the production of superoxide radicals.

Effects of MnSOD overexpression and a MnSOD mimic on cellular response to vitamin C

Vitamin C is an antioxidant that generally protects against oxidative stress. However, several studies demonstrated that vitamin C has anticancer activity through a prooxidant effect at high concentrations (24, 26, 42). One of these studies showed that vitamin C inhibited division and growth of human prostate cancer cells through the production of H_2O_2 (26). As shown in Fig. 5A, 2 m*M* vitamin C had a mild effect on the viability of

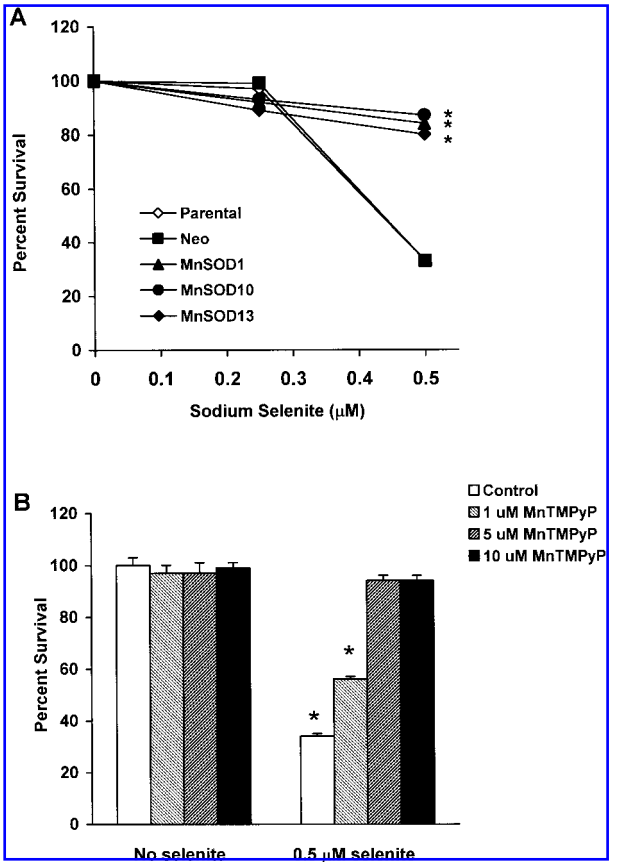

FIG. 4. MTT assay of the cytotoxic effect of sodium selenite in RWPE-2 cells. (A) Dose-dependent effect of sodium selenite in RWPE-2 parental, neo, and MnSOD-overexpressing cells. **(B)** Dose-dependent protective effect of the SOD mimic MnTMPyP on the cytotoxicity of sodium selenite in RWPE-2 parental cells. Cells were plated at 5,000 cells per well in 96-well plates overnight and treated with sodium selenite and/or MnTMPyP for 72 h. Percent survival is cell viability relative to 0 μ*M* sodium selenite in A or the control of no selenite in B. Data represent means ± SD ($n = 5$). *$p < 0.01$ compared with parental and neo cells at 0.5 μ*M* sodium selenite or compared with the control of no selenite.

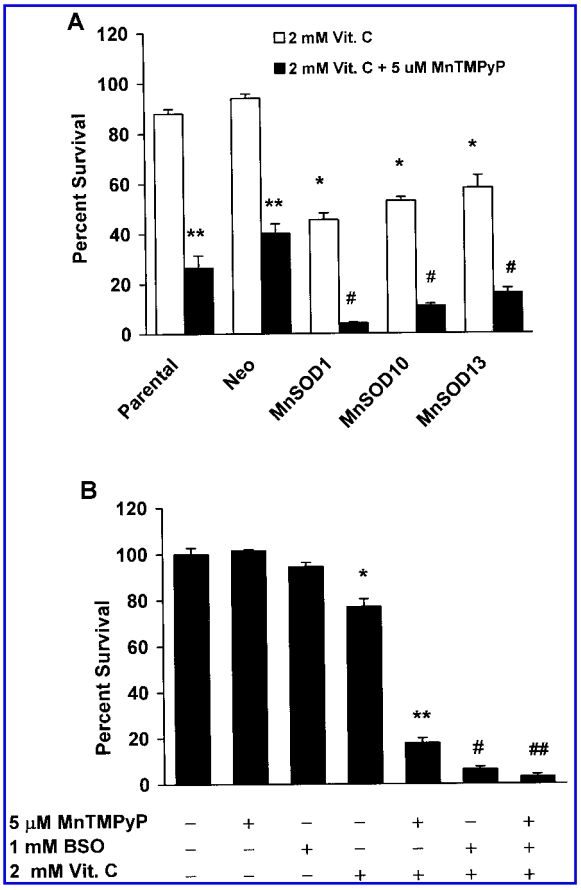

FIG. 5. (A) MTT assay of the effect of MnSOD overexpression and the SOD mimic MnTMPyP on vitamin C-induced cytotoxicity in RWPE-2 cells. Data represent means ± SD ($n = 5$). *$p < 0.01$ compared with parental and neo cells without MnTMPyP treatment; **$p < 0.01$ compared with parental and neo cells without MnTMPyP treatment; #$p < 0.01$ compared with corresponding cells without MnTMPyP treatment and parental and neo cells with MnTMPyP treatment. **(B)** Combined effects of MnTMPyP, vitamin C, and BSO in RWPE-2 parental cells. Data represent means ± SD ($n = 5$). *$p < 0.05$ compared with cells without treatment; **$p < 0.01$ compared with cells treated with MnTMPyP or vitamin C only; #$p < 0.01$ compared with cells treated with vitamin C or BSO only; ##$p < 0.01$ compared with cells treated with MnTMPyP plus vitamin C or BSO plus vitamin C. Cells were plated at 5,000 cells per well in 96-well plates overnight and treated with different agents for 72 h. Percent survival is cell viability relative to parental cells treated with vitamin C only in A or cells without treatment in B.

parental and neo cells, but significantly decreased the viability of all three MnSOD transfectants. Similar to the effect of MnSOD overexpression, parental and neo cells treated with vitamin C and the MnSOD mimic MnTMPyP showed a significant decrease in cell viability. MnTMPyP also enhanced the sensitivity of MnSOD-overexpressing cells to the cytotoxicity of vitamin C. When parental cells were treated with either MnTMPyP or BSO, they became more sensitive to vitamin C (Fig. 5B). In addition, MnTMPyP and BSO had a synergistic effect on cellular sensitivity to vitamin C (Fig. 5B). A significant increase in apoptosis was not detected in cells treated with these agents by Hoechst staining (data not shown).

DISCUSSION

MnSOD is a crucial antioxidant enzyme in biologic systems functioning to protect against oxidative stress, specifically superoxide radical stress or damage. MnSOD is also involved in cell redox regulation and controls signal transduction and gene expression (19, 32, 45, 47). The present study demonstrated that overexpression of MnSOD in RWPE-2 cells resulted in an increase in GSH and GSSG, a decrease in GSH:GSSG, cell growth inhibition, and increased levels of $p21^{Waf1}$. RWPE-2 cells with overexpression of MnSOD increased their sensitivity to the cytotoxicity of BSO. In addition, overexpression of MnSOD or treatment with an MnSOD mimic protected cells from the cytotoxicity

of selenium, but enhanced cell sensitivity to the cytotoxicity of vitamin C.

Accumulating evidence indicates that MnSOD is not simply an antioxidant enzyme, but also functions to regulate cell behavior. It has been postulated that MnSOD is also a tumor suppressor gene and that levels of MnSOD generally decreased in a variety of tumor cells compared with their normal counterparts (27, 38). Furthermore, overexpression of MnSOD by cDNA transfection suppressed the malignant phenotype of a variety of tumor types, including human prostate cancer cells (9, 22, 50). MnSOD can modulate cell redox state and, subsequently, signaling pathways, gene expression, and cell responses to other antioxidants and prooxidants (32, 45, 47, 49). Results from these studies strongly support the concept that overexpression of MnSOD may lead to an imbalance of antioxidant systems and subsequently to an oxidative shift in cell redox state, which may directly regulate cell growth by influencing gene expression, signal transduction, or mitochondrial metabolism.

The potential underlying mechanism of H_2O_2 accumulation by overexpression of MnSOD may be due to the imbalance between MnSOD and GPx in mitochondria, under which the production of H_2O_2 is greater than its removal. Alternatively, an increase in production of H_2O_2 by overexpression of MnSOD may result in an imbalanced increase in antioxidants and subsequent alteration of cell redox state. In the present study, levels of GSH were increased in MnSOD-overexpressing RWPE-2 cells. However, levels of GSSG were increased and GSH:GSSG was decreased, indicating a net oxidative shift in cell redox state by overexpression of MnSOD. Increased levels of GSH were likely to be a compensatory reaction of the cells to the prooxidative effects of overexpression of MnSOD. Cell death in MnSOD-overexpressing cells was significantly increased following GSH depletion by BSO, suggesting an increased production of H_2O_2 in MnSOD-overexpressing RWPE-2 cells because GSH is required for GPx to detoxify H_2O_2. RWPE-2 cells with MnSOD overexpression showed no changes or a slight increase in levels of CAT and GPx, which may not be sufficient to balance the prooxidative effects from MnSOD overexpression, particularly in mitochondria where levels of ROS are high.

Cyclin-dependent kinase inhibitor $p21^{Waf1}$ is a negative cell cycle regulator, which inhibits cell cycle progression through G_1/S or G_2/M checkpoints by inhibiting cyclin/cyclin-dependent kinase complexes. $p21^{waf1}$ is also known to be involved in stress response, apoptosis, and tumorigenesis (44). Studies have demonstrated that $p21^{Waf1}$ is a redox-sensitive gene that is up-regulated by a variety of oxidative stresses, including superoxide, H_2O_2, hyperoxia, and changes in GSH:GSSG status (31, 35). Our study demonstrated that overexpression of MnSOD increased levels of GSSG and decreased GSH:GSSG, indicating an oxidative shift in cell redox state in MnSOD-overexpressing cells. Therefore, up-regulation of $p21^{Waf1}$ by MnSOD overexpression may be through an oxidative-mediated mechanism. Cell growth inhibition by MnSOD overexpression may be due, at least in part, to the up-regulation of $p21^{Waf1}$. Cell growth inhibition can be due to cell cycle arrest or an increase in time of cell cycle progression. Our data demonstrated that overexpression of MnSOD in RWPE-2 cells inhibited cell growth mainly due to prolonged cell doubling time instead of cell arrest in a specific phase of the cell cycle. An up-regulation of $p21^{Waf1}$ may contribute to, but may not be the main regulator for cell growth inhibition following MnSOD overexpression.

Selenium is an essential trace element for humans and animals. Epidemiological studies have shown an inverse relationship between serum levels of selenium and the risk of prostate cancer (8, 43, 46). A recent clinical intervention trial demonstrated that selenium supplementation decreased the incidence of prostate, colon, and lung cancers and that selenium is a promising agent for cancer chemoprevention (10). Selenium is a key component for the enzymatic activity of GPx, which is responsible for detoxification of H_2O_2 and organic hydroperoxides in protecting cells against oxidative damage (34). Before natural chemical forms of selenium can be utilized for incorporation into GPx and other selenoproteins, they undergo thiol-dependent reductive metabolism with consumption of GSH and production of GSSG (11). In addition, hydrogen selenide, one of the intermediate metabolites, can be converted to elemental selenium with the production of superoxide radicals. Both consumption of GSH and production of superoxide radicals in selenium redox metabolism can cause an oxidative shift in cell redox state. Therefore, it is apparent that selenium can function as an antioxidant or a prooxidant.

Studies have demonstrated that selenium induced cell apoptosis in association with oxidative stress (36, 37, 48). Conversely, antioxidants protected cells from apoptosis induced by selenium (36, 48). Our previous study showed that sodium selenite induced apoptosis in LNCaP human prostate cancer cells and also decreased levels of GSH and increased levels of GSSG (48). Cell death induced by selenite was enhanced by cotreatment with BSO, but inhibited by cotreatment with the SOD mimic MnTMPyP or *N*-acetylcysteine. Additionally, it has been demonstrated that apoptosis induced by selenium was associated with mitochondrial injury and activation of caspase cascades (18, 48). The present study demonstrated that RWPE-2 cell death induced by selenite was inhibited by overexpression of MnSOD or the SOD mimic MnTMPyP. These results support the concept that selenium induces cell death by oxidative stress.

Epidemiological studies suggest that vitamin C, a water-soluble antioxidant, may have a protective effect against human tumors, but data are controversial (2, 17, 21, 25). It has been proposed that vitamin C protects against oxidative damage to DNA to reduce the risk of cancer by scavenging ROS. However, vitamin C can be autooxidized to produce semidehydroascorbate radicals or H_2O_2 and, therefore, also acts as a prooxidant, particularly in the presence of transition metal ions (20). Some studies have demonstrated that treatment with vitamin C increased DNA damage or lipid oxidation (20, 30). Whether vitamin C acts as an antioxidant or a prooxidant is dependent on the vitamin C concentration, the intracellular redox state, and/or levels of transition metals. The prooxidant effect of vitamin C has been proposed to be the potential mechanism of vitamin C in cancer treatment. Studies demonstrated that treatment with vitamin C reduced viability and DNA synthesis of human prostate cancer cells in a dose-dependent manner (24, 26, 42). The effects of vitamin C were inhibited by CAT, but not SOD, suggesting an involvement of H_2O_2 in vitamin C-induced cell death and growth inhibition. A recent study demonstrated that vitamin

C and dehydroascorbate induced cell cycle arrest at G_2/M phase, as well as potentiated oxidative stress induced by xanthine/xanthine oxidase, a superoxide-generating system (6). Consistent with these oxidative effects of vitamin C on human prostate cancer cells, our study demonstrated that a prooxidant state induced by treatment with BSO and the SOD mimic or overexpression of MnSOD enhanced prostate cancer cell sensitivity to vitamin C. The potential mechanism for the BSO effect is likely due to the depletion of GSH, which is required for ROS removal and conversion of vitamin C radicals to vitamin C. As a consequence of GSH depletion and H_2O_2 accumulation, cell killing was enhanced by the combined treatment of BSO with vitamin C. Increased sensitivity to vitamin C in cells with MnSOD overexpression is likely associated with oxidative stress due to an oxidative shift in cell redox state. Therefore, it is reasonable to postulate that effects of vitamin C on cancer cell killing or growth inhibition are due in part to a prooxidant mechanism or modulation of cell redox state. Therefore, levels of antioxidants and antioxidant enzymes can influence the anticancer efficacy of vitamin C.

The SOD mimic MnTMPyP is a stable manganese–porphyrin complex that has been demonstrated to catalyze dismutation of superoxide radical to H_2O_2, but unlike SOD, MnTMPyP acts like a NADPH/GSH:superoxide oxidoreductase *in vivo* (14). MnTMPyP also functions as a scavenger of H_2O_2 and nitric oxide (13, 29). It has been demonstrated that MnTMPyP protected *E. coli* or mammalian cells against superoxide damage by paraquat or pyocyanine (14, 16). MnTMPyP also protected mammalian cells against H_2O_2 toxicity (13), suggesting a CAT- or peroxidase-like activity. A lack of cell growth inhibition by MnTMPyP may due to its dual activities of scavenging superoxide and H_2O_2, which prevents accumulation of H_2O_2, the likely mechanism by which overexpression of MnSOD inhibits cell growth. This may be the reason why MnTMPyP alone only protected cells against oxidative damage from selenium without causing cell growth inhibition. On the other hand, one study demonstrated that MnTMPyP enhanced menadione-mediated cytotoxicity in mammalian cells (16). This study demonstrated that MnTMPyP enhanced the production of superoxide radical in cells in the presence of menadione through the redox cycling of MnTMPyP. Both MnTMPyP and menadione also potentiated the autooxidation of vitamin C. This may be the mechanism by which MnTMPyP enhanced vitamin C toxicity to cells in our study. The same study also demonstrated that MnTMPyP alone caused cell growth inhibition under 95% O_2 and 5% CO_2, but not under 95% air and 5% CO_2 (16). The cell growth inhibition by MnTPyP was cell density-dependent and was more effective in confluent cultures. Cell growth inhibition was not demonstrable in RWPE-2 cells treated with MnTMPyP in concentrations up to 50 μ*M* in our study (data not shown), suggesting that cell growth inhibition by MnTMPyP may be cell type- or cell redox state-dependent. Our data demonstrated that MnTMPyP functioned as an antioxidant or a prooxidant in RWPE-2 cells, depending on the cell redox state and the presence of other redox-modulating factors, but had no regulatory effects on cell growth, which was different from overexpression of MnSOD. This may also be due to the difference in subcellular localizations between MnSOD and MnTMPyP; the former is in mitochondria, whereas the latter is mainly in the cytosol (16).

Consistent with the results of previous studies, the present study demonstrates that overexpression of MnSOD altered cell redox state and sensitivity to oxidative stress, and inhibited cell proliferation in a k-*ras* oncogene-transformed human prostate cell line. The results suggest that cell redox state may play an important role in controlling cell behavior, such as proliferation and sensitivity to radiation and chemotherapy. Furthermore, the data indicate that effects of antioxidant enzymes and antioxidants on cell behavior are complex, depending on not only levels, but also the balance of antioxidant enzymes. MnSOD generally functions as an antioxidant by removing superoxide radicals. On the other hand, it can also function as a prooxidant by producing H_2O_2 through the dismutative catalysis of superoxide radicals when cellular peroxide-removing capability is inadequate, such as no corresponding increase in CAT or GPx or under circumstances of GSH depletion. Here, we demonstrate that overexpression of MnSOD protected against oxidative stress induced by a superoxide-generating compound, sodium selenite, but resulted in prooxidant effects as demonstrated by a decrease in GSH:GSSG and increased sensitivity to GSH-depleting agent BSO. Our data also demonstrate prooxidative effects of the SOD mimic MnTMPyP, selenium, and vitamin C, suggesting paradoxical effects of some antioxidants and antioxidant enzymes, which may depend on levels and the balance of antioxidant systems and the presence of other redox-modulating factors in biologic systems. To achieve successful therapies involving ROS generation, such as radiation and chemotherapy using free radical-generating agents, it is important to determine cell and organelle redox state and levels of antioxidant enzymes and other small molecular weight antioxidants. Future studies will be necessary to determine how to use gene transfection strategies to alter antioxidant enzymes and cell redox state or to use SOD mimics and other small molecular weight antioxidants or prooxidants to alter cell redox state and subsequently to lead to more successful cancer therapies.

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ABBREVIATIONS

BSO, buthionine sulfoximine; CAT, catalase; CuZnSOD, copper-zinc superoxide dismutase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, glutathione disulfide or oxidized form of glutathione; H_2O_2, hydrogen peroxide; MnSOD, manganese superoxide dismutase; MnTMPyP, manganese (III) tetrakis(1-methyl-4-pyridyl)porphyrin pen-

tachloride; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBT, nitro blue tetrazolium; PBS, phosphate-buffered saline; redox, reduction/oxidation; ROS, reactive oxygen species; SOD, superoxide dismutase; TTBS, Tris buffer with Tween 20.

REFERENCES

1. Abate C, Patel L, Rauscher FJ 3d, and Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science* 249: 1157–1161, 1990.
2. Abdulla M and Gruber P. Role of diet modification in cancer prevention. *Biofactors* 12: 45–51, 2000.
3. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, and Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* 272: 217–221, 1997.
4. Baker AM, Oberley LW, and Cohen MB. Expression of antioxidant enzymes in human prostatic adenocarcinoma. *Prostate* 32: 229–233, 1997.
5. Bello D, Webber MM, Kleinman HK, Wartinger DD, and Rhim JS. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. *Carcinogenesis* 18: 1215–1223, 1997.
6. Bijur GN, Briggs B, Hitchcock CL, and Williams MV. Ascorbic acid-dehydroascorbate induces cell cycle arrest at G_2/M DNA damage checkpoint during oxidative stress. *Environ Mol Mutagen* 33: 144–152, 1999.
7. Bostwick DG, Alexander EE, Singh R, Shan A, Qian J, Santella RM, Oberley LW, Yan T, Zhong W, Jiang X, and Oberley TD. Antioxidant enzyme expression and reactive oxygen species damage in prostatic intraepithelial neoplasia and cancer. *Cancer* 89: 123–134, 2000.
8. Brooks JD, Metter EJ, Chan DW, Sokoll LJ, Landis P, Nelson WG, Muller D, Andres R, and Carter HB. Plasma selenium level before diagnosis and the risk of prostate cancer development. *J Urol* 166: 2034–2038, 2001.
9. Church SL, Grant JW, Ridnour LA, Oberley LW, Swanson PE, Meltzer PS, and Trent JM. Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc Natl Acad Sci U S A* 90: 3113–3117, 1993.
10. Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL Jr, Park HK, Sanders BB Jr, Smith CL, and Taylor JR. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* 276: 1957–1963, 1996.
11. Combs GF Jr and Gray WP. Chemopreventive agents: selenium. *Pharmacol Ther* 79: 179–192, 1998.
12. Dalton TP, Shertzer HG, and Puge A. Regulation of gene expression by reactive oxygen. *Annu Rev Pharmacol Toxicol* 39: 67–101, 1999.
13. Day BJ, Fridovich I, and Crapo JD. Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. *Arch Biochem Biophys* 347: 256–262, 1997.
14. Faulkner KM, Liochev SI, and Fridovich I. Stable Mn(III) porphyrins mimic superoxide dismutase in vitro and substitute for it in vivo. *J Biol Chem* 269: 23471–23476, 1994.
15. Finkel T. Oxygen radicals and signaling. *Curr Opin Cell Biol* 10: 248–253, 1998.
16. Gardner PR, Nguyen DD, and White CW. Superoxide scavenging by Mn(II/III) tetrakis (1-methyl-4-pyridyl) porphyrin in mammalian cells. *Arch Biochem Biophys* 325: 20–28, 1996.
17. Jacobs EJ, Connell CJ, Patel AV, Chao A, Rodriguez C, Seymour J, McCullough ML, Calle EE, and Thun MJ. Vitamin C and vitamin E supplement use and colorectal cancer mortality in a large American Cancer Society cohort. *Cancer Epidemiol Biomarkers Prev* 10: 17–23, 2001.
18. Jiang C, Wang Z, Ganther H, and Lu J. Caspases as key executors of methyl selenium-induced apoptosis (anoikis) of DU-145 prostate cancer cells. *Cancer Res* 61: 3062–3070, 2001.
19. Kim KH, Rodriguez AM, Carrico PM, and Melendez JA. Potential mechanisms for the inhibition of tumor cell growth by manganese superoxide dismutase. *Antioxid Redox Signal* 3: 361–373, 2001.
20. Lee SH, Oe T, and Blair IA. Vitamin C-induced decomposition of lipid hydroperoxides to endogenous genotoxins. *Science* 292: 2083–2086, 2001.
21. Levi F, Pasche C, Lucchini F, and La Vecchia C. Dietary intake of selected micronutrients and breast-cancer risk. *Int J Cancer* 91: 260–263, 2001.
22. Li N, Oberley TD, Oberley LW, and Zhong W. Overexpression of manganese superoxide dismutase in DU145 human prostate carcinoma cells has multiple effects on cell phenotype. *Prostate* 35: 221–233, 1998.
23. Li S, Yan T, Yang JQ, Oberley TD, and Oberley LW. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res* 60: 3927–3939, 2000.
24. Maramag C, Menon M, Balaji KC, Reddy PG, and Laxmanan S. Effect of vitamin C on prostate cancer cells in vitro: effect on cell number, viability, and DNA synthesis. *Prostate* 32: 188–195, 1997.
25. McDermott JH. Antioxidant nutrients: current dietary recommendations and research update. *J Am Pharm Assoc* 40: 785–799, 2000.
26. Menon M, Maramag C, Malhotra RK, and Seethalakshmi L. Effect of vitamin C on androgen independent prostate cancer cells (PC3 and Mat-Ly-Lu) in vitro: involvement of reactive oxygen species-effect on cell number, viability and DNA synthesis. *Cancer Biochem Biophys* 16: 17–30, 1998.
27. Oberley TD and Oberley LW. Antioxidant enzyme levels in cancer. *Histol Histopathol* 12: 525–535, 1997.
28. Oberley TD, Zhong W, Szweda LI, and Oberley LW. Localization of antioxidant enzymes and oxidative damage products in normal and malignant prostate epithelium. *Prostate* 44: 144–155, 2000.
29. Pfeiffer S, Schrammel A, Koesling D, Schmidt K, and Mayer B. Molecular actions of a Mn(III)porphyrin superoxide dismutase mimetic and peroxynitrite scavenger: reaction with nitric oxide and direct inhibition of NO

synthase and soluble guanylyl cyclase. *Mol Pharmacol* 53: 795–800, 1998.
30. Podmore ID, Griffiths HR, Herbert KE, Mistry N, Mistry P, and Lunec J. Vitamin C exhibits pro-oxidant properties. *Nature* 392: 559, 1998.
31. Qiu X, Forman HJ, Schonthal AH, and Cadenas E. Induction of p21 mediated by reactive oxygen species formed during the metabolism of aziridinylbenzoquinones by HCT116 cells. *J Biol Chem* 271: 31915–31921, 1996.
32. Ranganathan AC, Nelson KK, Rodriguez AM, Kim KH, Tower GB, Rutter JL, Brinckerhoff CE, Huang TT, Epstein CJ, Jeffrey JJ, and Melendez JA. Manganese superoxide dismutase signals matrix metalloproteinase expression via H_2O_2-dependent ERK1/2 activation. *J Biol Chem* 276: 14264–14270, 2001.
33. Rodriguez AM, Carrico PM, Mazurkiewicz JE, and Melendez JA. Mitochondrial or cytosolic catalase reverses the MnSOD-dependent inhibition of proliferation by enhancing respiratory chain activity, net ATP production, and decreasing the steady state levels of H_2O_2. *Free Radic Biol Med* 29: 801–813, 2000.
34. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, and Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179: 588–590, 1973.
35. Russo T, Zambrano N, Esposito F, Ammendola R, Cimino F, Fiscella M, Jackman J, O'Connor PM, Anderson CW, and Appella E. A p53-independent pathway for activation of WAF1/CIP1 expression following oxidative stress. *J Biol Chem* 270: 29386–29391, 1995.
36. Shen HM, Yang CF, and Ong CN. Sodium selenite-induced oxidative stress and apoptosis in human hepatoma $HepG_2$ cells. *Int J Cancer* 81: 820–828, 1999.
37. Spallholz JE, Shriver BJ, and Reid TW. Dimethyldiselenide and methylseleninic acid generate superoxide in an in vitro chemiluminescence assay in the presence of glutathione: implications for the anticarcinogenic activity of L-selenomethionine and L-Se-methylselenocysteine. *Nutr Cancer* 40: 34–41, 2001.
38. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic Biol Med* 8: 583–599, 1990.
39. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, and Finkel T. Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* 270: 296–299, 1995.
40. Thannickal VJ and Fanburg BL. Activation of an H_2O_2-generating NADH oxidase in human lung fibroblasts by transforming growth factor beta 1. *J Biol Chem* 270: 30334–30338, 1995.
41. Toledano MB and Leonard WJ. Modulation of transcription factor NF-kappa B binding activity by oxidation-reduction in vitro. *Proc Natl Acad Sci U S A* 88: 4328–4332, 1991.
42. Venugopal M, Jamison JM, Gilloteaux J, Koch JA, Summers M, Hoke J, Sowick C, and Summers JL. Synergistic antitumour activity of vitamins C and K3 against human prostate carcinoma cell lines. *Cell Biol Int* 20: 787–797, 1996.
43. Vogt TM, Ziegler RG, Graubard BI, Swanson CA, Greenberg RS, Schoenberg JB, Swanson GM, Hayes RB, and Mayne ST. Serum selenium and risk of prostate cancer in U.S. blacks and whites. *Int J Cancer* 103: 664–670, 2003.
44. Weinberg WC and Denning MF. P21Waf1 control of epithelial cell cycle and cell fate. *Crit Rev Oral Biol Med* 13: 453–464, 2002.
45. Wenk J, Brenneisen P, Wlaschek M, Poswig A, Briviba K, Oberley TD, and Scharffetter-Kochanek K. Stable overexpression of manganese superoxide dismutase in mitochondria identifies hydrogen peroxide as a major oxidant in the AP-1-mediated induction of matrix-degrading metalloprotease-1. *J Biol Chem* 274: 25869–25876, 1999.
46. Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB, and Giovannucci E. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. *J Natl Cancer Inst* 90: 1219–1224, 1998.
47. Zhang HJ, Zhao W, Venkataraman S, Robbins ME, Buettner GR, Kregel KC, and Oberley LW. Activation of matrix metalloproteinase-2 by overexpression of manganese superoxide dismutase in human breast cancer MCF-7 cells involves reactive oxygen species. *J Biol Chem* 277: 20919–20926, 2002.
48. Zhong W and Oberley TD. Redox-mediated effects of selenium on apoptosis and cell cycle in the LNCaP human prostate cancer cell line. *Cancer Res* 61: 7071–7078, 2001.
49. Zhong W, Oberley LW, Oberley TD, Yan T, Domann FE, and St. Clair DK. Inhibition of cell growth and sensitization to oxidative damage by overexpression of manganese superoxide dismutase in rat glioma cells. *Cell Growth Differ* 7: 1175–1186, 1996.
50. Zhong W, Oberley LW, Oberley TD, and St. Clair DK. Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. *Oncogene* 14: 481–490, 1997.

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2. Junqiang Tian , Donna M. Peehl , Susan J. Knox . 2010. Metalloporphyrin Synergizes with Ascorbic Acid to Inhibit Cancer Cell Growth Through Fenton Chemistry. *Cancer Biotherapy & Radiopharmaceuticals* **25**:4, 439-448. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
3. Vasundara Venkateswaran, Laurence H. Klotz. 2010. Diet and prostate cancer: mechanisms of action and implications for chemoprevention. *Nature Reviews Urology* **7**:8, 442-453. [CrossRef]
4. Lorenzo Galluzzi, Eugenia Morselli, Oliver Kepp, Ilio Vitale, Alice Rigoni, Erika Vacchelli, Mickael Michaud, Hans Zischka, Maria Castedo, Guido Kroemer. 2010. Mitochondrial gateways to cancer. *Molecular Aspects of Medicine* **31**:1, 1-20. [CrossRef]
5. Huan Ling Liang, Jody Arsenault, Jordan Mortensen, Frank Park, Christopher P. Johnson, Vani Nilakantan. 2009. Partial attenuation of cytotoxicity and apoptosis by SOD1 in ischemic renal epithelial cells. *Apoptosis* **14**:10, 1176-1189. [CrossRef]
6. Nong Xiang, Rui Zhao, Weixiong Zhong. 2009. Sodium selenite induces apoptosis by generation of superoxide via the mitochondrial-dependent pathway in human prostate cancer cells. *Cancer Chemotherapy and Pharmacology* **63**:2, 351-362. [CrossRef]
7. R BHATTACHARYYA, B HUSBECK, D FELDMAN, S KNOX. 2008. Selenite Treatment Inhibits LAPC-4 Tumor Growth and Prostate-Specific Antigen Secretion in a Xenograft Model of Human Prostate Cancer. *International Journal of Radiation OncologyBiologyPhysics* **72**:3, 935-940. [CrossRef]
8. Farideh Mehraein-Ghomi, Elyse Lee, Dawn R. Church, Todd A. Thompson, Hirak S. Basu, George Wilding. 2008. JunD mediates androgen-induced oxidative stress in androgen dependent LNCaP human prostate cancer cells. *The Prostate* **68**:9, 924-934. [CrossRef]
9. E M Hurt, S B Thomas, B Peng, W L Farrar. 2007. Molecular consequences of SOD2 expression in epigenetically silenced pancreatic carcinoma cell lines. *British Journal of Cancer* **97**:8, 1116-1123. [CrossRef]
10. Guang-Xun Li, Hongbo Hu, Cheng Jiang, Todd Schuster, Junxuan Lü. 2007. Differential involvement of reactive oxygen species in apoptosis induced by two classes of selenium compounds in human prostate cancer cells. *International Journal of Cancer* **120**:9, 2034-2043. [CrossRef]
11. A BACSI, M WOODBERRY, M KRUZEL, I BOLDOGH. 2007. Colostrinin delays the onset of proliferative senescence of diploid murine fibroblast cells. *Neuropeptides* **41**:2, 93-101. [CrossRef]
12. W LO, M TSAI, Y TSAI, C HUA, F TSAI, S HUANG, C TSAI, C LAI. 2007. Identification of over-expressed proteins in oral squamous cell carcinoma (OSCC) patients by clinical proteomic analysis. *Clinica Chimica Acta* **376**:1-2, 101-107. [CrossRef]
13. Dr. Shinya Toyokuni . 2006. Novel Aspects of Oxidative Stress-Associated Carcinogenesis. *Antioxidants & Redox Signaling* **8**:7-8, 1373-1377. [Abstract] [Full Text PDF] [Full Text PDF with Links]
14. B. Husbeck, L. Nonn, D.M. Peehl, S.J. Knox. 2006. Tumor-selective killing by selenite in patient-matched pairs of normal and malignant prostate cells. *The Prostate* **66**:2, 218-225. [CrossRef]
15. Yijun Qi, Jen-Fu Chiu, Lidong Wang, Dora L. W. Kwong, Qing-Yu He. 2005. Comparative proteomic analysis of esophageal squamous cell carcinoma. *PROTEOMICS* **5**:11, 2960-2971. [CrossRef]
16. Shani Shilo , Michal Aharoni-Simon , Oren Tirosh . 2005. Selenium Attenuates Expression of MnSOD and Uncoupling Protein 2 in J774.2 Macrophages: Molecular Mechanism for Its Cell-Death and Antiinflammatory Activity. *Antioxidants & Redox Signaling* **7**:1-2, 276-286. [Abstract] [Full Text PDF] [Full Text PDF with Links]

17. Ivana Grivicich, Andréa Regner, Adriana B. da Rocha, Guilherme B. Kayser, Daniel P. Schunemann, Luciano B. Grass, Pedro A.G. Alves, João A.P. Henriques, Gilberto Schwartsmann. 2005. The Irinotecan/5-Fluorouracil Combination Induces Apoptosis and Enhances Manganese Superoxide Dismutase Activity in HT-29 Human Colon Carcinoma Cells. *Chemotherapy* **51**:2-3, 93-102. [CrossRef]
18. Terry D. Oberley . 2004. Mitochondria, Manganese Superoxide Dismutase, and Cancer. *Antioxidants & Redox Signaling* **6**:3, 483-487. [Citation] [Full Text PDF] [Full Text PDF with Links]