Published in final edited form as:

Prostate. 2012 June 15; 72(9): 1006–1012. doi:10.1002/pros.21506.

Inverse Association Between Glutathione Peroxidase Activity and Both Selenium Binding Protein 1 Levels and Gleason Score in Human Prostate Tissue

Anita Jerome-Morais^{1,2}, Margaret E. Wright^{1,2}, Rui Liu¹, Wancai Yang¹, Matthew I. Jackson³, Gerald F. Combs Jr.³, and Alan M. Diamond^{1,*}

¹Department of Pathology, University of Illinois at Chicago, Chicago, IL, USA

³Grand Forks Human Nutrition Research Center, USDA-ARS, Grand Forks, N.D., USA

Abstract

Background—Data from human epidemiological studies, cultured mammalian cells, and animal models have supported a potentially beneficial role of selenium (Se) in prostate cancer prevention. In addition, Se-containing proteins including members of the glutathione peroxidase (GPx) family and Selenium Binding Protein 1 (SBP1) have been linked to either cancer risk or development. For example, SBP1 levels are typically reduced in tumors compared to non-cancerous tissue, with the degree of reduction associated with increasingly poor clinical outcome.

Methods—In order to investigate inter-relationships between blood and tissue Se levels and GPx activity, tissue SBP1 levels, and disease aggressiveness using the Gleason Score, we measured these selenium status biomarkers in fasting serum and histologically normal prostate tissues obtained from 24 men undergoing radical prostatectomy for the treatment of localized prostate cancer.

Results—GPx enzyme activity was inversely correlated with SBP1 levels in prostate tissue as determined by densitometry of western blots obtained using anti-SBP1 antibodies (partial Spearman correlation coefficients and corresponding p-values overall and in African-Americans = −0.42 (0.08) and −0.53 (0.10), respectively), which is consistent with previous observations in cultured cells and mice. Of particular interest was the positive correlation between tissue GPx activity and Gleason Score, with this relationship achieving statistical significance among African Americans ($r=0.67$, $p=0.02$).

Conclusion—These studies support the continued investigation of the role of Se and selenoproteins in prostate cancer prevention, development and prognosis.

Keywords

Selenium; GPx; SBP1; prostate; Gleason score

INTRODUCTION

There has been considerable interest in studying the potentially beneficial effects of higher consumption of the essential trace element selenium (Se). This interest stems from a large body of *in vitro* and animal studies that have shown Se to have either an anti-cancer or

*Correspondence to: Alan M Diamond, Department of Pathology, College of Medicine, 840 S Wood St, Room 130 CSN, University of Illinois at Chicago, Chicago, IL, USA. Phone: 312 413-8747. Fax: 312 996-7586. adiamond@uic.edu.

²These two authors have contributed equally to this manuscript

chemopreventive benefit when provided at non-toxic levels. These benefits are supported by human epidemiological data that have often, albeit not exclusively, shown an inverse association between dietary Se intake and/or blood Se concentrations and cancer risk (1). In contrast, a large prostate cancer prevention trial designed to determine whether Se and/or vitamin E supplementation in men over 50 years of age reduced the incidence of prostate cancer was terminated early as there was no evidence of any benefit in either arm; furthermore, a non-significant increased risk of diabetes was observed among those randomized to receive Se (2). These findings have resulted in uncertainty as to whether Se intake can be developed into a strategy to prevent prostate cancer as well as other malignancies.

Within the cell, Se is specifically associated with two distinct classes of selenoproteins. One of these is a family consisting of 25 human Se-containing proteins in which selenocysteine is incorporated co-translationally in response to in-frame UGA codons in the selenoprotein mRNA (3). Among the best studied of these is the ubiquitously expressed cytosolic glutathione peroxidase, GPx-1. Over-expression of GPx-1 has been reported to result in protection against DNA damage (4) and a role for this protein in cancer etiology is supported by genetic data indicating the existence of functional polymorphisms that decrease enzyme activity and are also associated with increased risks of cancer at multiple sites (5,6). The other class of selenoprotein is typified by Se Binding Protein 1 (SBP-1) which does not contain selenocysteine, with the nature of the Se moiety still unknown (7). Examination of SBP1 levels in human tumors has revealed an inverse association with clinical outcome for several cancer types (8–13). GPx-1 and SBP1 regulate each others levels as ectopic expression of SBP1 results in a reduction in GPx-1 levels and increasing GPx-1 levels either by transfection of an expression construct *in vitro* or by increasing the Se intake of mice results in a consequential reduction in SBP1 (14).

We analyzed associations between several biomarkers of Se status measured in serum and normal prostate tissue samples obtained from 24 men who underwent radical prostatectomy for the treatment of localized prostate cancer. We additionally investigated the relation of these biomarkers to disease aggressiveness as measured using the Gleason Score. Finally, since prostate cancer incidence and mortality rates differ significantly between Caucasians and African Americans,(15) we examined whether the aforementioned relationships differed by race.

MATERIALS AND METHODS

Clinical Samples

Fasting blood samples and histologically normal appearing prostatic tissues were collected from 24 individuals enrolled in a larger parent study designed to assess relationships between diet and gene expression in normal prostatic tissue. Briefly, men were eligible to participate if they had a biopsy diagnosis of prostate cancer and were scheduled to undergo radical prostatectomy surgery at the University of Illinois Hospital or the Jesse Brown Veterans Affairs Medical Center in Chicago. There was no age limit for the study and no racial or ethnic groups were excluded from participation. Patients receiving exogenous hormones or androgen ablation therapy prior to surgery were excluded as these treatments might have affected gene expression levels in the prostate.

At research visits scheduled prior to surgery, participants completed dietary, medical history, and lifestyle questionnaires, had their height and weight measured, and provided a fasting blood sample. Collected blood was separated into serum, plasma, red blood cells, and white blood cells and stored at -80°C . Prostate specific antigen levels were also assessed prior to surgery. After radical prostatectomy, the prostate was received fresh and unfixed

and subsequently grossly examined by a member of the University of Illinois at Chicago Pathology department. Seminal vesicles, apex and base were removed and the prostate was serially sectioned at 2–3 mm intervals into several sections. A pre-specified number of sections were submitted for histological diagnosis and the remaining tissue was reserved for research. Slices of fresh prostate tissue obtained for research were snap-frozen in either liquid nitrogen or a dry ice/ethanol bath and then transferred to nitrogen vapor tank for storage. Gleason Score – a measure of disease aggressiveness based on morphologic characterization of the tumor compared to normal prostate cells (16)- was obtained from surgical pathology reports.

Hematoxylin and eosin staining was utilized for pathologic characterization of research tissue. Only normal tissues were selected for biomarker determination in the present study.

All subjects provided written informed consent and the study was approved by the UIC/ JBVAMC Institutional Review Board.

Analysis of Se in serum and prostate tissue

Samples (30µL) were diluted with 20µL deionized water and 150µL of matrix modifier (0.09% palladium chloride [Alfa Aesar, Ward Hill, MA] and 1.5% nickel nitrate [hexahydrate, Alfa Aesar, Ward Hill, MA]. A 20 µL aliquot of serum or prostate digest was mixed with 5 µL of 3% hydroxylamine hydrochloride [Eastman Kodak Company, Rochester, NY] and 5 µL of 18 megohm deionized water using an autosampler which transferred them to the L'Vov platform in the graphite furnace of a Perkin Elmer AAnalyst 800 instrument (Perkin Elmer Corp., Wellsley, MA). The temperature programming of the furnace was as follows: 1) to 90°C in 10 sec, held 30 sec under Ar; 2) to 150°C in 10 sec for 50 sec under Ar; 3) to 500°C in 5 sec under Ar, held for 19 sec under 100% O₂, held 4 sec under Ar; 4) to 1000°C in 5, held for 30 sec under Ar; 5) hold 2 sec at 1000°C with no gas flow; 6) to 2500°C in 1 sec, held for 6 sec with no gas flow; 7) read (196.0 nm, 2 nm slit) for 1 sec with no gas flow; 8) purged for 6 sec under Ar. The minimum detection limit of this method was 2 ng/ml. Serum selenium was measured in duplicate and the average pair-wise coefficient of variation (CV) was 1.6%. Selenium concentrations in prostate tissue were measured multiple times within and across batches; the average intra-batch and inter-batch CVs were 5% and 14.5%, respectively. Calibration standards including a blank, 5 ppb (ng/mL), 10 ppb and 25 ppb of selenium were sampled identically to the samples and validated with UTAK serum mineral control (UTAK Laboratories Inc., Valencia Ca.), and NIST certified standards (Bovine Liver SRM1577b and Natural Water SRM 1643e).

Measurement of GPx activity in serum and prostate tissue

GPx activity in serum and tissue was determined using a standard coupled spectrophotometric assay that determines the rate of reduction of hydrogen peroxide by peroxidase activity, using reducing equivalents from reduced glutathione coupled to the consumption of NADPH by glutathione reductase (17). Plasma was added directly to the assay. To prepare tissue for analysis, it was flash frozen in liquid nitrogen and minced using a mortar and pestle. RIPA lysis buffer (Cell Signaling Technology, Danvers, MA) was mixed with the powdered tissue and sonicated. The obtained data from serum is normalized to the volume added to the reaction, while GPx activity of tissue is normalized to the levels of protein added. Enzyme activity is presented as the nmoles of NADPH oxidized per minute. The pairwise CV for duplicate serum GPX measurements in a random sample of study participants was 15%. The CVs for GPX activity measured in triplicate in male K46 mouse +/- colon and liver tissues were 8% and 9%, respectively.

Determination of SBP1 levels in prostate tissue

Levels of the SBP1 Se-associated protein was determined by western blotting, quantified by densitometry and normalized to the levels of actin in the protein extract to control for loading variation and possible protein degradation. Tissue was suspended in RIPA lysis buffer (Cell Signaling Technology) containing a protease inhibitor cocktail (Sigma-Aldrich Corp. St. Louis, MO). After incubation on ice for 15 minutes, cell lysates were centrifuged at 12,000 rpm for 15 minutes at 4°C. The protein content of the supernatant was determined using the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories, Hercules, CA). Proteins were separated by 12% SDS-PAGE and transferred to PVDF membranes (Bio-Rad Laboratories, Hercules, CA). Blots were probed with antibodies specific for SBP1 and β -actin (Sigma-Aldrich Corp. St. Louis, MO). Signals were detected by enhanced chemiluminescence Plus reagents (Amersham Pharmacia, Piscataway, NJ.) and quantified using NIH Image software. CVs for triplicate measurements of SBP1 and actin in male K46 +/- mouse colon and liver tissues were 9% and 5%, respectively.

Statistical Analysis

Differences in participant characteristics and biomarkers of Se status between African-Americans and Caucasians were assessed using the Wilcoxon rank sum test for continuous variables and the chi-squared test for categorical variables. The Fisher's exact test was utilized to test for differences in categorical variables with cell counts of 5 or less. Spearman correlation coefficients adjusted for age, body mass index, smoking status, and PSA level were utilized to evaluate correlations between blood and tissue biomarkers of Se status and Gleason Score. All statistical analyses were performed using Statistical Analysis Systems software, version 9.2 (SAS, Inc.).

RESULTS

Characteristics of the study population – overall and by race – are shown in Table I. The median age in the sample was 65 years and many participants were obese (BMI ≥ 30 kg/m²) and/or currently smoked. The median pre-surgical PSA level and Gleason Score (obtained from pathology reports) was 5.6 ng/mL and 6.7, respectively, which was expected due to the fact that all of these men were enrolled into the parent study because they had been diagnosed with prostate cancer. Smoking status was the only characteristic that differed significantly by race, with 47% of African-Americans versus 63% of Caucasians reporting current smoking. Serum Se levels ranged from 119 to 199 μ g/L in this group of men, with a median of 156 μ g/L. Caucasians had higher serum Se and GPx activity, lower tissue GPx activity, and higher tissue SBP1 than African-Americans, although none of these differences achieved statistical significance. Prostatic tissue concentrations of Se did not differ by ethnicity.

Relationships between blood and tissue markers of Se status and biology, overall and by race, are presented in Tables IIa–c. There was no association between serum Se level and GPx activity, nor between serum Se and any of the biomarkers measured in normal prostatic tissue (including Se). Tissue Se levels were similarly unrelated to other biomarkers of Se status in the prostate, although there was a modest correlation between tissue Se levels and serum GPx-3 activity, the only GPx isoform found in serum, in African-Americans ($r=0.67$, $p=0.15$). A significant positive correlation was observed between serum and tissue GPx activity in all subjects ($r=0.55$, $p=0.02$), with a similarly strong yet borderline significant relation noted in African Americans ($r=0.47$, $p=0.15$).

Tissue GPx activity was suggestively inversely correlated with tissue SBP1 levels in all subjects ($r=-0.42$, $p=0.08$) and in African Americans ($r=-0.53$, $p=0.10$), which is consistent

with observations previously reported in cell culture and animal tissues.(14) Although tissue GPX activity and SBP1 levels (as well as tissue GPX and Gleason Score) were also correlated in Caucasians, the small sample size ($n=7$) lessens the potential impact of these findings. Unexpectedly, there was a positive association between prostatic GPx activity and Gleason Score, with this number approaching significance in all subjects ($r=0.38$, $p=0.12$) and achieving it among African Americans ($r=0.67$, $p=0.02$). Consistent with this finding, tissue SBP1 levels were inversely associated with Gleason Score in all participants and in the subset of African-Americans, although these findings did not reach statistical significance ($r=-0.25$, $p=0.30$ overall and $r=-0.47$, $p=0.14$ in African Americans)).

DISCUSSION

The present study was initiated in order to gain a better understanding of the interrelationships between Se and selenoprotein levels measured in blood and prostate tissue, and to evaluate how these markers of Se status relate to prostate cancer aggressiveness. One surprising result from this effort is the lack of association between serum Se levels and any of the parameters examined. Of particular note is the lack of any correlation between serum Se status and GPx activity measured in either serum or prostate tissue. Serum GPx activity represents the activity of GPx-3 secreted into the blood from the proximal tubule of the kidney, and is often used as a general indicator of Se status. Our data are consistent with that reported by Takata et al. who also failed to detect a correlation between serum Se levels and prostate GPx activity among men without detectable prostate cancer (18). We observed a relationship between serum and prostate tissue GPx activity, although statistical significance was only achieved when all participants were considered. Given the possible importance of GPx enzyme activity in prostate cancer risk and development, both as an anti-oxidant enzyme and as a potential factor in prostate carcinogenesis, determination as to whether serum Se or GPx can be used as a surrogate biomarker to predict that enzyme's activity in the prostate merits further investigation. Other approaches for determining Se status may be better correlated to prostate GPx activity and these methods have recently been reviewed (19).

Our study indicates for the first time that GPx activity is inversely correlated with SBP1 levels in normal prostate tissue. Previous work has shown that increasing GPx-1 levels in either MCF-7 breast cancer or HCT116 colon cancer cells, either by transfection of GPx-1 expression construct or supplementing the tissue culture media with low levels of sodium selenite, resulted in lower SBP1 levels.(14) In that same study, it was also shown that increasing the levels of GPx in the colon and duodenum by feeding mice a Se deficient, adequate or supplemented diet resulted in a consequential decline in SBP1 (14). As seen in Table II, the inverse relationship between GPx and SBP1 may be extended to human prostate tissue, although these findings require confirmation in larger studies.

Lower levels of SBP1 have been shown to be an indicator of poor prognosis of colon cancer (12,13) and as seen in our study, its reduced levels are suggestively associated with increased Gleason Score - a histopathological grading system in which higher numbers are an indication of worse clinical outcome. Importantly, this observation approached statistical significance only in African Americans – a group that has one of the highest incidence rates of prostate cancer in the world (20). While the biological function of SBP-1 is unknown, its over-expression can inhibit cancer cell migration *in vitro* and inhibit tumor growth in nude mice (21). In contrast to the aforementioned inverse correlation between SBP1 levels and disease aggressiveness, prostate tissue GPx activity was positively associated with higher Gleason Score; again, this result was most pronounced in African-Americans. While it might seem paradoxical that elevated levels of an anti-oxidant protein could contribute to tumor progression, transgenic mice that over-express GPx-1 exhibit enhanced skin cancer

development following exposure to TPA (22). Elevated GPx-1 enzyme activity can suppress apoptosis (23–25) and it is possible that the same anti-oxidant enzyme can be beneficial in preventing cancer over the course of a lifetime by protecting against oxidative damage and also detrimental by protecting pre-malignant and malignant cells from being cleared; this possibility has been recently discussed (26).

CONCLUSIONS

The results presented above report on the determination of Se concentration and GPx activity in serum and normal prostatic tissue, as well as tissue SBP1 levels, obtained from men with localized prostate cancer. The lack of a correlation between Se content and GPx activity in both serum and matched normal prostate tissue indicates the need for caution in using any of these endpoints as biomarkers for the other. In addition, the findings expand on our previous observations in cell culture and mice and now indicate an inverse association between GPx-1 and SBP1 in histologically normal human tissues. Of particular note is the association between higher prostate GPx activities with increased aggressiveness of prostate cancer. Conclusions regarding these data should take into account the relatively small sample size and the cross-sectional study design, which leaves open the possibility that the presence of disease affected the measured endpoints. Additional studies will be required to assess the significance of these observations.

Acknowledgments

Grant sponsor: NIH Grant 1R01CA127943 to AMD, a post-doctoral fellowship from the American Institute of Cancer Research to AJM, DOD Grant W81XWH-06-1-0414 to MEW, and GFHNRC CRIS project #5450-51000-036-00D.

We gratefully acknowledge the expert technical contributions of Erika Enk-Reuter, UIC, and Craig Lacher, GFHNRC. This work was supported by NIH Grant 1R01CA127943 to AMD, a post-doctoral fellowship from the American Institute of Cancer Research to AJM, DOD Grant W81XWH-06-1-0414 to MEW, and GFHNRC CRIS project #5450-51000-036-00D.

Abbreviations

Se	selenium
GPx	glutathione peroxidase
SBP1	Selenium binding protein 1

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Table ICharacteristics¹ of the study sample, overall and by race

	All subjects	African-American	Caucasian	p-value ²
No.	24	15	8	
Age (years)	64.5 (60, 67.5)	62.0 (59.0, 71.0)	64.5 (60.5, 66.0)	0.99
Body mass index (kg/m ²)	30.0 (26.6, 34.5)	30.6 (28.2, 36.1)	27.7 (25.7, 31.3)	0.30
Smoking status				0.03
Never	4 (16.7)	1 (6.7)	3 (37.5)	
Former	7 (29.2)	7 (46.7)	0	
Current	13 (54.2)	7 (46.7)	5 (62.5)	
Cigarettes per day ³	10.0 (6.5, 20.0)	10 (7.0, 20.0)	20 (10.0, 20.0)	0.41
PSA (ng/mL)	5.6 (4.3, 10.7)	5.6 (3.9, 14.5)	5.5 (4.4, 9.4)	0.80
Gleason grade	7.0 (6.0, 7.0)	7.0 (6.0, 7.0)	6.5 (6.0, 7.0)	0.21
Serum Se (ng/mL)	156 (141, 168)	149 (139, 161)	173 (154, 183)	0.09
Tissue Se ⁴ (ng/g)	0.18 (0.13, 0.25)	0.18 (0.13, 0.25)	0.18 (0.10, 0.23)	0.90
Serum GPx ⁵	282 (231, 300)	260 (228, 298)	294 (289, 309)	0.14
Tissue GPx ^{5,6}	16.2 (9.4, 25.0)	17.6 (8.2, 27.6)	10.0 (9.4, 11.5)	0.17
Tissue SBP1 ⁷	0.85 (0.61, 1.22)	0.77 (0.59, 0.96)	1.08 (0.84, 1.44)	0.15

¹ Medians (25th percentile, 75th percentile) for continuous variables and number (%) for categorical variables² Significance test for the difference between African-Americans and Caucasians: Wilcoxon rank sum test for continuous variables and Fishers exact test for categorical variables³ Among former and current smokers only⁴ Based on 19 subjects with available measurements (10 African-Americans and 8 Caucasians)⁵ GPx activity is expressed as nmoles of NADPH oxidized per minute. For serum, these numbers are expressed per mL of serum while tissue GPx is expressed per mg of protein.⁶ Based on 23 subjects with available measurements (15 African-Americans and 7 Caucasians)⁷ SBP1 levels are expressed as arbitrary values obtained from densitometry and normalized to the value obtained from measuring the actin signal on the western blot.

Adjusted¹ Spearman correlation coefficients and corresponding *p*-values among all study subjects (n=24)

Table IIa

	Tissue Se ²		Serum GPx		Tissue GPx ³		Tissue SBP1		Gleason Score	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Serum Se	-0.16	0.58	-0.08	0.75	-0.25	0.33	-0.09	0.72	0.006	0.98
Tissue Se ²			0.12	0.69	-0.05	0.88	0.22	0.46	0.22	0.44
Serum GPx					0.55	0.02	0.18	0.44	0.07	0.78
Tissue GPx ³							-0.42	0.08	0.38	0.12
Tissue SBP1									-0.25	0.30

¹ Adjusted for age, body mass index, smoking status, PSA level, and race

² Based on 19 subjects

³ Based on 23 subjects

⁴ Adjusted for age, body mass index, smoking status, and PSA level

⁵ Based on 10 subjects

⁶ Based on 7 subjects

Adjusted⁴ Spearman correlation coefficients and corresponding *p*-values among African-Americans (n=15)

Table IIb

	Tissue Se ⁵		Serum GPx		Tissue GPx		Tissue SBP1		Gleason Score	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Serum Se	-0.47	0.35	-0.35	0.29	-0.15	0.67	-0.06	0.85	-0.02	0.95
Tissue Se ⁵			0.67	0.15	0.11	0.84	0.13	0.81	0.30	0.56
Serum GPx					0.47	0.15	-0.07	0.83	0.23	0.50
Tissue GPx							-0.53	0.10	0.67	0.02
Tissue SBP1									-0.47	0.14

¹ Adjusted for age, body mass index, smoking status, PSA level, and race

² Based on 19 subjects

³ Based on 23 subjects

⁴ Adjusted for age, body mass index, smoking status, and PSA level

⁵ Based on 10 subjects

⁶ Based on 7 subjects

Adjusted⁴ Spearman correlation coefficients and corresponding *p*-values among Caucasians (n=8)

Table IIc

	Tissue Se		Serum GPx		Tissue GPx ⁶		Tissue SBP1		Gleason Score	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
Serum Se	-0.65	0.35	0.16	0.84	0.24	0.84	0.26	0.74	-0.60	0.40
Tissue Se			0.61	0.39	0.26	0.83	-0.23	0.77	0.92	0.08
Serum GPx					0.55	0.63	0.24	0.76	0.67	0.33
Tissue GPx ⁶							0.99	0.09	0.99	0.05
Tissue SBP1									0.17	0.83

¹ Adjusted for age, body mass index, smoking status, PSA level, and race

² Based on 19 subjects

³ Based on 23 subjects

⁴ Adjusted for age, body mass index, smoking status, and PSA level

⁵ Based on 10 subjects

⁶ Based on 7 subjects