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Preliminary Study of Selenium (Se) Toxicity in Human Prostate Carcinoma (PC3) Cells with the Overexpression of Selenocysteine Synthase (*SecS*) Gene

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Abstract

Selenium (Se) is a trace element required for normal body function. Its supplementation of human diet at standard optimum amount prevents oxidative damages in cells and could be a viable method in the prevention of diseases related to DNA damage, including cancer, neurodegenerative diseases and aging. While Se anticancer properties have been linked to its ability to remove excess Reactive Oxygen Species (ROS) in cells, the underlying molecular mechanism remains unknown. Recent studies have shown that the removal of ROS alone cannot account for Se anticancer properties. To really comprehend the molecular basis of Se anticancer properties, current researches now focus on the metabolism of Se in the cell, especially Se-containing amino acids. Selenocysteine (Sec) is a novel amino acid and one of the selenium-containing compounds in the cell. It is essential in the maintenance of the integrity of its parent proteins, some of which include enzymes such as Glutathione Peroxidases (GPXs) and Thioredoxin Reductases (TrXs). We propose in this study that the overproduction of Sec via the overexpression of Selenocysteine synthase (*SecS*) gene and Se supplementation induced cell death in Prostate Carcinoma (PC-3) cells. Although the mechanism underlying the cell death induction is unknown, we propose it could be due to the random incorporation of Sec into proteins at high concentration, causing premature protein degradation and cell death. The outcome of this study showed that increasing the concentration of intracellular Se-containing amino acids may provide important clinical implications for the treatment of cancer.

Keywords

Selenium, Reactive Oxygen Species, Selenocysteine, Prostate Carcinoma,

Selenocysteine Synthase Gene

1. Introduction

Selenium (Se) is important for normal body function because of its antioxidant properties that are very essential in the prevention of cancer, aging and neurodegenerative diseases. In prokaryotes and eukaryotes, Se is found as a constituent of a novel amino acid, Selenocysteine (Sec) [1] [2]. In this amino acid, the constituent sulfur is replaced by Se (**Figure 1**). Sec is a constituent of about 25 naturally occurring Selenoproteins in the human body [2]. Prominent among these Selenoproteins are Glutathione Peroxidases (GPXs), Thioredoxin Reductases (TrXs), Formate and Glycine Dehydrogenases, Iodothyronine Deiodinases and Selenoprotein P (SelP) [2]. Except in breast and skin cancer, Se has been shown to reduce the incidence rate of various forms of cancer. This is especially true for prostate and colorectal cancer [3]. A Se supplement of 200 μg/day was shown to reduce the incidence rate of these forms of cancer in USA [4]. Apart from its ability to remove excess Reactive Oxygen Species (ROS), the mechanism by which Se prevents cancer development is still unknown [5]. Many theories are now being proposed, some of which have implicated Se in the regulation of p 53 protein, a notable tumor suppressor protein, and inhibition of apoptosis [6]. Se supplementation in human has been shown to reduce the incidence rate of various types of cancer, but this is true only when the reference intake of Se is not above the optimum amount [4] [7]. To avoid or prevent Se toxicity, 400 μg of Se is still considered safe for consumption [8]. However, additional supplementation of dietary Se in any form beyond the safe amount could result in selenosis or Se poisoning [9]. The underlying mechanism of Se toxicity is yet to be fully understood. It was initially believed that the toxic effect of Selenocompounds, including Selenocysteine (Sec) and Selenomethionine, may be due to their random incorporations into proteins, which can induce structural and functional complications and ultimately results in cell death [10]. Contrary to this belief, replacement of the 150th methionine residue of β galactosidase by Selenomethionine was not harmful [11]. This may however be attributed to the insignificant roles played by methionine residues in the determination of structure cum function of proteins. It was suggested that the replacement of cysteine with Sec could be responsible for Se toxicity. It was presumed that cysteine, unlike methionine, plays critical roles in providing disulfide bonds, which is one of the bonds that stabilize protein native structure. Some recent studies attributed Se toxicity to the induction of the expression of Selenoproteins, especially GPXs, by Selenocompounds. The overexpression of GPX1 in endothelial cell line (ECV304) has been shown to inhibit cell growth [12]. This observation was presumably attributed to increased rate of conversion of reduced glutathione to the oxidized form, exposing the cell to oxidative stress [12]. Another study proposed that Se toxicity is due to the induction of apoptosis by Selenocompounds, especially Methylselenocysteine, MSC [13]. Apoptosis was shown to be triggered in human promyelocytic leukemia cell

line (HL-60) treated with MSC via the caspase 3 pathway [13]. Although this study provided much information about selenium-induced programmed cell death, it could not clearly explain the connection between Selenocompounds and the apoptotic machineries. Because previous reports have shown that low molecular weight Selenocompounds are capable of selectively inducing apoptosis in cells [13] [14], we presumed that high concentration of Sec in cells may also induce apoptosis. Our hypothesis is that the high concentration may induce the random incorporations of Sec into proteins and eventually destroy the structure and function of parent Selenoproteins through an unknown mechanism. In this study, we overexpressed the gene encoding selenocysteine synthase(*SecS*) (Figure 2) in human prostate carcinoma (PC3) cells to increase the production of Sec and presumably its subsequent random incorporation into proteins in PC3 cells. This was predicated on the fact that SecScatalyses the rate limiting step in the biosynthesis of Sec, *i.e.* the addition of activated Se to an activated phosphoseryl-tRNA (Figure 2). Thus, overexpression of *SecS* and Se supplementation is expected to activate this pathway and the overproduction of Sec. This will provide a model to study and observe the effect of Se toxicity on a typical and prominent prostate cancer cell line. Our decision to use PC3 cells as model for this study was based on the sensitivity of prostate tumor to Se treatment. Studies have shown more than 60% decrease in prostate carcinoma incidence rate in men taking Se as food supplements [15]. We believe that testing this hypothesis in PC3 cells is a step in the right direction as prostate cancer is one of the most prevalent cancer in the United States.

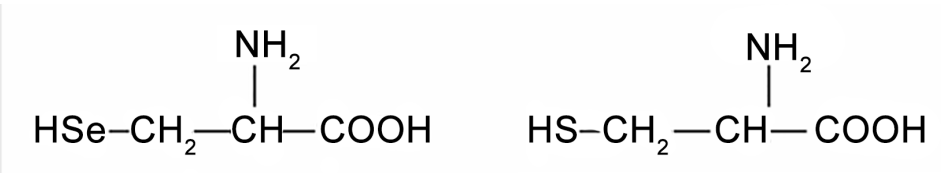

Figure 1. Structures of selenocysteine and cysteine.

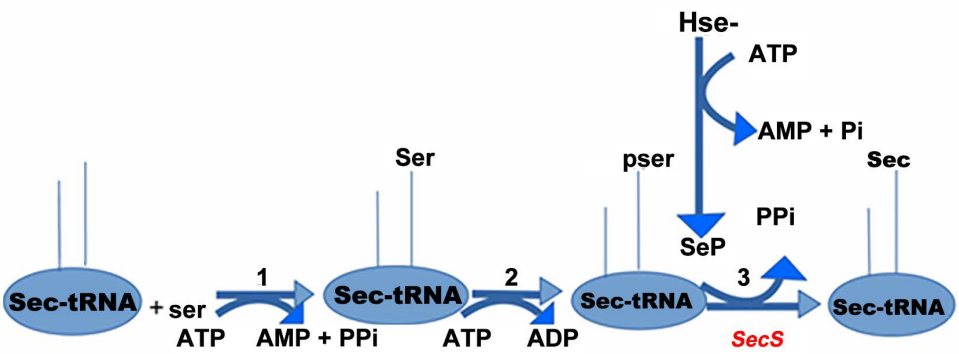

Figure 2. Biosynthetic pathway of selenocysteine on it tRNA (Modified from Sec Biosynthesis in Eukaryotes by [16]. Selenocysteine biosyntheisis; 1: Seryl-tRNA synthentase (SERS); 2: Phopsphoseryl-tRNA Kinase (PSTK); 3: Selenocysteine synthase (SecS); 4: Selenophophate synthetase 2 (SPS2).

2. Methods

2.1. Plasmid Constructs

Coding region of human (SecS) (Accession number MGC-14307) was obtained as clones in *E. coli* cells from the American Type Culture Collection (ATCC, Manassas, VA, USA). The clones were provided as *E. coli* cultures in LB medium with 10% glycerol. SecS was cloned into vector pOTB7 with chloramphenicol resistant marker gene (25 μg/mL). To isolate plasmid DNA from the *E. coli* culture, SecS coding region in pOTB7 was amplified using Polymerase Chain Reaction (PCR) for subcloning. The forward primer (SecF), 5'-GGATCCCTCGCATGAGCAC-3' was used to introduce the Bam HI restriction cutting site, while the reverse primer (SecR) was 5'-TCATGAAGAAGCATCCTGGTA-3'. Subsequently, the amplified 1.26 kb SecS fragments were ligated into a Zero Blunt TOPO PCR Cloning Vector (Invitrogen Inc.) for sequencing to confirm the fidelity of the open reading frame (ORF). Thereafter, Bam HI and Eco RI restriction enzymes were used to cut *SecS*from the Zero Blunt TOPO PCR Cloning Vector and further ligated to pCMV to generate pCMV-SecS. Similarly, pEGFP was digested with Sac I and Not I restriction enzymes to liberate the EGFP fragment, which was further cloned into the pCMV-Script to generate pCMV-EGFP to monitor gene expression. Both SecS and *EGFP* inserts were confirmed using PCR and restriction digestion enzyme analysis.

2.2. Transfection of PC3 Cells with PCMV-*SecS*, PCMV-*EGFP* and PCMV-Script Vector

Transfection of PC3 cells with pCMV-*SecS*, pCMV-*EGFP*, and pCMV-Script vector only (as a control) were carried out using Fugene HD (Promega) according to manufacturer instruction to generate stable expression line.

2.3. Screening of Transfected Cell Lines

Transfected cell lines were screened by dilution in 96 well plates to ensure that transfected cell lines were derived from a single transfection event.

2.4. Confirmation of Ransgenes in Transfected Cell Lines

To confirm the expression of genes in the transfected cell line, various techniques were used, including restriction enzyme digestion analysis, polymerase chain reaction (PCR) and reverse transcription PCR (RT-PCR). Specifically, the RT-PCR was usedto confirm the SecS overexpression using 18S rRNA as a normalisation gene (**Figure 3**).

2.5. Toxicity Testing

The control (pCMV-Script) and transfected cell lines were tested with sodium selenite at different concentrations in 96 well plates to determine their sensitivity and MTT reagents (Promega) were used to test cells viability according to manufacturer instructions. Cell viability was determined by Try pan Blue stain.

2.6. Analysis

All data collected were analyzed statistically and significances determined using Microsoft excel package to make possible statistical inferences and conclusions.

3. Results

3.1. Transfection of PC3 Cells

PC3 cell lines were transfected with pCMV-SecS, pCMV-GFP and pCMV-Script vector using fugene HD reagent (Figure 4). The pCMV-script transfected cell lines were used as vector control while the pCMV-GFP transfected cells were used to monitor and determine the transfection efficiency.

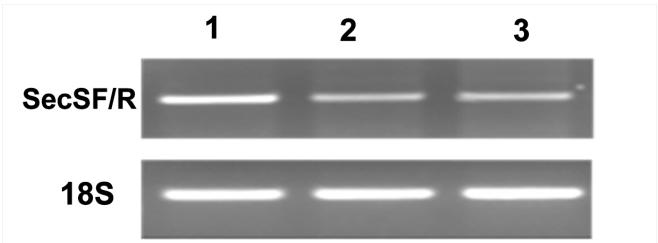

Figure 3. RT-PCR with SelcF/SelcR to measure SecS transcript levels of different transformed PC3 cell lines. 1: cDNA of SecS + PC3; 2: cDNA of pCMV-Script + PC3; 3: cDNA of PC3; PCR with 18S primers was used to show equal amount of RNA used.

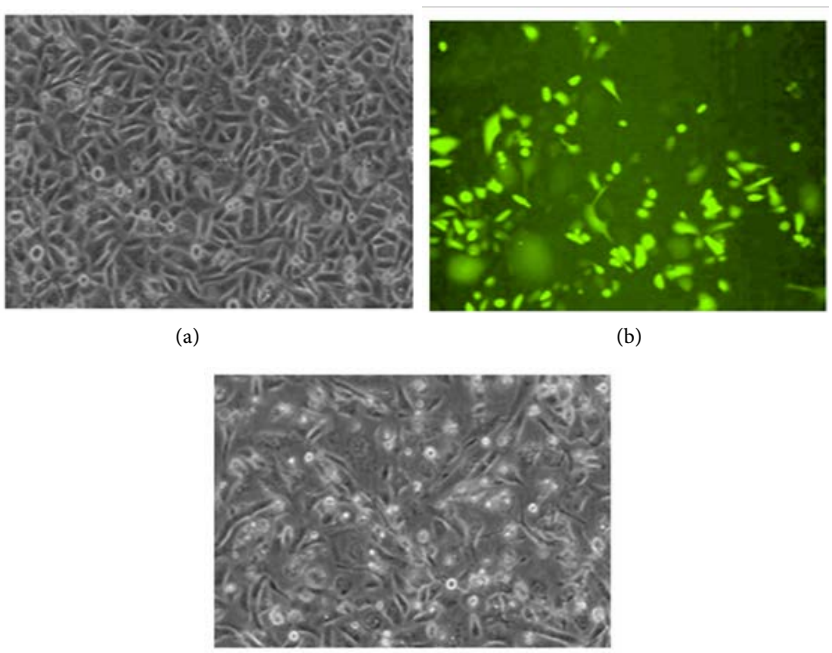

Figure 4. TransfectedProstate Carcinoma (PC3) cells. (a) pCMV-SecS transfected PC3 cells using fugene HD; (b) pCMV-GFP transfected PC3 cells with fugene HD; (c) pCMV-Script vector transfected PC3 cells with fugene HD.

3.2. Toxicity Testing

Using the MTT assay, the viabilities of control PC3 cells and both pCMV-SecS and pCMV-Script transfected PC3 cell lines were determined after exposure to sodium selenite at different concentrations (Figure 5). At 5 μM concentration of sodium selenite, there were no significant changes in the viabilities of all the cells, although the transfected cell lines (pCMV-Scriptand pCMV-SecS) had high viabilities. However, at 10 μM concentration of sodium selenite, the viabilities of SecS transfected PC3 cells dropped significantly, while control PC3 cells and pCMV-Script transfected PC3 cells showed no significant difference in terms of viabilities. The drop in viabilities of SecS transfected PC3 cells was about 80%. The significant drop in viability of pCMV-SecS transfected PC3 cells suggests that pCMV-SecS transfected PC3 cells were more sensitive to sodium selenite at 10 μM concentration than control and pCMV-Script (vector) transfected PC3 cells. At concentration greater 40 μM of sodium selenite, the growths of both control PC3 and pCMV-Script transfected PC3 cells were inhibited.

4. Discussion

The toxicity of Se in PC3 cells with the overexpression of *SecS* has been studied. It has been shown that the overexpression of *SecS* increased the sensitivity of transfected PC3 cells to sodium selenite. As hypothesized, the upregulation of *SecS* induced cell death in transfected cells at low concentration compared to control cells (Figure 5). A model, *SecS*-transfected PC3 cells, has been created. This model can be used to investigate the

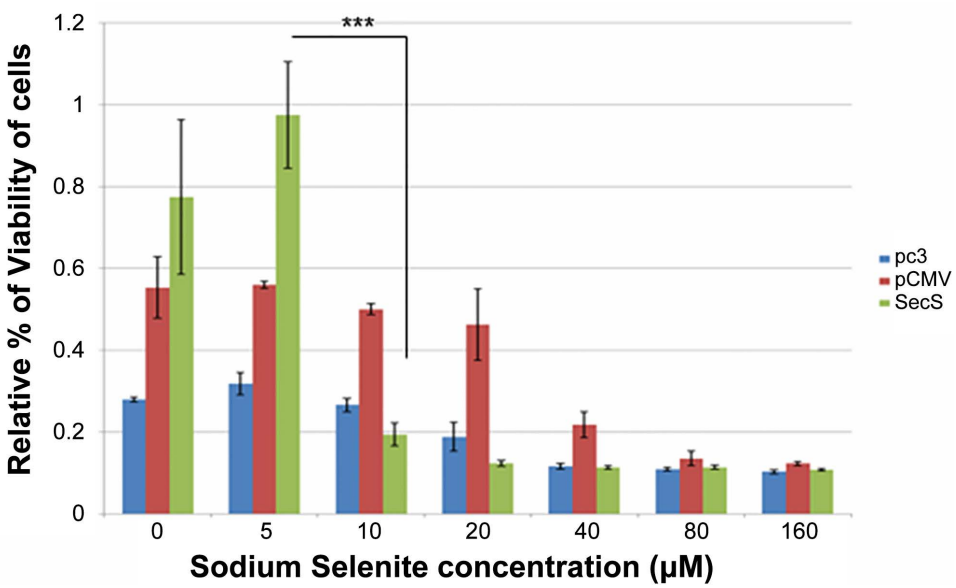

Figure 5. Measurement of the viabilities of control and transfected PC3 cells after exposure to sodium selenite. About 5000 cells were plated in 96 well plates for 24 hours in RPMI media supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin and the appropriate concentration of sodium selenite. The optical density (OD), which is a measure of the viability, was determined at a wavelength of 550 nm.Significance was determined using ANOVA one way. The data shown is an average of 3 assays + Standard Deviation (SD). P value <0.0001.

effects of this gene on the downstream regulation of vital cellular processes, such as apoptosis. This primary data also showed that potential overexpression of *SecS* in certain type of cancer cells via gene therapy and combination with Se supplementation may provide an alternative approach to cancer treatment. Future work on this project will be geared towards the determination of the expression level of pro-apoptotic genes such as the caspases. Measuring the expression levels of these genes and others would definitely provide more information about the mechanism of Se toxicity. Also, *SecS*-transfected PC3 cells would be tested with other forms of Se like selenomethionine and sodium selenide. Finally, *SecS* would be overexpressed in other cancer cells to see if this observation is ubiquitous or peculiar to only prostate cancer cells.

5. Conclusion

The characterization of Seas, a cancer chemo preventive agent has generated a lot of controversies in recent time. While several animal and *in vitro* studies have shown that Se supplementation enhanced cancer prevention [17] [18], some clinical trials in human have proven otherwise [19]. The reason for the disparities in the outcome of these various studies could be attributed to the fact that knowledge is lacking regarding the underlying molecular mechanism of Se. It is therefore important that more studies should be directed towards the unraveling of the molecular activities of Se, especially its metabolism and incorporation into various selenoproteins via the Se-containing amino acids. These pathways can be manipulated to alter Se and selenoproteins activities intra cellularly vis-a-vis their molecular function. This present study is focused on one of the major pathways involved in Se metabolism (**Figure 2**). This pathway produces Sec, a prominent Se-containing amino acids. Up-regulation of this pathway via the over expression of *SecS* and Se supplementation induced cell death in PC3 cells. Unlike previous studies that oversimplified Se supplementation studies, this study provided a mechanistic approach to the use of Se in the treatment of cancer.

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