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14. ABSTRACT For this postdoctoral fellowship the specific role of selenoproteins (SP) in prostate cancer (PCa) was investigated. First, the response of varying allelic identities in the SP glutathione peroxidase (GPx-1) to selenium (Se) supplementation was determined. GPx-1 has either a leucine (leu) or proline (pro) amino acid at the 198 codon, and cancer risk has been shown to vary depending on the allelic identity. Our investigation showed that the prostate cell line LNCaP containing leu at the 198 codon was more responsive to Se supplementation although its baseline GPx activity was lower compared to cells with a pro at the same codon. These studies have increased our understanding of the effect of genetic variations in GPx-1 on the response to dietary Se and are relevant to the observed effects of Se in the SELECT trial. Second, using the siRNA technique, levels of GPx-1 were significantly reduced in LNCaP cells and the effect on DNA damage investigated. Compared to control transfectants, cells with reduced GPx-1 levels were found to be more susceptible to UVC induced DNA damage as assessed by micronuclei formation. Se Supplementation was more effective in attenuating micronuclei formation in the control compared to GPx knock-down transfectants, suggesting that maximum benefits were achieved through its regulation of GPx-1. Studies in transgenic mice that express reduced levels of SP and an increased risk for PCa indicated that Se supplementation attenuated the progression to high-grade prostatic intraepithelial neoplasia (PIN) in the wildtype, but not in the SP deficient animals. This benefit however was not observed with histopathological progression to low-grade PIN or microinvasion. Collectively, these studies suggest that Se effects in PCa may in part be mediated through SP.					
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ANNUAL SUMMARY

INTRODUCTION

Epidemiological and experimental data over several years have provided new information indicating that selenium is an effective, non-toxic means to prevent several types of cancer, including cancer of the prostate. It is unknown whether the mechanisms of selenium action are mediated through selenium-containing proteins although genetic evidence supports a role for two selenoproteins, GPx-1 and Sep15 in this process. Loss of one of two copies of the GPx-1 gene is a common event in several cancers, and a polymorphic variant of GPx-1 was found to be associated with cancer risk. Similarly, allelic loss at the Sep15 locus occurs in several cancer types as well, and functional genetic variations in this selenoprotein gene may be involved with cancer risk as well. GPx-1 has anti-oxidant activity, while the function of Sep15 is unknown. It is hypothesized that GPx-1 and/or Sep15 levels are associated with prostate cancer risk and the increase in the activity of these proteins when humans are provided dietary selenium supplements help prevent that disease. This concept is particularly relevant to the SELECT trial, the largest prostate cancer prevention trial ever conducted, designed to investigate if selenium supplementation can prevent prostate cancer when provided to cancer-free men.

This post-doctoral fellowship application proposed to investigate the role of these selenoproteins in prostate cancer using cell culture and transgenic animal model systems. In the cell culture model, the specific role of GPx-1 in prostate cancer was addressed using the small interference RNA (siRNA) technique, by which levels of GPx-1 were significantly reduced in prostate cancer cells (LNCaP) and the biological consequence of this reduction examined. In the transgenic animal model, in which levels of most selenoproteins are significantly reduced, the effect of selenium supplementation on the histopathological progression of prostate glands was assessed. Together, both these models have provided information on the possible role selenoproteins may have in the progression of prostate carcinogenesis.

BODY

RESEARCH ACCOMPLISHMENTS (According to tasks outlined in the statement of work)

Description of research accomplishments associated with completed tasks as reported in Annual Summary-I is unchanged. Research data accrued in the last 1 year is described in detail in the following sections.

- 1) Task I of this proposal aimed at exploring the biological consequence of Sep15 polymorphism in human prostate cell lines as a function of selenium availability. Specifically, the aim was to determine whether the naturally occurring polymorphic alleles of Sep15 produce differing amounts of the corresponding protein as a function of selenium availability in human prostate cells.

a) Screening cell lines for Sep15 and GPx-1 alleles (Completed in year 1).

To fulfill this aim, prostate cell lines were screened, in order to identify the Sep15 genotypes. In addition to identifying Sep15 alleles, the nucleotide identity at the polymorphic 198 codon of the GPx-1 gene of these same cells was also determined. The Sep15 gene was genotyped for the only reported polymorphisms at positions: CG/TA, CG/CG and TA/TA. The GPx-1 gene was genotyped to identify the nucleotide at the codon 198 polymorphism.

Nine independent prostate cell lines were screened in order to identify the genotypes for Sep15 and GPx- 1 selenoproteins. The identified genotypes are listed below in table 1:

Table 1: Selenoproteins Sep15 and GPx genotype in prostate cell lines.

Name of cell line	Genotype	
	Sep15	GPX198
1532NTPX	CG/TA	198P
1532CTPX	CG/TA	198P
1535NTPX	CG/CG	198P
1535CTPX	CG/CG	198P
1542NTPX	CG/TA	198P
1542CTPX	CG/TA	198P
LNCaP	CG/CG	198L
Du145	CG/CG	198L
PC3	CG/CG	198L

The following technical difficulties were encountered in our investigation of the role of Sep15 in prostate carcinogenesis, due to which the proposed work on Sep15 in these prostate cell lines could not be effectively pursued.

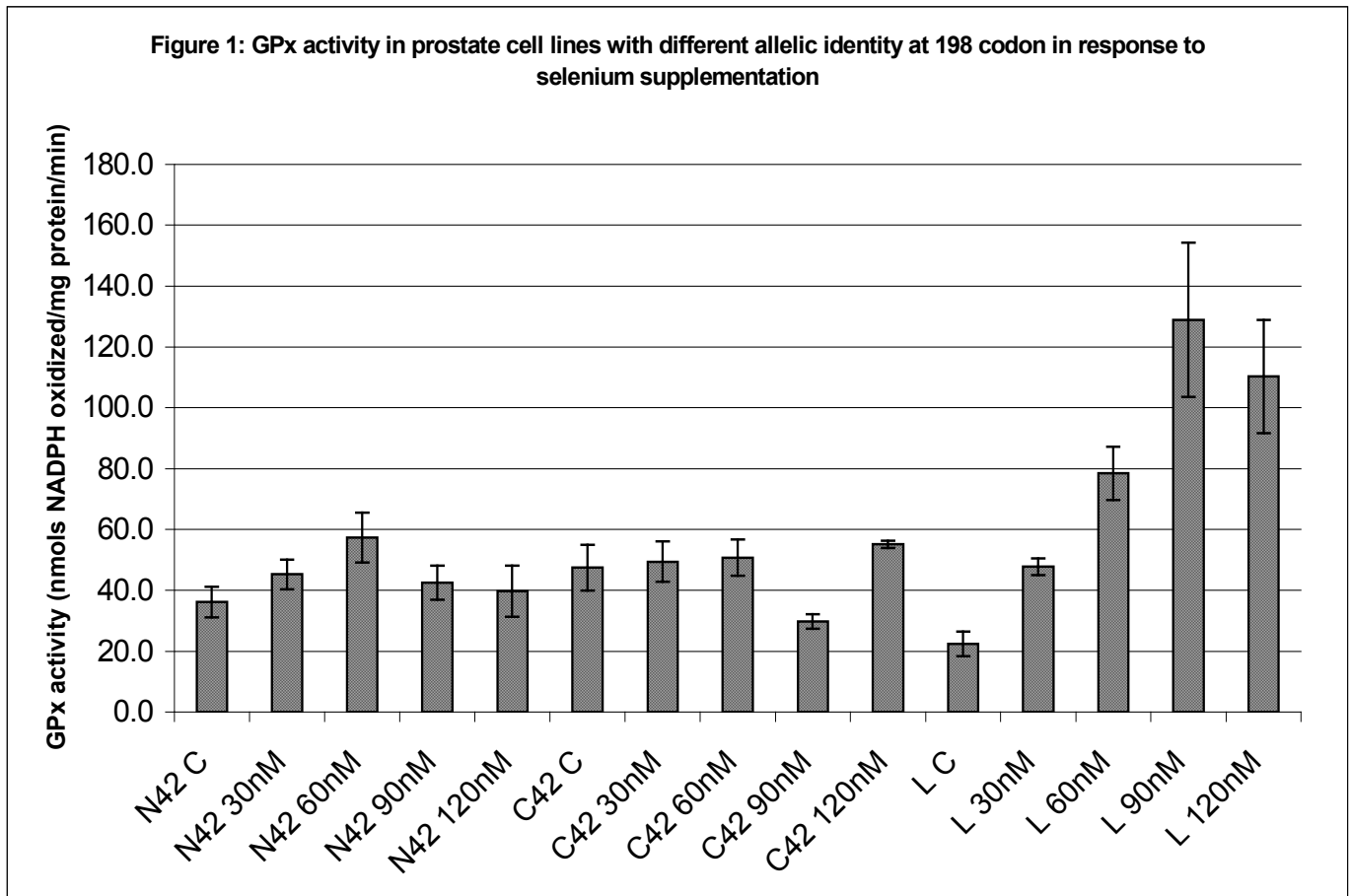
- 1) None of the screened cell lines displayed the TA/TA allele for the Sep15 gene, which we had proposed to investigate.
- 2) Good, stable antibodies to detect the expression of Sep15 were unavailable, which limited any further investigations on the function of this selenoprotein.

Future studies instead used transgenic animals that express reduced Sep15 levels, along with reduced levels of other selenoproteins, to address this protein's role in prostate cancer risk and development.

b) Experiments with selenium supplementation of human prostate cell lines differing in GPx-1 gene allele identity (Completed in year 1).

Allelic identity of the GPx-1 gene was determined in prostate cancer cell lines. Among the screened cell lines, three (LNCaP, NPTX-42 and CPTX-42) were selected to investigate whether the presence of a leucine or proline containing allele affected GPx enzyme activity as a function of selenium availability. LNCaP and CPTX-42 are both prostate cancer cell lines, while NPTX-42 represents a normal cell line derived from normal tissue of the same individual from which the CPTX-42 line was derived. LNCaP cells contain the Leu allele, and both NPTX-42 and CPTX-42 cell lines contain the pro allele (Table 1)

GPx activity was assessed at 4 different concentrations of selenium (30, 60, 90 and 120nM) compared to untreated control cells. Response to 3 day selenium treatment in the cell lines is shown in Figure 1.



N42 = NPTX42 Normal prostate cell line with Proline at GPx 198 codon

C42 = CPTX42 Prostate cancer cell line with Proline at GPx 198 codon

L = LNCaP Prostate cancer cell line with Leucine at GPx 198 codon

C = Control untreated cells

30nM = 30 nM Selenium supplementation

60nM = 60 nM Selenium supplementation

90nM = 90 nM Selenium supplementation

120nM = 120 nM Selenium supplementation

Key observations:

- 1) Baseline GPx activity was found to be lower in the LNCaP cells (Leu) compared to NPTX42 and CPTX42 cell lines (Pro). This difference was significant compared to CPTX42 cell line ($P = 0.04$). No difference in baseline GPx activity was observed between NPTX42 and LNCaP or NPTX42 and CPTX42 cells.

- 2) While both NPTX42 and CPTX42 cell lines did not show a significant increase in GPx enzyme activity following selenium supplementation at several doses, a significant induction in GPx activity was found in LNCaP cells supplemented with selenium at 30nM ($p < 0.001$), 90nM ($p < 0.05$) and 120 nM ($p < 0.05$) compared to untreated control cells.

These results indicate that although the baseline GPx activity was lower in the leu-containing LNCaP cells, the response to selenium supplementation in these cells was significant when compared to pro-containing NPTX42 and CPTX42 cells. These observations are relevant to the SELECT trial. Future efforts may need to be directed towards genotyping individuals participating in prostate cancer clinical trials with selenium supplementation, in order to determine whether differences in allelic distribution influence the extent to which the benefits of selenium are realized.

2) Task II of this proposal aimed at assessing the consequence of reduced levels of GPx-1 on DNA damage in human prostate cells.

- a) Reduction of GPx-1 levels in human prostate cells using the siRNA technique (Completed in year 1).

In order to reduce GPx-1 levels using interfering RNA technology, 5 mRNA target sequences for the GPx-1 gene were identified using Ambion's siRNA target finder tool. The following table lists the sequences of the siRNAs.

Table 2: SiRNA target sequences for GPx-1 gene silencing

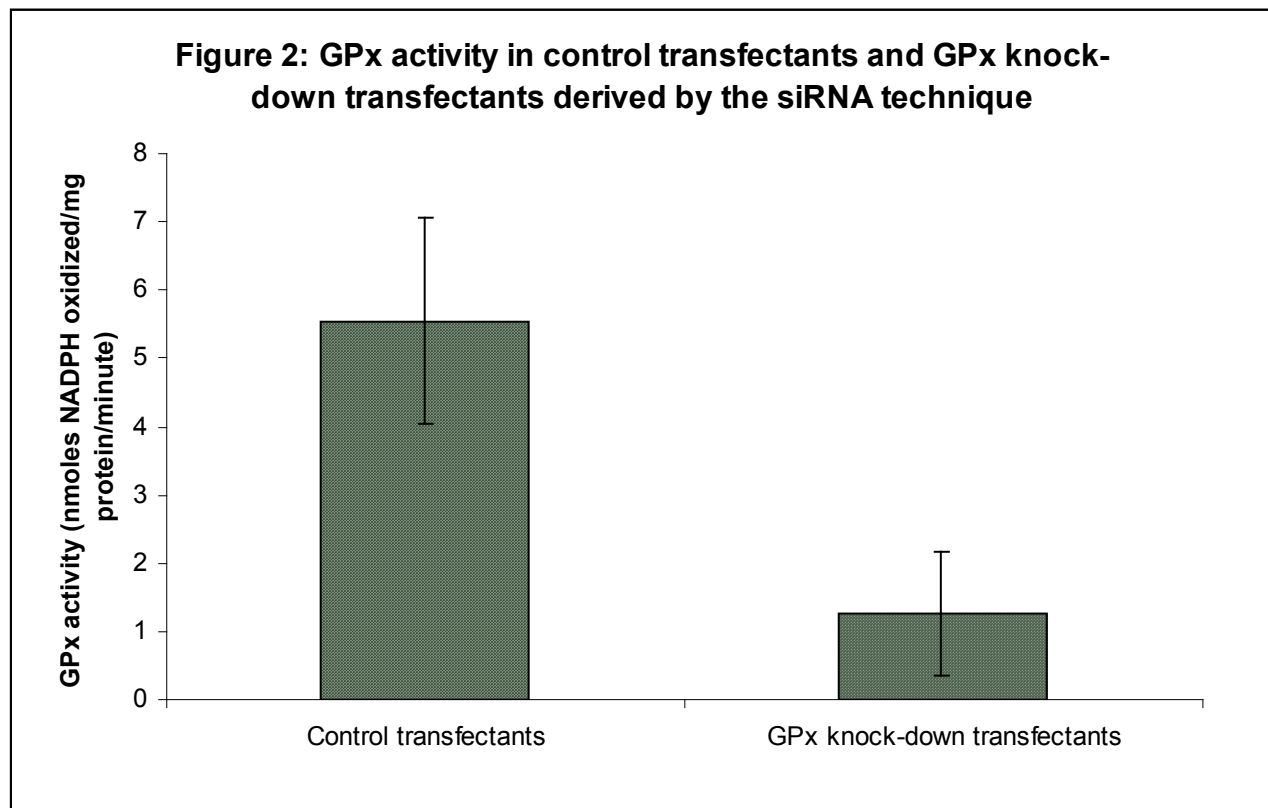
Target Sequence Number	Sequence	Position in gene sequence
TS 4	AAGGTACTACTTATCGAGAAT	192
TS 12	AATCTCCCTTGTTTGTGGTTA	536
TS 15	AAGAACGAAGAGATTCTGAAT	621
TS 16	AACGAAGAGATTCTGAATTCC	624
TS 17	AAGAGATTCTGAATTCCTCA	628

- Using each target sequence, hairpin siRNA template oligonucleotides were synthesized for ligation into Ambion's pSilencer vector. As a negative control, the pSilencer vector without an inserted oligonucleotide was used.
- The hairpin siRNAs were cloned into Ambion's psilencer 2.1-U6 hygromycin siRNA expression vector.
- Clones with siRNA inserts were identified by restriction enzyme digestion following amplification in a bacterial host.

Plasmids containing the target sequence (TS 15) or the control vector were transfected by electroporation into LNCaP cells using Amaxa biosystem's nucleofector. Transfected cells were selected with hygromycin B and 12 colonies were picked for each plasmid.

b) Efficacy of GPx-1 siRNA gene silencing (Completed in year 1).

Each of the transfectants were expanded and screened for GPx enzyme activity in order to select a cell line with the reduced GPx activity as compared to control transfectants. Figure 2 shows the GPx activities obtained using extracts prepared from these transfectants. GPx activity in the control transfectants was significantly higher ($P < 0.001$) than that in the GPx knock-down cells. This difference between the two transfectants was 5 fold, a physiological difference, which is more likely to be seen in the human population.



c) Effect of reduced selenoprotein levels on DNA damage and cell proliferation (Completed in year 2).

In order to investigate the effects of reduced GPx-1 levels on DNA damage, prostate cancer cells derived by the siRNA technique described above were subjected to a UVC dose of 12 J/M^2

and the presence of potentially mutagenic DNA damage was assessed by the micronuclei assay which is described below. A UVC dose of 12 J/M² was selected based on previous studies in our lab in which this dose was found to cause significant DNA damage in breast cancer cells without producing substantial cell death.

The following treatment groups were included for the control and GPx knock-down transfectants:

- 1) No treatment group
- 2) Cells treated with 30nM sodium selenite for 5 days (Se only group)
- 3) Cells treated with a UVC dose of 12 J/M² at 5 days (UV only group)
- 4) Cells treated with 30nM sodium selenite for 5 days + UVC dose of 12 J/M² at 5 days (UV + Se group)

c-1) GPx activity was measured in these samples in order to determine the effect of UVC exposure on enzyme activity in either the control or the GPx knock-down transfectants. As seen in Figure 3, in both the control and GPx knock-down transfectants, UVC treatment did not affect GPx activity. In the untreated groups, GPx activity in the control transfectants was significantly higher than that in the GPx knock-down cells ($P < 0.001$). Interestingly, while supplementation with 30nM selenium for 5 days resulted in an 8-fold increase in GPx activity in the control transfectants, this increase was only marginal (3-fold) in the GPx knock-down cells. Protein levels as assessed by western blot analysis (Figure 4) reflected the change in GPx activity suggesting that silencing of the GPx gene was not completely reversed by selenium supplementation. Therefore this knock-down model allowed the investigation of the independent roles of selenium and GPx-1 in prostate cancer.

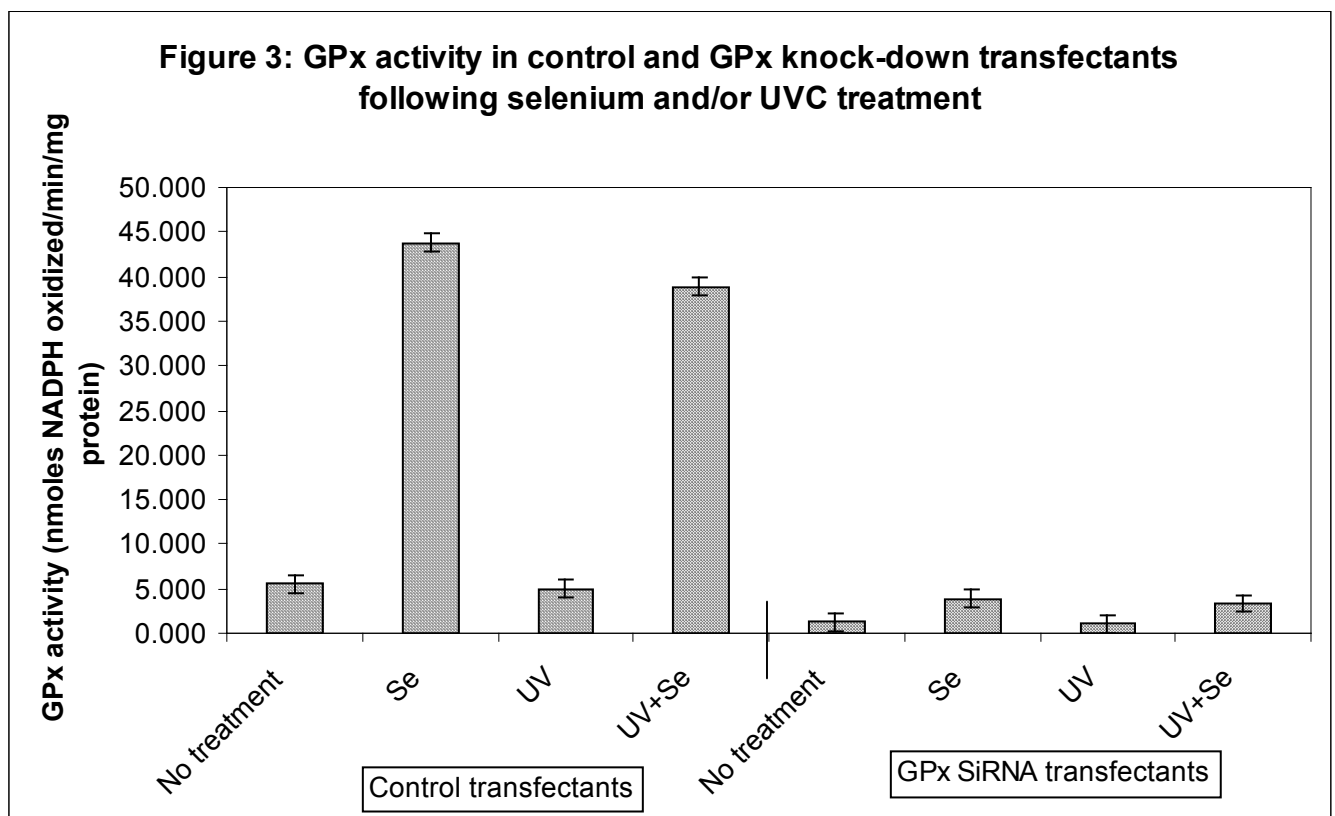
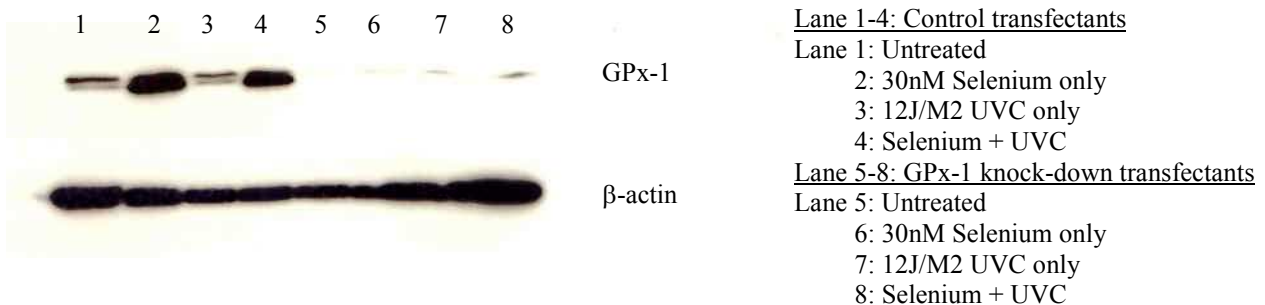
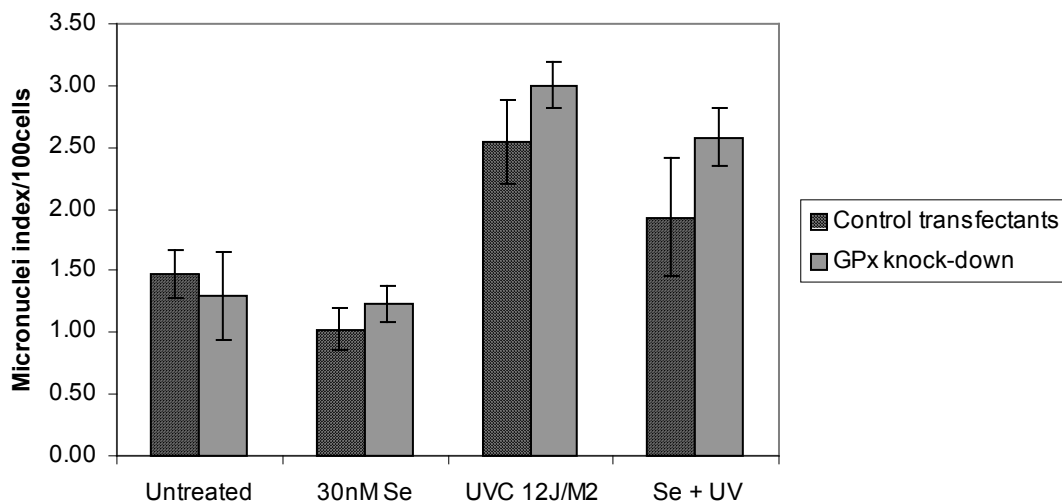


Figure 4: GPx-1 expression in control versus GPx-1 knock-down transfectants following 12 J/M² UVC and 30nM Selenium treatment



c-2) DNA damage following exposure to UVC in these cells was determined by scoring micronuclei formation, an assay commonly used to assess chromosomal damage in mitogen stimulated cells. Micronuclei are small extranuclear bodies that arise from aberrant replication. Previous work in our laboratory has demonstrated a decrease in UVC induced micronuclei frequency in breast cancer cells genetically engineered to overexpress GPx-1. Micronuclei frequency was further reduced with selenium supplementation in these cells, suggesting that the chemoprotective benefits conferred by selenium are likely mediated through this selenoprotein (Baliga et al., 2006 accepted for publication). Our work with GPx-1 knock-down prostate cancer cell line corroborate these findings; results are presented below in Figure 5.

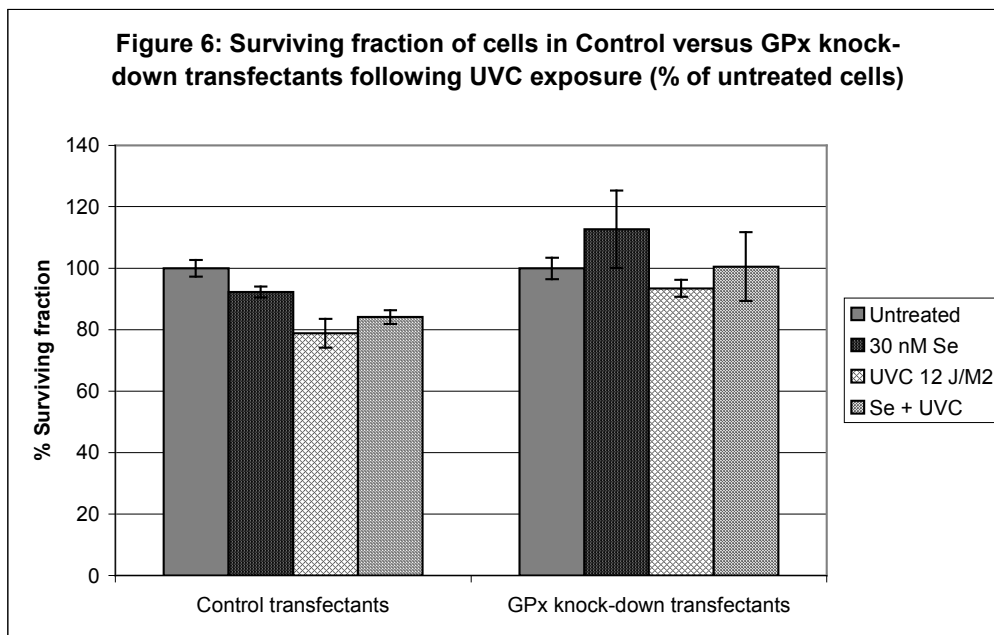
Figure 5: Micronuclei frequency/100 cell in LNCaP control versus GPx-1 knock-down transfectants



Baseline micronuclei frequency was similar in the control transfectants and GPx-1 knock-down cells, which did not change significantly with selenium supplementation in either of the groups, although a marginal decrease in baseline micronuclei levels was observed in the control transfectants with selenium. UVC exposure increased micronuclei frequency significantly in both the transfectants compared to the respective untreated groups. However GPx-1 knock-down cells seemed more susceptible to the effects of UVC exposure and showed significantly higher micronuclei frequency compared to control transfectants ($P = 0.028$). More importantly, selenium supplementation was more effective in reducing DNA damage in the control transfectants (24%) ($P = 0.042$) than in GPx-1 knock-down cells (14%) ($P = 0.066$) compared to the respective untreated controls (Figure 5). In addition, with both the UVC exposure and selenium treatment the micronuclei frequency in the control transfectants was significantly lower than that in the GPx knock-down cells ($P = 0.025$).

c-3) The surviving fraction of cells following UVC exposure were assessed using the clonogenic assay.

As seen in figure 6, following UVC treatment, surviving fraction of cells in the control transfectants decreased significantly ($p < 0.001$) compared to its untreated group. Supplementation with selenium prior to UV treatment made these cells less susceptible to the effects on cell survival ($p = 0.066$). In the GPx knock-down cells however, UVC exposure did not affect cell survival significantly, nor did selenium supplementation.



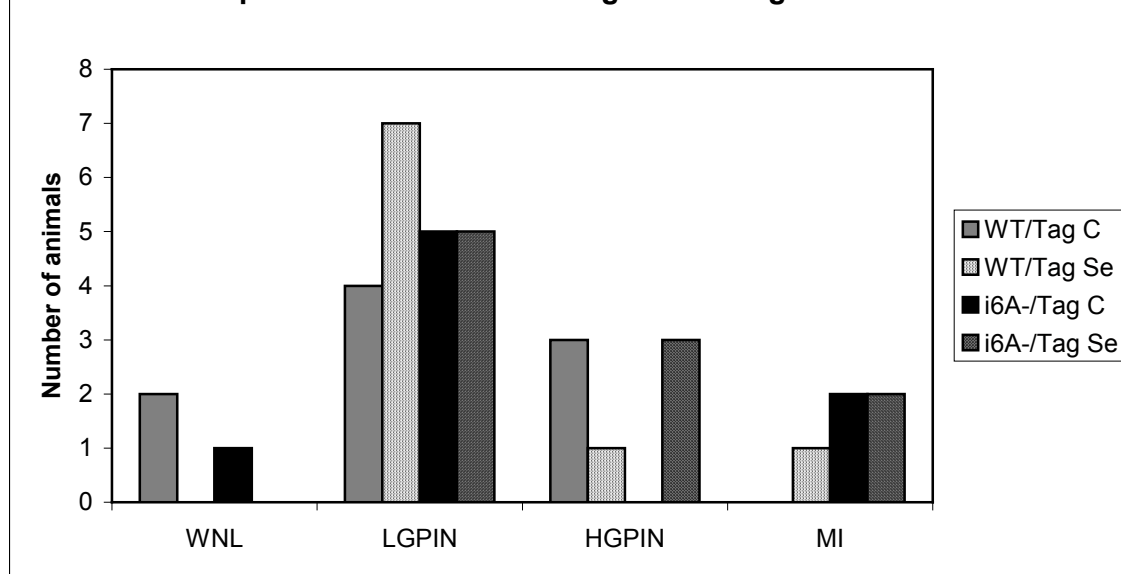
The greater surviving fraction and high micronuclei frequency in prostate cancer cells with reduced GPx levels indicates that low levels of this selenoprotein likely supports the viability of potentially mutagenic cells that can possibly proliferate to advanced stages of prostate cancer. In the presence of adequate levels of GPx, such as in the control transfectants, the micronuclei levels in the cells were lower, as was also the fraction of surviving cells following irradiation with UVC. This may suggest that perhaps elimination of damaged cells through apoptosis or DNA repair was more effective in these cells. The specific repair pathways affected by GPx-1 warrants further investigation.

- 3) Task III Assess the role of selenium and selenoproteins in prostate cancer progression using a transgenic mouse model in which levels of most selenoproteins are reduced.

This study is an extension of previously published work from our laboratory in which selenoprotein deficient transgenic mice were used in order to investigate the role of selenoproteins in prostate carcinogenesis in the absence of a variation in selenium intake (Diwadkar-Navsariwala et al., 2006). These mice, referred to as i⁶A⁻/Tag, express reduced levels of selenoproteins due to the presence of a mutant selenocysteine tRNA, and an increased risk for prostate cancer due to the directed expression of the SV40 large T and small t oncogenes in the prostate. The i⁶A⁻/Tag mice were compared to control WT/Tag for the presence, degree and progression of prostatic intraepithelial neoplasia (PIN). A clear difference in prostate histopathology was observed, with the selenoprotein deficient mice displaying an increased predisposition towards higher-grade lesions with time. In the early weeks significantly more prostates were normal in the WT/Tag compared to the i⁶A⁻/Tag mice, however as time elapsed a greater shift from low-grade (LGPIN) to high-grade PIN (HGPIN) was apparent in the selenoprotein deficient mice indicative of an accelerated development towards prostate cancer (Diwadkar-Navsariwala et al., 2006). These data implicate selenoprotein levels in prostate cancer progression.

In order to determine whether selenium supplementation is able to attenuate the rapid progression to HGPIN in the selenoprotein deficient mice, animals from both groups were fed a diet containing 5 mg/kg selenium. Selenium was provided as selenomethionine, the form that is being used in the SELECT trial. Figure 7 shows the incidence of histopathological progression of 20-week prostates in WT and i⁶A⁻ animals fed selenomethionine.

Figure 7: Effect of selenium supplementation on incidence of prostate lesions in WT/Tag vs i6A-/Tag animals



Comparison between WT and i⁶A⁻ animals that were not supplemented with selenium showed that more animals in the WT group had prostates within normal limits (WNL), and more animals in the i⁶A⁻ group had LGPIN compared to the WT group. Although the incidence of HGPIN was lower in the i⁶A⁻ animals compared to the WT mice, the incidence of microinvasion was higher. Comparison between WT and i⁶A⁻ animals that were supplemented with selenium showed that less number of i⁶A⁻ animals displayed LGPIN compared to WT, however the incidence of HGPIN and MI was higher in the i⁶A⁻ animals compared to the WT.

When within group comparisons were made, in both the WT and i⁶A⁻ mice more prostates were found to be WNL in animals that were not fed the selenium supplemented diet. Interestingly, no prostates from the selenium supplemented animals, WT or i⁶A⁻, were WNL. Similarly, in both the groups, selenium supplementation did not confer any protection against the progression to LGPIN, however, in the WT group, selenium supplemented animals had a lower incidence of HGPIN, while this protection from selenium was not observed in the i⁶A⁻ animals. This suggested that selenium was effective in attenuating the progression to HGPIN only in the WT animals with sufficient levels of selenoproteins. Selenium supplementation was not able to protect prostates from either the WT or the i⁶A⁻ animals from acquiring microinvasive (MI) lesions.

These findings with selenium supplementation suggests that selenium may likely be affecting the molecular mechanisms that occur during the progression of prostates from LGPIN to HGPIN through its ability to regulate the levels and activities of various selenoproteins. It appears however that selenium as selenomethione is unable to prevent the initiation of LGPIN or the advancement to microinvasion. Future studies would need to focus on the molecular mechanisms affected by selenium and/or selenoproteins during the histopathological progression of prostates. In addition it would be useful to study whether different effects are seen due to supplementation with other forms of selenium such as sodium selenite.

Due to limited tissue availability, assays to detect protein expression of total and phosphorylated Akt could not be conducted as proposed in the statement of work.

Methodologies used for accomplishing research tasks:

Glutathione peroxidase activity:

GPx activity was measured by a standard coupled spectrophotometric method. Briefly, cells were washed twice with ice cold PBS and harvested to recover cell pellet. Sodium phosphate buffer (0.1 M Na₂HPO₄, pH 7.5), was added to the cell pellet before disruption of cells by sonication (Ultra sonic homogenizer 4710 series; Cole-Parmer Instrument Co., Chicago, IL). Protein levels were quantified in the supernatant using the Dc Protein Assay Kit (Bio-Rad Laboratories, Richmond, CA, USA). GPx activity was determined using 100 - 400 µl of the supernatant (130 – 328 µg proteins), assayed in a 1 ml reaction volume containing NADPH, reduced glutathione reductase, sodium azide, and H₂O₂. The oxidation of NADPH to NADP was monitored at 339 nm and expressed as nmol of NADPH oxidized / mg protein /min.

Western blot analysis:

Protein extracts (30 µg) were electrophoresed on a 14% SDS-Polyacrylamide gel, and transferred onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore Corp., Bedford, MA). To evaluate GPx protein levels, membranes were blocked with 5% non-fat dry milk in TBST (Tris buffered saline with 0.1% Tween-20) overnight at 4°C, and then incubated with the primary antibody for GPx-1 (diluted 1:1000 in blocking solution) for 1 hour at room temperature (RT). Antibodies were affinity purified from eggs of hens immunized with a KLH-conjugated peptide representing amino acid residues 83-100 of the human GPx-1 gene, CZHQENAKNEEILNSLKYVR (Aves Labs, Inc., Tigard, OR). Membranes were washed 3 times with TBST and incubated with a rabbit anti-chicken horseradish peroxidase-conjugated secondary antibody (diluted 1:5000 in 5% non-fat dry milk in TBST) for 1 hour at RT and washed 3 times with TBST.

Micronuclei assay:

The micronuclei assay was carried out according to the method of Fenech and Morley (1985) and the protocol described by Krisch-Volders et al. (2000). Briefly, a fixed number (10×10^5) of exponentially growing cells were inoculated into several individual culture plates and allowed to attach and grow. At 30-40% confluence cells were treated either with 30 nM of selenium containing media for 5 days. After five days, the medium was removed, the cells were washed with cold PBS and cells were exposed to UVC in the presence of 1 ml of PBS (to keep cells hydrated during). The PBS was removed and cultures were replenished with complete medium containing cytochalasin B (3 µg/ml) for 48 h post-irradiation. After 48 hours, the media containing cytochalasin-B was removed and the cells were washed with PBS. The cells were dislodged by trypsin EDTA treatment, centrifuged and subjected to mild hypotonic treatment (0.7% ammonium oxalate) for 5 min at 37°C, and centrifuged again. The resultant cell pellet was fixed in Carnoy's fixative (3:1 methanol, acetic acid) overnight at 4°C. The next day, after centrifugation, the cells were resuspended in a small volume of Carnoy's fixative and dropped on

to slides and air dried. The slides containing the cells were stained with 10% (v/v) Giemsa for 30 min, washed, dried and scored for the presence of micronuclei in binucleate cells according to the criteria of Krisch-Volders et al. (2000). A minimum of 1000 binucleate cells with well preserved cytoplasm were scored from each culture and the frequency of micronucleated binucleate (MNBNC) cells were determined.

Statistical analysis:

Statistical significance of differences within-group were analyzed by one-way ANOVA, followed by post hoc analysis to determine all pairwise differences. Student's t-test was used for specific between-group comparisons. All statistical tests were performed using SigmaStat and differences were considered significant at $P \leq 0.05$.

KEY RESEARCH ACCOMPLISHMENTS

1. The difference in response to selenium supplementation observed in prostate cells with varying allelic identity of GPx-1 at the 198 codon suggests, that individual differences in the benefits due to selenium supplementation may depend on GPx-1 allelic identity. Therefore in order to maximize the effects of selenium supplementation, future human clinical trials may need to tailor individual needs based on the GPx-1 genotype.
2. LNCaP cells with reduced GPx-1 levels that have been derived through the work funded by this fellowship are a good resource to study the role of GPx-1 in prostate carcinogenesis. In particular, using these cells it will be determined in the future whether GPx-1 and /or selenium have a role to play in the advancement of prostate cancer from the hormone sensitive to the hormone insensitive stage. Whether the benefits of Se could be extended to its use from a chemopreventive to a therapeutic agent to reduce the advancement of disease to the hormone refractory stage could have significant consequence. Use of the GPx knock-down cells will involve studies that will investigate the possibility of using selenium supplementation as an adjuvant to androgen ablation therapy in the treatment of prostate cancer.
3. Based on the micronuclei studies conducted on the GPx-1 knock-down cells, it appears that this selenoprotein may have a role to play in attenuating DNA damage. Whether this occurs through reduced DNA damage or enhanced DNA repair mechanisms needs further investigations. The GPx-1 knock-down cells will be useful in investigating the specific DNA damage and repair pathways that may be affected by GPx-1 in prostate cancer.
4. Data from the animal study indicate that selenium (as selenomethionine) via its ability to regulate selenoproteins may retard the histopathological progression of prostates especially to HGPIN. Selenomethionone supplementation did not slow down the progression to LGPIN or microinvasion in this model. It will be determined in future whether selenium supplemented in the form of sodium selenite is able to retard initiation of LGPIN or progression to microinvasion and the possible molecular mechanisms that may involved in this process.

LIST OF REPORTABLE OUTCOMES

- 1) Manuscript: A manuscript is in preparation to describe the role of GPx-1 in prostate carcinogenesis based on the data accrued on the GPx-1 knock-down cells during this fellowship period.
- 2) Glutathione peroxidase knock-down cells generated during this fellowship period will be used for future studies to assess the role of GPx-1 and/or selenium in the progression of prostate cancer from hormone sensitive to hormone insensitive stage. A proposal to this effect has been submitted to funding agencies.

CONCLUSIONS

My efforts supported by the awarded postdoctoral fellowship have helped address the specific role of selenoproteins, including GPx-1 in prostate cancer. I have genotyped several human prostate cancer cell lines and established a relationship between GPx-1 genotype and the levels/induction of that protein. These studies increase our understanding of the effect of genetic variations in GPx-1 on the response of that protein to dietary selenium levels.

Using the siRNA technique we were able to significantly reduce levels of GPx-1 in the prostate cancer cell line LNCaP. These cell lines were used to determine the effect of reduced GPx-1 on DNA damage induced by UVC. GPx knock-down cells were found to be significantly more susceptible to UVC induced DNA damage and less responsive to the protective effects of selenium supplemented at 30nM concentration. These studies indicate that selenium's protective effects against DNA damage are in part mediated through this selenoprotein. Future investigations will examine the specific pathways involved in this process.

Collectively the cell culture and animal studies indicate that selenium likely affects the course of prostate carcinogenesis through its regulation of selenoproteins and GPx-1 is a likely mediator of these effects.

REFERENCES

Baliga MS, Wang H, Zhuo P, Schwartz JL and Diamond AM. Selenium and GPx-1 over-expression protect MCF-7 Cells from UV-induced DNA damage (Accepted for publication)

Diwadkar-Navsariwala V, Prins GS, Swanson SM, Birch LA, Ray VH, Hedayat S, Lantvit DL, Diamond AM. Selenoprotein deficiency accelerates prostate carcinogenesis in a transgenic model. *Proc Natl Acad Sci U S A*, 2006, 103(21):8179-84.

Fenech M. and Morley AA. Measurement of micronuclei in lymphocytes. *Mutat Res*, 1985. 147(1-2): 29-36.

Kirsch-Volders M, Sofuni T, Aardema M, Albertini S, Eastmond D, Fenech M, Ishidate M Jr, Lorge E, Norppa H, Surralles J, von der Hude W, and Wakata A. Report from the In Vitro Micronucleus Assay Working Group. *Environ Mol Mutagen*, 2000. 35: 167-172.