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ORIGINAL ARTICLE

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Correlations of SELENOF and SELENOP genotypes with serum selenium levels and prostate cancer

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Background: Selenium status is inversely associated with the incidence of prostate cancer. However, supplementation trials have not indicated a benefit of selenium supplementation in reducing cancer risk. Polymorphisms in the gene encoding selenoprotein 15 (SELENOF) are associated with cancer incidence/mortality and present disproportionately in African Americans. Relationships among the genotype of selenoproteins implicated in increased cancer risk, selenium status, and race with prostate cancer were investigated.

Methods: Tissue microarrays were used to assess SELENOF levels and cellular location in prostatic tissue. Sera and DNA from participants of the Chicago-based Adiposity Study Cohort were used to quantify selenium levels and genotype frequencies of the genes for SELENOF and the selenium-carrier protein selenoprotein P (SELENOP). Logistic regression models for dichotomous patient outcomes and regression models for continuous outcome were employed to identify both clinical, genetic, and biochemical characteristics that are associated with these outcomes.

Results: SELENOF is dramatically reduced in prostate cancer and lower in tumors derived from African American men as compared to tumors obtained from Caucasians. Differing frequency of SELENOF polymorphisms and lower selenium levels were observed in African Americans as compared to Caucasians. SELENOF genotypes were associated with higher histological tumor grade. A polymorphism in SELENOP was associated with recurrence and higher serum PSA.

Conclusions: These results indicate an interaction between selenium status and selenoprotein genotypes that may contribute to the disparity in prostate cancer incidence and outcome experienced by African Americans.

KEYWORDS
genotype, polymorphisms, prostate carcinogenesis, racial disparities, SELENOF, selenoproteins

1 | INTRODUCTION

Selenium is an essential trace element shown to reduce the incidence of a wide variety of cancer types in experimental animal models. While selenium status has been observed to be inversely associated with the risk of prostate cancer, randomized controlled studies have not revealed any consistent benefit of selenium supplementation in reducing the disease.[1] Most of the biological consequences of selenium intake are considered to be mediated by its role as a constituent of selenium-containing proteins. One of these, SELENOF (previously referred to as Sep15) was originally identified as a human T cell 15 kDa protein that was labeled with Se^{75} and was expressed at high levels in the prostate.[2,3] It belongs to the family of selenium-containing proteins, that is, selenoproteins, in which selenium is inserted co-translationally in response to a UGA codon in the corresponding mRNA.[4] The molecular insertion of selenocysteine is determined by sequences in the 3′-untranslated region (3′-UTR) of the mRNA referred to as a SECIS (selenocysteine insertion sequence) element.[5,6] The SELENOF gene is polymorphic in the 3′-UTR at positions 811 and 1125, a region that is critical for the recognition of UGA codons as the amino acid selenocysteine.[2] We have shown that these genetic variations are functional and contribute to determining the amount of SELENOF protein made as a function of selenium availability.[3,7] Focusing on these polymorphisms to investigate genetic changes that occurred prior to or during cancer development, we reported that there was loss of heterozygosity (LOH) at the SELENOF locus in breast cancers and cancers of the head and neck among African Americans, but not Caucasians.[7] Several other studies have also indicated an association between genetic variants of SELENOF and the risk of colorectal cancer[8,9] while the knockout of SELENOF has been shown to inhibit the in vitro growth and in vivo tumorigenicity and metastasis of human colon cancer cells,[10,11] and to protect mice against chemically induced aberrant crypt foci.[12] While the function of SELENOF remains unresolved, it has been reported to reside in the endoplasmic reticulum (ER) and to associate there with the UDP-glucose:glycoprotein glucosyltransferase (UGTR), likely playing an important role in disulfide bond formation and protein quality control in that organelle.[13–16] SELENOF is unusual in that it contains an ER-localization sequence but does not contain an ER-retention signal, and retention of SELENOF in the ER has been postulated to occur due to its interaction with UGTR.[13]

In 2010, Penney et al[17] reported that there was a statistically significant association between polymorphisms in the gene for SELENOF, plasma selenium levels and importantly, prostate cancer mortality, but not risk. In this study, polymorphisms previously shown as functional, (forming a haplotype such that a C at residue 811 always corresponded to a G at 1125 and a T at 811 always corresponded with an A at 1125) were associated with prostate cancer-specific mortality with marginal significance ($P = 0.10$). The population examined in this nested case-control study consisted of self-reported Caucasians obtained from the Physicians Health Study, and the allele frequency for the 811 AA genotype was 4.8% for cases of prostate cancer ($n = 1195$) and 4.6% for controls ($n = 1186$), in good agreement with the low frequency we previously reported among Caucasians.[7] In contrast, the frequency we reported for African Americans for the AA genotype was much higher at 31%.[7] To evaluate whether racial disparity in prostate cancer incidence and outcomes may involve race-specific differences in SELENOF levels, we investigated the involvement of SELENOF in prostate cancer using cultured cells and human prostate cancer tissue microarrays, DNA samples, and human data.

2 | MATERIALS AND METHODS

2.1 | Cell culture, plasmid construction, and western blotting

A doxycycline-inducible SELENOF expression construct was generated by the insertion of the PCR amplified SELENOF open reading frame generated using cDNA derived from RNA obtained from the PC3 prostate cancer cell line as the template and ligation into the pRetroX-Tight-Pur vector (Clontech, Mountain View, CA). For amplification, two oligonucleotides containing *NotI* and *MluI* restriction sites were synthesized; a forward primer (5′-GCAGCAGCGGCCGC-GATCAGGCTCTGGAGTGGAC-3′) and reverse primer (5′-GCAG-CAACGCGTGAGCAGGCAATCTGTTGAGG-3′). The amplification product was digested with *NotI* and *MluI* and directionally ligated into the vector. The inducible expression system includes the pRetroX-Tight-Pur-TetOn-Advanced plasmid containing the Tet-On transactivator gene. The Tet-On gene codes for a protein that binds to the promoter region of the pRetroX-Tight-Pur plasmid and induces transcription of the gene downstream of the promoter in response to doxycycline. The SELENOF gene was inserted downstream of the doxycycline responsive promoter region in the pRetroX-Tight-Pur plasmid yielding the pRetroX-Tight-Pur-SELENOF. The human PC3, LNCaP, and RWPE-1 prostate cell lines were obtained from ATCC (Manassas, VA) and authenticated by analyzing 15 autosomal short tandem repeat loci and the sex specific amelogenin locus to identify gender (Genetica DNA Laboratories, Burlington, NC). Human primary prostate epithelial cells obtained from tissue after prostatectomy were prepared as previously described.[18] Western blotting was performed on obtained LNCaP and PC3 protein extracts using antibodies specific for SELENOF (rabbit, Abcam, Cambridge, MA) and β-Actin (rabbit, Abcam). Induction of ectopic expression of SELENOF was achieved by treatment of transfectants with 1.0 ug/mL doxycycline for 72 h.

2.2 | Confocal microscopy

Prostate cancer derived cell lines LNCaP and PC3 were plated onto MatTek 1.5 mm glass-bottomed culture dishes (MatTek Corporation, Ashland, MA). The cells were allowed to grow to 80% confluence and were washed twice with PBS and then incubated with ER-Tracker Green dye (Thermo Fisher Scientific #E34251, Waltham, MA) at a concentration of 1 μM in HBSS/Ca/Mg (Gibco) at 37°C and 5% CO_2 for 30 min. The cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min. After fixation, cells were permeabilized using 100% ice cold methanol for 15 min. After permeabilization, the cells were again

washed with PBS and blocked using 5% BSA in 1% TBS-T for 45 min. Following the blocking step, the cells were washed and incubated with SELENOF primary antibody (Abcam, # ab124840) at 1:100 in 1% BSA in 1% TBS-T in a humid chamber to prevent drying. Secondary antibody (Alexafluor-647) was then incubated at 1:200 in 1% BSA in 1% TBS-T for 2 h at room temperature in a dark humid chamber. The cells were incubated with DAPI (50 μM) for 30 min with agitation in the dark. Following DAPI incubation, the cells were washed, and PBS was added to a final volume of 2 mL. RWPE-1 and primary prostate cells were fixed as described above. However, the cells were permeabilized and washed using 0.1% Saponin-TBST (Sigma-Aldrich, St. Louis, MO) for 10 min at 37°C. After the blocking step with background sniper (BIOCARE Medical, Pacheco, CA), the cells were incubated with SELENOF (Abcam, # ab124840) and E-Cadherin (Abcam # ab76055) antibodies diluted in Diamond Antibody Diluent (Cell Marque, Rocklin CA). Images were obtained using a LSM510UV confocal microscope (Zeiss).

2.3 | Genotyping at the SELENOF and SELENOP loci

The desired regions of SELENOF and SELENOP were amplified using gene specific primers (Integrated DNA Technologies, Coralville, IA) and the amplified DNA was sequenced across the polymorphic region. PCR and sequencing primers used in the analysis as well as the size of the amplicon are found in Supplemental Table S1. All sets of PCR reactions included a control without template DNA. The sizes of the PCR products were assessed by electrophoresis using a 1% agarose gel and bands were visualized with ethidium bromide (Bio-Rad, Hercules, CA). Sequencing was performed by the UIC DNA Core. Single-nucleotide heterozygosity was apparent when 2 peaks were visualized at the same location on the chromatogram that corresponded to the polymorphism of interest.

2.4 | Selenium levels

Selenium levels in plasma were determined using a Pinaacle 900T Graphite Furnace Atomic Absorption Spectrometer (PerkinElmer, Waltham, MA 02451). The minimal detection limit was 25 μg/L, with a linear correlation coefficient of 0.999898 and 98.7% average recovery of the UTAK serum standard, lot #8315 for Trace Metals (Utak Laboratories Inc., Valencia, CA 91355).

2.5 | Source of clinical samples

Prostate cancer outcome tissue microarrays (TMAs) were obtained from the Cooperative Prostate Cancer Tissue Resource (CPCTR), a multi-institutional consortium to bank prostatectomy tissue, with detailed uniform annotation of patient demographics, surgical pathology data and follow-up history.[19,20] The TMA used in this study included prostate cancer tissue cores of 0.6 mm diameter in quadruplicate from 200 men ("cases") who experienced biochemical recurrence (a single post-surgery prostate specific antigen (PSA) value above 0.4 ng/mL or two consecutive PSAs above 0.2 ng/mL) after radical prostatectomy and 200 non-recurrent controls matched by age at surgery (±5 years), year of surgery, race, Gleason sum, and pathological stage.

DNA and clinical data were obtained from the Adiposity and Outcomes of Clinically Localized Prostate Study, a prospective cohort study examining the mechanistic basis for the association of obesity with biochemical failure and other adverse prognostic outcomes after surgery. Participants were predominantly middle-aged (mean = 60 yrs) non-Hispanic white men (66.7%), and all had undergone radical prostatectomy. Before surgery, biospecimens were collected (serum, plasma, and buffy coat DNA) and body weight status, lifestyle, and clinical factors assessed. Pathologic tumor diagnoses were determined by a single pathologist with 10% of the cases reviewed by a second pathologist. All of the patients have consented to 10 years of follow-up (up to 2019-2023, depending on year of enrollment) and the protocol was approved by the UIC Office for the Protection of Research Subjects.

2.6 | Immunohistochemistry

Immunohistochemistry was performed by the UIC Research Histology and Tissue Imaging Core using the Bond™ polymer refine detection HRP (Leica Biosystems, DS9800) method. Deparaffinization and antigen retrieval (pH6; 20 m) was performed on-line using Leica Bond-Max (Leica Biosystems, Buffalo Grove, IL). The TMA sections were washed with Bond Dewax solution (Leica Biosystems, AR9222) at 72°C followed by a 100% ethanol wash. The slides were subsequently washed with bond wash solution and target antigen were unmasked by incubation in Bond ER 1 Solution (pH 6) for 20 min at 100°C (Leica Biosystems, AR9640). Following a last wash with bond wash solution, immunostaining for the primary antibody SELENOF (Abcam, Cat# ab124840, rabbit monoclonal NCIR128A) or SELENOM (Abcam, Cat # ab133681, rabbit monoclonal EPR8279) diluted 1:1000 was performed by incubating the primary antibody for 15 min at room temperature. Following primary antibody incubation, tissue sections were incubated with non-conjugated secondary rabbit anti-mouse IgG for 8 min at room temperature. Subsequently, the slides were washed and the tissue sections were incubated with HRP conjugated polymer anti-rabbit poly-HRP-IgG for 8 min at room temperature. Then, endogenous peroxidase activity was blocked with hydrogen peroxide for 5 min. Immunoreactivity was visualized with 3, 3-diaminobenzidine (DAB) Chromogen for 10 min at room temperature. Finally, sections were counterstained with Mayer's Hematoxylin for 5 min, dehydrated by graded alcohol to xylene, and mounted and cover slipped. Tissues known to express SELENOF (prostate cancer tissue TMA) were used as a positive control. As a negative control, sections were treated as described above omitting the primary antibody. Verification of SELENOF antibody specificity was obtained by blocking the primary SELENOF antibody with a 10-fold excess of synthetic peptide and immunostaining as described above.

2.7 | Identification and recruitment of subjects of the adiposity and outcomes of clinically localized prostate study cohort

The objective of the Adiposity Study was to elucidate mechanisms linking obesity to worse treatment outcomes after surgery for clinically

localized prostate cancer. After obtaining institutional review board approvals, patients awaiting radical prostatectomy for histologically confirmed, organ-confined prostate cancer (AJCC 7th Ed., Stage I-II, $cT_{1b\text{-}2c}N_0M_0$) were identified through medical record review at four Chicago medical centers between 2009 and 2013 (Loyola University Medical Center, Maywood, IL; University of Illinois Hospital & Health Sciences System, Chicago, IL; Edward Hines, Jr. VA Hospital, Hines, IL; John H. Stroger, Jr. Hospital of Cook County, Chicago, IL). Eligible patients were approached by their urologist either in clinic or by telephone or letter ($n = 530$). Of these, 307 declined or did not respond, and 223 were consented to participate. Baseline clinical and demographic data were collected by self-administered questionnaires and medical record review. Demographic data included date of birth, self-reported race/ethnicity, and family history of prostate cancer. Clinical data collected included initial PSA levels, date of prostate cancer diagnosis on prostate biopsy, biopsy Gleason score, and clinical stage. Of the 223 subjects enrolled, 12 were subsequently excluded due to their surgery being cancelled for medical reasons, aborted surgery, no-show for surgery, change to non-surgical treatment per subject request (active surveillance or external beam radiation), lost to follow-up, or withdrawal of consent, leaving 201. Sera and DNA from a random sample of 126 of these subjects were made available in the present study.

2.8 | Pathologic staging

Immediately after surgical removal (or at the time of extraction for robotic prostatectomy), the prostate gland was placed into sterile saline and delivered to the medical facility's pathology department. The entire surface of the prostate was inked using distinct colors for the left and right lobes. The apex and base were amputated at a thickness of 2-3 mm, and subsequently cut in sagittal sections and submitted in entirety in paraffin embedding. The remaining prostate was sectioned at 3 mm intervals, creating parallel transverse sections perpendicular to the long axis. Every other slice was submitted for microscopic evaluation, with the remaining specimens retained for further study. Following institutional reporting, slides for each subject were submitted to a single pathologist for review, with pathologic findings reported consistent with published recommendations.[21] Histologic grade was reported according to the Gleason grading system,[22] and Gleason sum groups were defined as 2-6 (group 1), 7(3 + 4) (group 2), 7 (4 + 3) (group 3), 8 (group 4), and 9-10 (group 5).[23] Anatomic stage/prognostic group (I, IIA, IIB, III, and IV) was reported according to the American Joint Committee on Cancer, 7th edition.[24]

2.9 | Follow-up

Post-operatively, subjects were instructed to follow-up with the treating urologist with a serum PSA test every 3 months for the first year, every 3-6 months the second year, and annually thereafter. Alterations to the follow-up protocol were allowed at the discretion of the treating clinician. Serum PSA measurements were performed using the Tandem PSA monoclonal antibiotic assay (Hybritech, San Diego, CA). Treatment failure within 2 years was defined as detectable (≥0.1 ng/mL) and subsequently rising serum PSA ("biochemical failure") or receipt of androgen deprivation therapy post-operatively for high progression-risk disease.

2.10 | Statistical analysis

Multivariate logistic and ordinal logistic regression models analyzed dichotomous outcomes (treatment failure, yes vs no) and ordinal outcomes (Gleason sum groups and anatomic stage/prognostic groups), respectively. Linear regression models analyzed baseline serum PSA levels. PSA levels were log-transformed as regression diagnostics showed normality violation in error distribution. Backward model selections were performed for each outcome analysis, first among demographic and clinical characteristics (age, race/ethnicity, family history of prostate cancer, body mass index), then among biochemical/molecular variables (selenium, SELONOF, SELENOP rs3877579, and SELENOP rs3877899 polymorphisms). For each outcome, estimates of effects, standard errors, odds ratios, and corresponding 95% confidence intervals reported are based on the final backward-selected model. All tests shown are two-sided tests, controlling for a Type I error probability of 0.05.

3 | RESULTS

3.1 | SELENOF expression in human cell lines

SELENOF levels were assessed in protein extracts derived from the human prostate cancer cell lines PC3 and LNCaP by western blotting (Supplemental Figure S1). Relatively high levels of expression were observed, consistent with the levels previously reported.[25] In order to verify the specificity of the antibody used for these and subsequent studies, we generated a doxycycline inducible SELENOF expression construct that was introduced into the MCF-7 cell line which does not normally express this protein. As illustrated in Supplemental Figure S1, an obvious band of the anticipated 15 kDa size is apparent in the cells treated with doxycycline but not in cells treated with just vehicle. Using this same antibody to examine the SELENOF distribution in PC3 and LNCaP cells by confocal microscopy, the images obtained were indicative of a predominantly ER localization of the protein (Figure 1), consistent with what has been published previously.[13]

3.2 | SELENOF localizes to the plasma membrane in normal human prostate tissues, but not in tissues derived from other sources

Using this validated antibody, SELENOF was visualized in tissue microarray cores obtained from prostatectomy specimens. Such cores typically contain both tumor tissue and adjacent benign tissue. In benign tissue, the SELENOF protein was expressed in the epithelial cells, but not the stroma, and was predominantly localized to the lateral and basal membranes of prostate epithelial cells with reduced expression on the apical border (Figure 2A and Supplemental

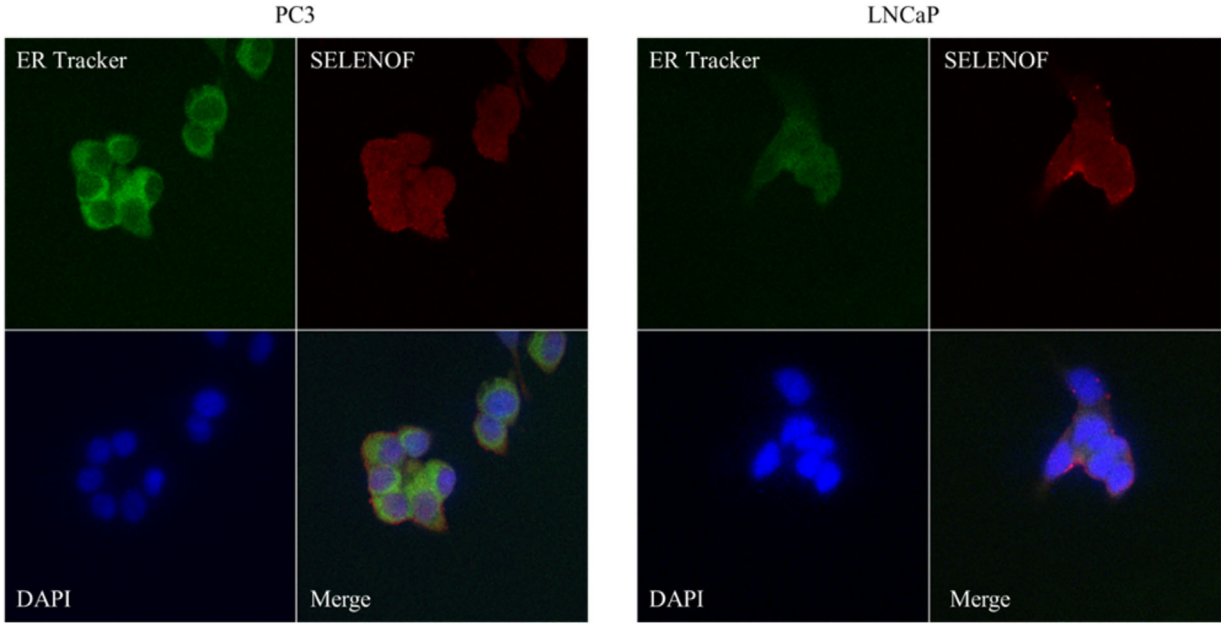

FIGURE 1 SELENOF levels and cellular location in human cell lines. PC3 and LNCaP cells were fixed, permeabilized and immunostained with SELENOF antibodies. SELENOF was visualized with Alexa Fluor 647. ER and nuclei were visualized with ER Tracker Green and DAPI, respectively

Figure S2). This plasma membrane localization was unexpected given previous reports in the literature describing SELENOF as an ER-localized protein. To address the possibility that SELENOF plasma membrane localization is a general feature of human tissues, we examined SELENOF localization in benign human breast, colon, and kidney tissue (Figure 2B). The levels of SELENOF in these tissues were generally low, with a diffuse SELENOF staining throughout the cytoplasm. This is consistent with ER localization and clearly distinct from the membranous staining observed in benign prostate epithelium. Of note is the distinct pattern observed in normal human kidney where expression was generally low with the exception of sporadic tubular staining within that tissue.

SELENOF belongs to a distinct subgroup of selenoproteins that includes SELENOM (previously referred to as SELM), which is also located in the ER and shares 31% sequence homology with SELENOF.[14,26] We next examined SELENOM expression in the prostate, breast, colon, and kidney and observed variable levels of expression in all tissues examined. SELENOM distributed in perinuclear areas, consistent with ER localization and not in the plasma membrane as was observed for SELENOF in non-cancerous prostate (Figure 2C).

3.3 | SELENOF levels are dramatically reduced in prostate cancers as compared to histologically normal tissue adjacent to the tumor, but are not associated with clinical grade or cancer recurrence

In order to investigate the association between SELENOF levels and prostate cancer, we examined human prostatic tissue cores that included benign sections in addition to tumor. This was done using immunohistochemistry staining of a tissue microarray containing cores obtained from tissues of 200 prostate cancer patients who experienced biochemical (PSA) recurrence after prostatectomy and 200 matched control patients whose cancer did not recur. We found no significant associations between SELENOF levels and either tumor grade or cancer recurrence (data not shown). However, the pattern observed in tumor was dramatically different from that observed in non-cancerous tissue, with SELENOF expression being much lower in tumor tissue and generally not in the plasma membrane (See Figure 3A for a representative image). Among 85 tissue cores with clear tumor and benign epithelium regions randomly selected for analysis, the difference in staining was highly significant ($P = 1 \times 10^{-7}$). Since SELENOF staining of the tumor derived cell lines exhibited ER staining (Figure 1) and the staining of benign prostatic tissue was predominantly in the plasma membrane (Figure 2), we examined the subcellular location of SELENOF in non-transformed prostate epithelial cells. SELENOF localization was predominantly in the plasma membrane of both RWPE-1 immortalized human prostate epithelial cells and patient-derived primary prostate epithelial cell cultures (Figure 3B). In contrast to what was observed for prostatic tissue, no apparent differences in SELENOF localization or levels were observed between benign and cancerous regions of the other types of tissues examined (data not shown).

3.4 | Lower SELENOF levels in tumor tissue derived from African Americans than tissue from Caucasian men

We previously reported an approximately fivefold higher frequency of a functional polymorphism in the 3′-UTR of the SELENOF mRNA

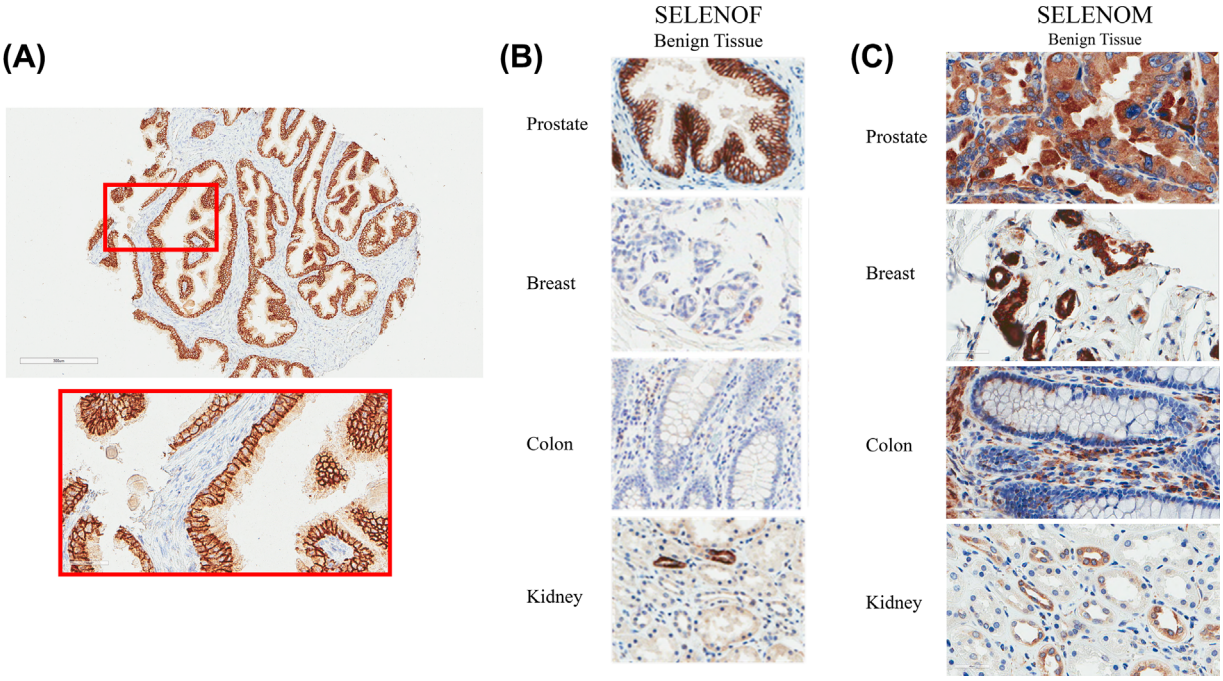

FIGURE 2 Localization of SELENOF and SELENOM in human tissues. (A) A representative image of benign prostatic tissue immunostained with anti-SELENOF antibodies. Nuclei were counterstained with hematoxylin. A greater magnification of this image is presented in Supplemental Figure S2. (B) SELENOF immunostaining of benign tissue derived from the prostate, breast, kidney, and colon. (C) SELENOM immunostaining of benign tissue derived from the prostate, breast, colon, and kidney. Nuclei were counterstained with hematoxylin

predicted to result in lower SELENOF levels among African Americans as compared to Caucasians.[7,25] The levels of SELENOF in the tumor tissue obtained from 33 African American men in the TMA were lower than in tissue cores obtained from Caucasians (n = 295), regardless as to whether signals obtained from the entire cell (0.086, SD = 0.038 normalized counts (NC) vs 0.101, SD = 0.041 NC, P < 0.02) or just the plasma membrane (0.142, SD = 0.061 NC vs 0.165, SD = 0.059 NC, P < 0.01) were evaluated.

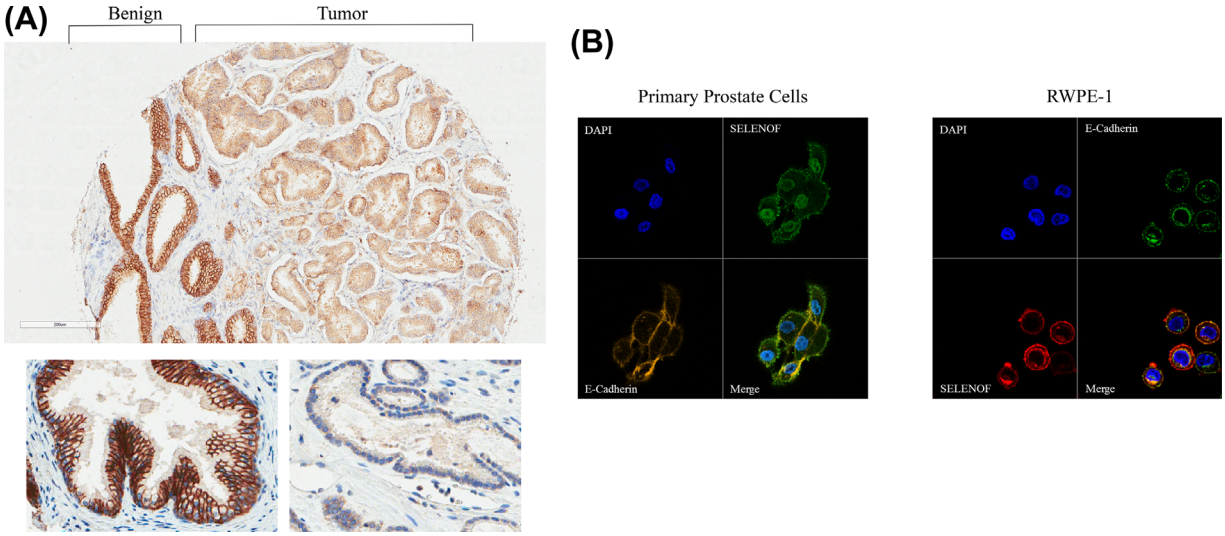

FIGURE 3 SELENOF levels are lower in prostate tumors compared to benign glands and non-tumorigenic cells in culture. (A) A representative image of a tissue core composed of both tumor and benign glands immunostained with anti-SELENOF antibodies. Nuclei were counterstained with hematoxylin. (B) Primary prostate cells and RWPE-1 cells were fixed, permeabilized, and co-immunostained with SELENOF and E-Cadherin antibodies. SELENOF was visualized with Alexa Fluor 488 (primary prostate cells) or with Alexa Fluor 647 (RWPE-1). E-Cadherin was visualized with Alexa Fluor 647 (primary prostate cells) or with Alexa Fluor 588 (RWPE-1). Nuclei were visualized with DAPI

3.5 | Serum selenium levels are lower and the frequency of a SELENOF polymorphism is higher in African American men with prostate cancer as compared to Caucasian men

The study presented above using TMAs was limited as neither genotype nor selenium status was available from the archived tissues used. The impact of those variables was addressed in the 126 subjects from the Adiposity Study. The mean age of study participants was 61.1 years (SD = 6.6 years, range 44-75 years), 28 (22%) were African Americans, 90 (71%) were Caucasian, and eight (6%) were classified as "other" race/ethnicity. The mean BMI was 29.2 kg/m^2 (SD = 4.3 kg/m^2, range 18.7-41.8 kg/m^2). The mean pre-operative serum PSA concentration was 7.5 ng/mL (SD = 6.3 ng/mL, range 1.2-50 ng/mL), with the majority of subjects having tumors with Gleason sums of 7 or higher (n = 73, 58%) or locally advanced stage disease (any pT3, n = 72, 57%) at the time of surgery. Mean selenium levels in serum samples were 134.2 ng/mL (SD = 24.8 ng/mL) with a median of 132 ng/mL and ranged from 80.4 to 230.7 ng/mL. As indicated in Supplemental Figure S3, mean serum selenium concentrations were significantly lower in African-American subjects compared to Caucasian subjects (mean = 116.6 ng/mL and 138.3 ng/mL in African Americans and Caucasians, respectively, $P < 0.0001$). Subjects classified as "other" race/ethnicity had mean levels of 148.1 ng/mL ($P = 0.0008$ for the difference with Caucasians).

DNA samples were genotyped to determine allelic identity at both the SELENOF locus as well as for polymorphisms in the gene for the SELENOP selenium carrier protein, previously shown to be associated with the risk for advanced prostate cancer.[27] The allele frequencies are presented in Table 1. A significant difference in the distribution in SELENOF allele frequencies for position 1125 was observed between African Americans and Caucasians, with the TT homozygous allele being 10 times more frequent in African Americans, an even greater difference than we previously reported in another cohort comprised of cancer-free individuals in the Chicago area.[7] It is noteworthy that this genotype is expected to result in lower levels of the SELENOF protein and our analysis of the TMAs described above indicated a much lower level of SELENOF protein in prostatic tissue obtained from African Americans as compared to Caucasians. Significant racial differences in allele frequencies for the SELENOP polymorphisms were not detected (Table 1).

3.6 | Gleason sum group positively associates with serum selenium levels and negatively associates with SELENOF genotype

For each ng/mL increase in serum selenium (median = 132 ng/mL, ranged from 80.4 to 230.7 ng/mL), the odds of a higher Gleason sum group increased by 2% (OR [95%CI] = 1.02 [1.001, 1.03], $P = 0.034$) after accounting for SELENOF genotype (Table 2). In contrast, the SELENOF^{CC} genotype was associated with a fivefold decrease in the odds of a higher Gleason sum group after accounting for serum selenium concentration ($P = 0.0018$).

3.7 | Treatment failure within 2 years after surgery positively associates with the serum selenium SELENOP rs3877899 AA genotype

We next searched for associations between serum selenium levels and polymorphisms in the selenium carrier protein, SELENOP, previously

TABLE 1 Distributions of the selenoprotein genotypes overall and by race/ethnicity

		By race/ethnicty[a]		
Genotype	**Overall, n = 126**	**AA, n = 28**	**CA, n = 90**	***P*-value**
SELENOF[b]				
TT	13 (11.1%)	10 (35.7%)	3 (3.4%)	
CT	51 (40.5%)	15 (53.6%)	35 (40.2%)	
CC	59 (48.4%)	3 (10.7%)	49 (56.3%)	<0.0001[c]
SELENOP rs3877579				
GG	71 (56.6%)	20 (71.4%)	48 (53.3%)	
GA	46 (36.4%)	8 (28.6%)	35 (38.9%)	
AA	9 (7.0%)	0 (0%)	7 (7.8%)	0.75[d]
SELENOP rs3877899				
GG	77 (61.2%)	18 (64.3%)	52 (57.8%)	
GA	41 (32.6%)	8 (28.6%)	33 (36.7%)	
AA	8 (6.2%)	2 (7.1%)	5 (5.6%)	0.72

AA, African American; CA, Caucasian.
[a]Data for eight subjects categorized as "other" race/ethnicity not shown.
[b]SELENOF genotype was characterized in 123 of the 126 subjects (n = 28 AA, 87 CA, and eight "other").
[c]Chi-squared test at $\alpha < 0.05$.
[d]Fisher's exact test at $\alpha < 0.05$.

linked to cancer risk. The rs3877899 polymorphism results in either an alanine or threonine at position 284 of the protein with the threonine-encoding allele associated with higher tissue selenium levels while the alanine-encoding allele was associated with lower breast cancer incidence and higher levels of anti-oxidant proteins.[28,29] A significant association of selenium and SELENOP rs3877899 was found for risk of treatment failure within the first two years after surgery (Table 2). The odds of treatment failure increased with increasing serum selenium levels (OR [95%CI] = 1.02 [1.003, 1.05], per ng/mL increase, $P = 0.037$). Furthermore, compared to subjects with SELENOP^{GG} genotype (encoding an alanine), those with SELENOP^{AA} genotype (encoding a threonine) experienced a nearly sixfold increase in their odds of treatment failure within the first 2 years after surgery OR [95%CI] = 5.75 [1.09, 30.5], $P = 0.021$).

4 | DISCUSSION

The studies described above were initiated to investigate the possible correlations of specific polymorphisms in selenoprotein-encoding genes with prostate cancer. This work was motivated by previous data indicating that SELENOF polymorphisms are associated with the risk of dying from prostate cancer[17] and that functional polymorphisms in the SELENOF 3′-UTR are represented at a much higher frequency among African Americans,[7] a group with a greater likelihood of succumbing to the disease. We observed a marked reduction in SELENOF expression and altered subcellular localization in prostate cancer tissue as compared with adjacent benign tissue, indicating that this protein may play a role in prostate cancer development. An intriguing finding was the previously unreported subcellular localization of SELENOF in benign prostate epithelium and its shift in localization in cancer. The biological significance of these observations is not clear and merits further investigation.

A dramatic difference in the SELENOF genotype frequency was found between African Americans and Caucasians, consistent with our previous data obtained from a different Chicago cohort. Based on data using a specialized reporter construct developed to specifically assess the impact of sequence variations in the SECIS element on the read-through of the selenocysteine-encoding UGA codon in SELENOF mRNA,[4,7] we anticipated that individuals who expressed the SELENOF^{TT} genotype would produce less SELENOF protein. While this could not be directly assessed in this study because neither of the two sources of patient material included both DNA and tissue, the data obtained is consistent with this prediction because African Americans had a statistically higher frequency of the SELENOF^{T} allele among patients participating in the Adiposity Study and lower SELENOF protein levels in the TMAs.

A significant increase in the odds of a patient presenting with a Gleason sum score of 7 or higher occurred among individuals who were heterozygous for the rs25197579 SELENOF polymorphism compared to those who were homozygous for the C allele of SELENOF. Because this allele is located in the 3′-UTR of the gene in a region critical for the recognition of the UGA in-frame codon as selenocysteine, we anticipated that carriers of the C-expressing allele would have lower levels of SELNOF under selenium-replete conditions.[7] The impact of reduced SELENOF in the etiology of prostate cancer is unknown, but the association of the rs25197579 allele with higher tumor grade and the dramatic loss of SELENOF levels observed in tumor tissue as compared to adjacent benign regions indicates that this protein might contribute to disease severity.

A somewhat unexpected result was that higher pre-prostatectomy serum selenium levels were significantly associated with higher tumor grade, an indication of an increased likelihood of recurrent disease following prostatectomy. These results are consistent with the possibility of an association between the frequency of polymorphisms in the selenium carrier protein SELENOP, which has been linked to higher selenium status,[30] and adverse outcome. While selenium intake and serum/plasma levels have been associated with reduced prostate cancer risk,[1] increased prostate cancer incidence was observed among men with high baseline selenium levels who were supplemented with selenomethionine in the SELECT randomized clinical trial.[31] It is therefore conceivable that higher dietary selenium intake through the course of a lifetime may be beneficial but higher selenium status later in life, as with men participating in the SELECT trial, may be associated with increased risk of prostate cancer recurrence after prostatectomy and perhaps more aggressive disease. This notion is consistent with the results presented herein which included older participants who had undergone prostatectomy.

Prostate cancer is disproportionally greater for African American men who have both the highest incidence and mortality as compared

TABLE 2 Associations of pathological tumor gleason grade group and treatment failure with selenium and selenoprotein genotypes

	Gleason Grade Group[a]	Treatment Failure[b]
	OR (95%CI)[c]	OR (95%CI)[d]
Selenium	1.02 (1.001, 1.03)	1.02 (1.003, 1.05)
SELENOF		
TT	Ref.	
CT	0.55 (0.17, 1.83)	–
CC	0.19 (0.06, 0.67)	
SELENOP rs3877899		
GG		Ref.
GA	–	0.69 (0.27, 1.78)
AA		5.75 (1.09, 30.5)

OR, odds ratio; CI, confidence interval.

[a]Gleason sum = 2-6 (group 1), 7 (3 + 4) (group 2), 7 (4 + 3) (group 3), 8 (group 4), and 9-10 (group 5) .

[b]Defined as a detectable (≥0.1 ng/mL) and rising serum PSA or receipt of additional therapy ($n = 32$).

[c]Ordinal logistic regression; final model after backwards selection procedure: Gleason grade group (1 thru 5) = β_1SE + β_2SELENOF + ε.

[d]Logistic regression model; final model after backwards selection procedure: Treatment failure (yes vs no) = β_1SE + β_2SELENOP rs3877899 + ε.

to other racial groups.[32] The reasons for this disparity are likely multifactorial, including reduced access to care, as well as other socioeconomic factors. As recently reviewed, there are also a host of biological differences in disease presentation and clinical outcome, which along with environmental modifiers, are likely to account for the differences observed between African American and Caucasian men.[33,34] Here, we identify several possible factors that may contribute to this disparity, including selenium status and SELENOF allele distribution. Determining what impact these factors have on prostate cancer risk and outcome will require future investigation as will the role of SELENOF in prostate cancer.

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CONFLICTS OF INTEREST

None.

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REFERENCES

1. Vinceti M, Dennert G, Crespi CM, et al. Selenium for preventing cancer. *Cochrane Database Syst Rev*. 2014;3:CD005195.
2. Gladyshev VN, Jeang K-T, Wootton JC, Hatfield DL. A new human selenium-containing protein. Purification, characterization and cDNA sequence. *J Biol Chem*. 1998;273:8910–8915.
3. Kumaraswamy E, Malykh A, Korotkov KV, et al. Structure-expression relationships of the 15-kDa selenoprotein gene. Possible role of the protein in cancer etiolgy. *J Biol Chem*. 2000;275:35540–35547.
4. Kumaraswamy E, Korotkov KV, Diamond AM, Gladyshev VN, Hatfield DL. Genetic and functional analysis of mammalian Sep15 selenoprotein. *Methods Enzymol*. 2002;347:187–197.
5. Berry MJ, Banu L, Chen Y, et al. Recognition of UGA as a selenocysteine codon in Type I deiodinase requires sequences in the 3' untranslated region. *Nature*. 1991;353:273–276.
6. Driscoll DM, Copeland PR. Mechanism and regulation of selenoprotein synthesis. *Annual Rev Nutr*. 2003;23:17–40.
7. Hu YJ, Korotkov KV, Mehta R, et al. Distribution and functional consequences of nucleotide polymorphisms in the 3'-untranslated region of the human Sep15 gene. *Cancer Res*. 2001;61:2307–2310.
8. Meplan C, Hughes DJ, Pardini B, et al. Genetic variants in selenoprotein genes increase risk of colorectal cancer. *Carcin*. 2010;31:1074–1079.
9. Sutherland A, Kim DH, Relton C, Ahn YO, Hesketh J. Polymorphisms in the selenoprotein S and 15-kDa selenoprotein genes are associated with altered susceptibility to colorectal cancer. *Genes Nutrit*. 2010;5:215–223.
10. Tsuji PA, Naranjo-Suarez S, Carlson BA, Tobe R, Yoo MH, Davis CD. Deficiency in the 15kDa selenoprotein inhibits human colon cancer cell growth. *Nutrients*. 2011;3:805–817.
11. Irons R, Tsuji PA, Carlson BA, et al. Deficiency in the 15-kDa selenoprotein inhibits tumorigenicity and metastasis of colon cancer cells. *Cancer Prev Res*. 2010;3:630–639.
12. Tsuji PA, Carlson BA, Naranjo-Suarez S, et al. Knockout of the 15kDa selenoprotein protects against chemically-induced aberrant crypt formation in mice. *PLoS ONE*. 2012;7:e50574.
13. Korotkov KV, Kumaraswamy E, Zhou Y, Hatfield DL, Gladyshev VN. Association between the 15-kDa selenoprotein and UDP-glucose:glycoprotein glucosyltransferase in the endoplasmic reticulum of mammalian cells. *J Biol Chem*. 2001;276:15330–15336.
14. Labunskyy VM, Hatfield DL, Gladyshev VN. The Sep15 protein family: roles in disulfide bond formation and quality control in the endoplasmic reticulum. *IUBMB Life*. 2007;59:1–5.
15. Labunskyy VM, Yoo MH, Hatfield DL, Gladyshev VN. Sep15, a thioredoxin-like selenoprotein, is involved in the unfolded protein response and differentially regulated by adaptive and acute ER stresses. *Biochem*. 2009;48:8458–8465.
16. Kasaikina MV, Fomenko DE, Labunskyy VM, et al. Roles of the 15-kDa selenoprotein (Sep15) in redox homeostasis and cataract development revealed by the analysis of Sep 15 knockout mice. *J Biol Chem*. 2011;286:33203–33212.
17. Penney KL, Schumacher FR, Li H, et al. A large prospective study of SEP15 genetic variation, interaction with plasma selenium levels, and prostate cancer risk and survival. *Cancer Prev Res (Phila Pa)*. 2010;3:604–610.
18. Qiu X, Yuan Y, Vaishnav A, Tessel MA, Nonn L, van Breemen RB. Effects of lycopene on protein expression in human primary prostatic epithelial cells. *Cancer Prev Res (Phila)*. 2013;6:419–427.
19. Melamed J, Datta MW, Becich MJ, et al. The cooperative prostate cancer tissue resource: a specimen and data resource for cancer researchers. *Clin Cancer Res*. 2004;10:4614–4621.
20. Kajdacsy-Balla A, Geynisman JM, Macias V, et al. Practical aspects of planning, building, and interpreting tissue microarrays: the Cooperative Prostate Cancer Tissue Resource experience. *J Molec Histol*. 2007;38:113–121.
21. Amin MB, Grignon D, Bostwick D, Reuter V, Troncoso P, Ayala AG. Recommendations for the reporting of resected prostate carcinomas. Association of Directors of Anatomic and Surgical Pathology. *Amer J Clin Path*. 1996;105:667–670.
22. Gleason DF. In: Tannenbaum M, ed. *Histologic Grading of and Staging of Prostatic Carcinoma*. Philadelphia: Lea & Febiger; 1977.
23. Epstein JI, Zelefsky MJ, Sjoberg DD, et al. A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *Euro Urol*. 2016;69:428–435.
24. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17:1471–1474.
25. Kumaraswamy E, Malykh A, Korotkov KV, et al. Structure-expression relationships of the 15-kDa selenoprotein gene. Possible role of the protein in cancer etiology. *J Biol Chem*. 2000;275:35540–35547.
26. Korotkov KV, Novoselov SV, Hatfield DL, Gladyshev VN. Mammalian selenoprotein in which selenocysteine (Sec) incorporation is supported by a new form of Sec insertion sequence element. *Mol Cell Biol*. 2002;22:1402–1411.

27. Geybels MS, van den Brandt PA, Schouten LJ, et al. Selenoprotein gene variants, toenail selenium levels, and risk for advanced prostate cancer. *J Nat Can Inst*. 2014;106:dju003.
28. Meplan C, Crosley LK, Nicol F, et al. Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). *FASEB J*. 2007;21:3063–3074.
29. Meplan C, Dragsted LO, Ravn-Haren G, Tjonneland A, Vogel U, Hesketh J. Association between polymorphisms in glutathione peroxidase and selenoprotein P genes, glutathione peroxidase activity, HRT use and breast cancer risk. *PLoS ONE*. 2013;8: e73316.
30. Ekoue DN, Zaichick S, Valyi-Nagy K, et al. Selenium levels in human breast carcinoma tissue are associated with a common polymorphism in the gene for SELENOP (Selenoprotein P). *J Trace Elem Med Biol*. 2017;39:227–233.
31. Klein EA, Thompson IM, Tangen CM Jr., et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA*. 2011;306:1549–1556.
32. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin*. 2013;63:11–30.
33. Martin DN, Starks AM, Ambs S. Biological determinants of health disparities in prostate cancer. *Current Opin Oncol*. 2013;25:235–241.
34. Farrell J, Petrovics G, McLeod DG, Srivastava S. Genetic and molecular differences in prostate carcinogenesis between African American and Caucasian American men. *Intl J Mol Sci*. 2013;14: 15510–15531.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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