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Enhancement of Radiation Sensitivity by Seleno-Methionine

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Abstract

Since selenium has been widely known for their anti-proliferative and anti-growth effect, we used selenium compounds to investigate their effect on cellular radiation response. First we used western blot analysis to screen for differential protein expression by selenium treatment. Expression of proteins involved in radiation response, cell cycle and cell proliferation showed significant change by selenium treatment. P53, a major mediator of radiation response, increased its protein level by treatment of cancer cell lines with selenium compound. Increased p53 protein was correlated with the higher activity as shown by reporter assay. Consistent with the increased expression and activity of p53, which causes anti-proliferative effect on cells, proteins involved in cell proliferation decreased. Akt and cyclin D1, cell proliferation related proteins, decreased significantly. Decreased Akt expression could be seen through all three isotypes. Increased expression of anti-proliferative, cell death-causing p53 proteins significantly reduced cell viability when the compound treatment and ionizing gamma irradiation were combined. The enhanced cell killing or reduced cell survival effect by combined treatment of cells with radiation and selenium compounds provides a

paradigm where pretreatment of cells with cell death enhancing drugs and subsequent radiation could lead to a better radiation therapy.

Introduction

Selenium is an essential trace element. Selenium is incorporated into cellular protein post-translationally and mostly involved in cellular redox control. Selenium has been known for cancer-protective effect. Human epidemiological evidence indicates inverse relationship between selenium intake and the risk of cancer overall, particularly in men (1). In terms of cancer types, the evidence for cancer-protective effect is strong for lung, esophageal, prostate and gastric cancer, but there is very little information about selenium's effect on radiation therapy. Selenium supplementation in the form of selenized yeast, either alone or with other antioxidants reduces the risk of cancer. Possible mechanisms have been proposed to explain the cancer-protective effects of selenium compounds. Chemopreventive effect of selenium might be mediated by selenoproteins, GPX (glutathione peroxidase) and TR(thioredoxin reductase) which remove cancer-promoting reactive oxygen species. Another mechanism involves growth inhibition and apoptosis-inducing activity of selenium (2,3).

The objective of this study was to take advantage of selenium's cancer-protective effect to enhance radiation treatment of cancer cells. We show here that combined treatment of cells with selenium compounds and ionizing radiation specifically decreased cell proliferating proteins whereas pro-apoptotic protein p53 increased. These data are consistent with those of cell viability where co-treatment of cancer cell lines with both selenium and irradiation reduced cell viability.

Material and method

Cell culture and treatment: HeLa cells and NCI-H460 (human lung cancer cell lines) were grown with DMEM+10% FBS. For selenium treatment, seleno-DL-methionine (SeMet,

Sigma) was added to cultured cells and incubated upto 2 days.

For western analysis, cells were harvested at the indicated time points and processed to make whole cell lysate. Equal amount of cell extracts were used to run SDS-PAGE. Proteins in SDS-PAGE gel were transferred to nitrocellulose membrane to detect specific protein with antibodies. Antibodies were purchased from Santa Cruz or from Signal Transduction. For detection of protein bands, we used ECL solution from Perkin Elmer.

Reporter assay: transcriptional activity of p53 was measured using reporter assay. p53 reporter construct was made to have multiple copies of p53 binding site adapted from p21 promoter. Cells were transfected with reporter construct and beta-galactosidase for normalization purpose. After one or two days of protein expression with the constructs, cells were fed with fresh media and SeMet and then incubated further. We followed Promega manual for cell extract preparation and reporter assays. Measurement of reporter activity was done with Promega kit. Reporter activity was expressed as ratio of specific reporter activity over beta-galactosidase activity.

Results and discussion

Since p53 is one of the main regulators of radiation response, we followed p53 protein after SeMet treatment of human lung cancer cell lines, NCI-H460.

P53 protein was moderately increased after 24 to 48 hours by SeMet treatment (see Fig.1).

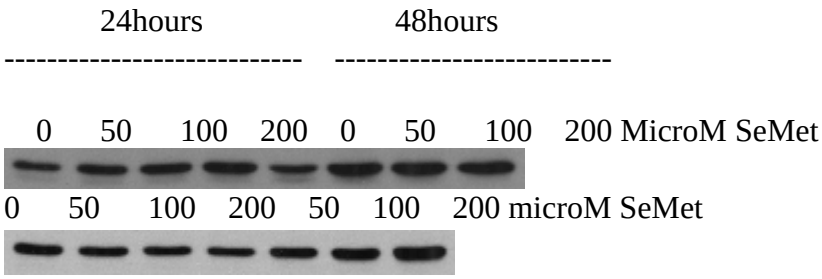

Figure 1. H-460 cells were treated with the indicated concentration of SeMet. After24 or 48

hours of incubation with the chemical, cells were washed and processed for western analysis. p53 antibody was used to detect cellular level of p53 protein. Increased p53 protein can be seen in higher concentration (200 microM) of SeMet.

We failed to detect significant increase of p53 until 24 hours of incubation. This result suggests that the effect of selenium on cellular protein level might be caused by selenium metabolite. Requirement of one or two days of incubation time before seeing any effect on protein level might reflect a time period for selenium metabolism to occur. The increase was dose-dependent with higher dose producing more p53.

p53 is involved in cell cycle, death, and growth inhibition by selectiveegulating genes which involved in aforementioned activities. Activity of p53 is modulated mainly by post-translational mechanism which involves phosphorylation-induced stabilization of the protein. To test whether the increase in p53 protein was correlated with its phosphorylation, we analysed phosphorylated p53 (Fig.1). We observed moderate increase of p53 phosphorylation at serine 15 residue. The increase was clear after 36 hours of incubation with 100uM SeMet. Increased phosphorylation might have been a reason for the increased protein level.

We reasoned if the abundance of p53 is to play a major role in cell killing, then increased p53 protein level should cause equivalent increase in transcriptional activity. To test the possibility, we used reporter assay(Fig. 2).

p53 reporter constructs are specific to p53 since it is made with multiple copies of p53-binding sequence from p21 promoter. Reporter activity increased with dose-dependent fashion. Increased reporter activity which is consistent with protein level was measured after 48 hour incubation with SeMet.

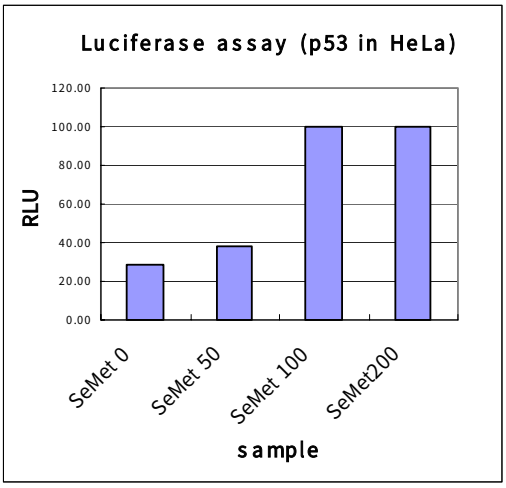

Figure 2. Reporter assay to measure p53's transcriptional activity. HeLa cells were treated with indicated amount of SeMet and p53 promoter fusion reporter construct was used to measure reporter activity. SeMet increased p53's transcriptional activity.

Increased level of pro-apoptotic proteins is often accompanied by decreased level of proteins involved in cell growth and proliferation. We screened for differential protein expression of selected cell proliferation proteins. Among them, cyclin D1 and Akt showed dramatically reduced protein levels by SeMet treatment (Fig. 3).

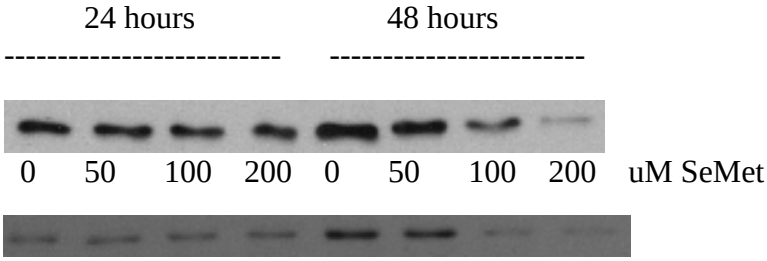

Figure 3. Western analysis of Akt and Cyclin D1. Upper panel shows Akt and lower, cyclin D1,

Cyclin D1 is involved in G1/S phase transition of cell cycle. Decreased cyclin D1 probably causes G1 cell cycle arrest. Unlike a previous report which indicated that selenium modulate cell cycle by inducing expression of p21 and p27 rather than by Cyclin D1 change, our results

revealed that p21 and p27 levels were constant through various concentration of SeMet. This difference might have been caused by the different cell lines used. We used human lung cancer cell line whereas other group used prostate cancer cell lines (4). Despite the difference in cell line, we could observe that SeMet caused similar cell cycle arrest at G1 phase.

Akt protein is a main proliferation-signaling molecule in cells. All three isotypes of Akt were decreased by SeMet treatment. The decrease was pronounced at higher doses (>100uM) after 48 hours of incubation which is consistent with other expression pattern.

We tested whether SeMet-induced condition of increased proapoptotic protein and decreased proliferating signal leads to reduced cell viability by further irradiation with ionizing radiation (Fig. 4). Combined treatment of cancer cell lines by SeMet and irradiation caused decreased cell viablity as assayed by colony forming assay. Decrease of cell viability by combined treatment was more pronounced than radiation alone.

Colony forming assay required about 7 days to complete. In this time frame, the beginning one or two days of SeMet treatment was enough to cause a significant effect on cell viability.

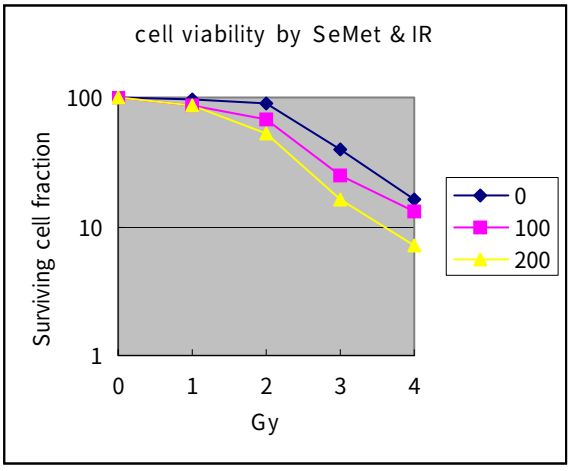

Figure 4. Enhanced cell killing effect of combined treatment of H-460 cells. Cells were treated either IR alone (0 seMet) or IR+SeMet(100 or 200 microM).

Less irradiation was used when combined with SeMet treatment to produce comparable result with higher dose. We may design similar scheme to devise a better radiation therapy to treat cancer.Effective drugs that can make cell environment pro-apoptotic could be combined with irradiation to treat cancer.

References

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