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Differential actions of selenium on Tumor vs. Normal increase radiosensitivity

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1. Introduction

Selenium is an essential trace element required for normal health and is also a promising agent for preventing cancer. In clinical trials, selenium has significantly protective effects against lung, prostate, colon, and head and neck cancer (1). Solid tumors in hypoxic and hypoxic/reoxygenation condition have long been considered a problem in cancer therapy. Hypoxic tumor cells were shown to be more resistant to radiotherapy (RT) and many conventional chemotherapeutic agents than their normoxic counterparts (2,3). Lung cancer is the leading cause of cancer death in both men and women in the United States. Non-small cell lung cancer (NSCLC) accounts for more than 75% of all lung cancers. RT is the routine treatment modality for these lung cancer patients. The goal of RT is to deliver cytotoxicity to the tumor site, while minimizing cytotoxicity to the surrounding normal tissues. Peroxiredoxin I (Prx I) has been reported to be highly elevated in lung cancer (4,5) compared to that in normal tissues. NF-E2-related factor 2 (Nrf2) assumed as one of the major transcription factors of Prx I. Nrf2 plays a critical role in regulating expression of antioxidant and phase II drug-metabolizing enzymes, thereby contributing to detoxification, elimination, and protection of tissues or cells against environmental oxidative stress or xenobiotics including medicine (6,7).

In the present study, we demonstrate that pretreatment with selenium has differential effect on tumor and normal tissue that might be associated with different regulation of Nrf2 under the circumstances of surrounding microenvironment.

2. Materials and Methods

2.1 Animals

Female athymic nude mice (nu/nu, body weight, 20–25 g), 6–12 weeks of age, were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and kept 5 mice to a cage. Mice were fed with water and food ad libitum according to Institutional Animal Care and Use Committee approval.

2.2 Drug doses and schedules.

SeMet was administered per orally (p.o.) once a day for 21 days at 0.2 mg/mouse and the first dose given 7 days before radiation therapy 8 Gy/day × 5 days. For molecular study, SeMet was given p.o. of 0.2 mg/mouse/day for 7 days. Each experiment was repeated at least twice.

2.3. Electrophoretic mobility shift assay (EMSA).

Double-stranded oligonucleotide probes containing Nrf2 binding NF-E2 consensus sequences 5'-TGG-GGAACCTGTGCTGAGTCACTGGAG-3', AS 5'-CTCCAGTGACTCAGCACAGGTTCCCCA-3' (Santa Cruz Biotechnology, Santa Cruz, CA) and EpRE sequence were end-labeled with [γ-^{32}P] ATP using T4 polynucleotide kinase. The nuclear extract was incubated at room temperature for 15 min with [γ-^{32}P]-labeled probe in a binding buffer and DNA/nuclear protein complexes were electophoresed on 4% nondenaturing acrylamide gel. The gel was dried and exposed overnight to an X-ray film at -80°C. The film was developed for analysis.

2.4. Western blot analysis.

Cell lysates were prepared by extracting proteins with RIPA supplemented with protease. Equal amounts of the proteins were separated on 8 or 12% SDS-PAGE for Nrf2 and Akt or Prx I, respectively and transferred to nitrocellulose membranes (Bio-Rad, CA). The membranes were blocked with 5% skim milk in Tris-buffered saline and then incubated with primary antibodies for 1 h at room temperature. Blots were developed by peroxidase-conjugated secondary antibody, and proteins were visualized by enhanced chemiluminescence (ECL) reagents according to the manufacturer's recommendation (Amersham Biosciences, England). The experiments were repeated at least three times.

3. Results

3.1 Selenium is a potential radiosensitizer to nude mice bearing A549 tumor xenograft.

To determine whether selenium sensitize radiation treatment on A549 human lung cancer cells, antitumor activity was measured by tumor size of xenografted mice. Mice were daily p.o. given with selenium at 0.2 mg/mouse for 21 d from 7 d before RT (8 Gy/day for 5 days) and the tumor size was monitored for 50 days. Combined treatment of selenium with RT was dramatically inhibited tumor growth compared to selenium alone or RT alone. In addition, apoptosis in A549 tumor tissues of the mice treated with selenium and RT was also more increased.

3.2 Selenium differentially regulates Prx I and Nrf2 in A549 lung tumor tissues vs. normal lung tissues on xeno -graft nude mice.

We hypothesized that selenium pretreatment lead to molecular level changes in tumor microenvironment to sensitize radiation. Since selenium is well accepted as a strong antioxidant, we first examined the expression of Prx I protein and its transcription factor Nrf2 by western blot analysis or EMSA. A549 xenograft nude mice were administered with selenium (0.2 mg/mouse/day) for 7days and they were sacrificed 24 h after selenium treatment. Selenium significantly decreased Prx I in the tumor tissue, whereas Prx I and Nrf2 was increased following treatment with selenium in normal tissue. Therefore, selenium differentially regulates Prx I and Nrf2 in A549 lung tumor tissue compared to normal lung tissue.

3.3 Selenium differentially regulates Prx1 and Nrf2 under normoxia vs. hypoxia.

We questioned why selenium has a differential effect on Prx I and its transcription factor Nrf2 in tumor and normal. Since hypoxic condition is one of the characteristics of tumors, we employed normoxic and hypoxia/reoxygenation conditions in order to mimic the hypoxic tumor microenvironment and tested whether selenium differentially regulates Nrf2 binding activity in *in vitro* system. Transient hypoxia/reoxygenation increased Nrf2 binding activity but the treatment of selenium was remarkably decreased under hypoxia and the level reached a maximum significance at 2h reoxygenation after hypoxia for 4h. In contrast, the Nrf2 binding activity was further increased when cells were under normoxic condition, in the presence of selenium at the same time point. The results suggested that the differential role of selenium might be associated with Nrf2 regulation under hypoxia/reoxygenation.

4. Conclusion

Our studies based on human lung cancer xenograft in preclinical models, which showed selenium pretreatment can sensitize to tumor tissues with radiotherapy but not to normal tissues. We found that selenium differentially regulates Prx I and Nrf2, a regulator of detoxifying enzymes, depending on their microenvironment and render radiosensitivity of tumor. These *in vivo* and *in vitro* studies provide convincing rationale for differential effect of selenium on tumor vs. normal. Although we currently do not understand the sophisticated signaling mechanisms of the regulation of Nrf2 by selenium, our findings demonstrate that selenium may be exploited as adjuvant agent with radiotherapy or chemotherapy.

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