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TITLE: The Contribution of SELENOF to the Disproportionate Mortality Experienced by African American Men Due to Prostate Cancer

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CONTRACTING ORGANIZATION: University of Illinois at Chicago

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14. ABSTRACT

Prostate cancer disproportionately affects African American men, and our laboratory has determined that an at-risk polymorphism in the gene for SELENOF is 10-times more frequent in the genomes of African Americans and this genetic variation is likely to result in lower SELENOF levels in tissues. Consistent with these observations is data indicating that the levels the SELENOF protein are lower in the prostate tumors of African American men as well. We have established that reduced levels of SELENOF are likely contributing to cancer progression. These data, along with observations on human tissues, provide solid evidence that the loss of SELENOF is mechanistically linked to prostate cancer aggressiveness and contribute to the disparity in disease outcome experienced by African Americans. Moreover, our recent data indicate the potential for a commercially available compound to enhance SELENOF levels, indicating that this approach can be developed into a new therapeutic strategy to impact prostate cancer mortality, especially among African American men.

15. SUBJECT TERMS

Prostate cancer, health disparity, tumor suppressor

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1. Introduction

Prostate cancer is the second leading cause of death among American men and the disease is disproportionally greater among African American men who experience the highest incidence and mortality from PCa world-wide as compared to other racial groups in the US. The identification of risk factors that predominate among African American men is a critical step in reducing the risk of dying of PCa in that population and may lead to identifying risk factors in the US male population at large. It is hypothesized that the SELENOF protein plays a role in the disparity in PCa incidence and outcome between African American and Caucasian men and our broad goal is to determine this role. Among the data supporting this hypothesis are results indicating 1) the dramatic reduction of SELENOF in prostate tumors compared to adjacent benign tissue, 2) an association between specific *SELENOF* alleles and the risk of getting prostate cancer or dying from the disease, 3) a 10-fold higher frequency of the at-risk allele in African Americans and 4) lower levels of SELENOF in prostate cancers from African Americans as compared to Caucasians. The proposed studies included genetically engineering human prostate derived cells to over- and under-express SELENOF to interrogate mechanistically the consequences of its activity. Human tissues will be examined, both as tissue microarrays and formalin fixed, to determine associations between race, SELENOF genotype and levels, selenium levels and clinical parameters of prostate cancer. An animal model for the impact of the loss of SELNOF on prostate carcinogenesis will be developed by breeding asymptomatic SELENOF knock-out mice with mice that that develop prostate cancer. Collectively, the investigation of the impact of the reduction of SELENOF levels on prostate cancer has generated new information about the disease and the disparity in incidence and mortality experienced by African American men.

2. Keywords

Prostate; cancer; selenoprotein; polymorphism; disparity; cell culture; tissues; mouse models; regulation; transcription; selenium

3. Accomplishments

- **What were the major goals of the project?**

Below are the aims presented in the awarded grant:

Aim 1. Determine the differences in levels of SELENOF between African American and Caucasian men and establish whether there is an association between SELENOF serum and tissue levels and clinical parameters including PSA levels, tumor sage and grade, and outcome.

Aim 2. Determine whether the absence of SELENOF in the prostate reduces the time to the appearance of prostate cancers, the incidence of these tumors, and their severity in mouse models genetically engineered to develop prostate cancer.

Aim 3. Determine the mechanism by which reduced SELENOF levels contribute to a higher prostate cancer risk and poorer clinical outcome. We will reduce the levels of SELENOF in immortalized and primary human prostate epithelial cells as well as increase SELENOF in human tumor cell lines cell lines. These derivative cells will be examined for features associated with the transformed phenotype.

- **What was accomplished under these goals?**

Aim 1, Major Task 1. Quantify SELENOF levels in ethnicity arrays

Subtask 1: Obtain ethnicity array from PCBN.
Subtask 2: Obtain ethnicity array from CPCTR.
Subtask 3: Optimize staining.

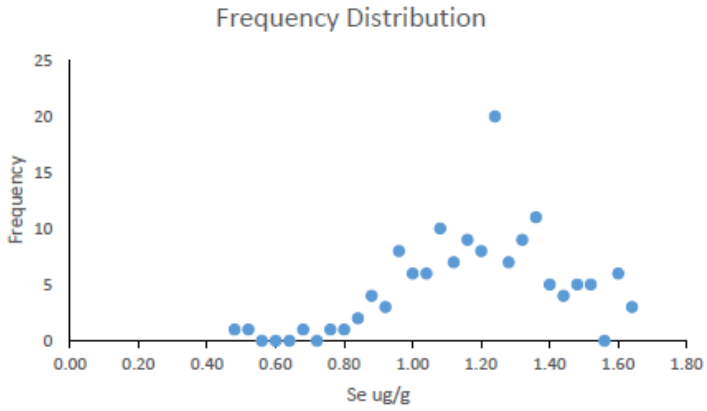
Please see Hong et al. IJMS 2021 for details.

Major Task 3: Determine the levels of selenium and DNA in obtained tissues.

Subtask 1: Obtain small sections of tissues.

We obtained all the needed 145 archived prostate tissues from African American and Caucasian men from which we will determine 1) levels of SELENOF, 2) SELENOF genotype and 3) selenium levels. Each tissue was recovered from the UIC tissue bank, demographic information collected, examined by a pathologist to determine benign and cancerous sections for collection and individual "pieces" of tissue collected for preparation of DNA for genotyping and another segment for selenium analysis.

Subtask 2: Frozen aliquots of all the samples were sent to our collaborator John Brockman at the University of Missouri Research Reactor Center for selenium analysis by Instrumental Neutron Activation. Descriptive data on the selenium levels obtained are presented below in Figure 1.



Mean	Median	Mode	Range
1.19	1.21	1.24	1.16

Figure 1. Selenium levels in obtained prostate cancer tissues.

Subtask 3: Isolate DNA and genotype for SELENOF and SELENOP.

DNA was isolated from most of the frozen prostate samples and genotyped to identify SELENOF allele identity and frequency. The data is presented below in Figure 2 and allele distribution in African Americans and Caucasians is presented in Figure 3 No difference in the *SELENOP* allele distribution between African Americans and Caucasians was found.

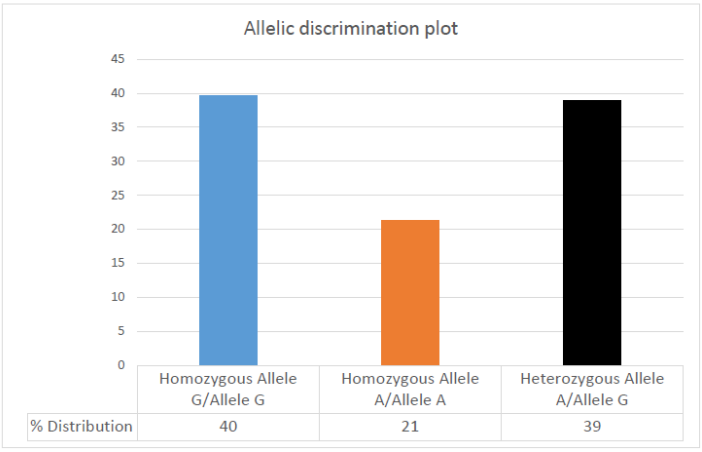


Figure 2. *SELENOF* allele distribution among the obtained clinical samples.

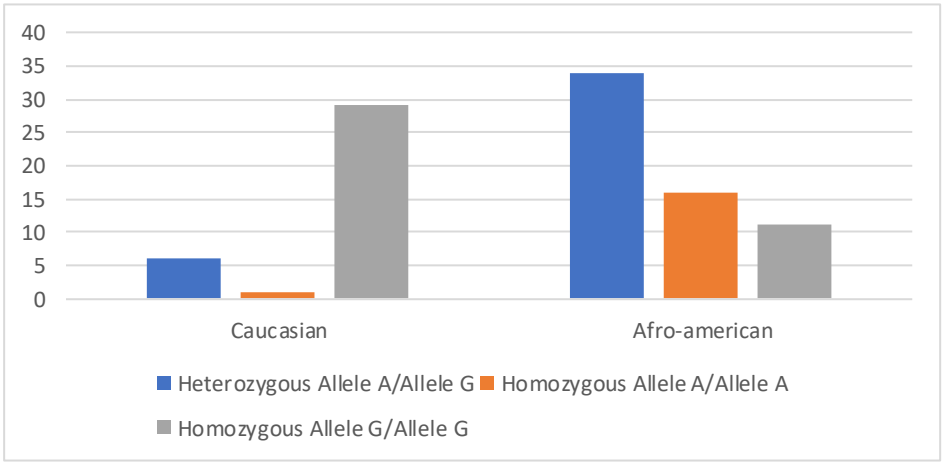


Figure 3. *SELENOF* allele distribution among African Americans and Caucasians

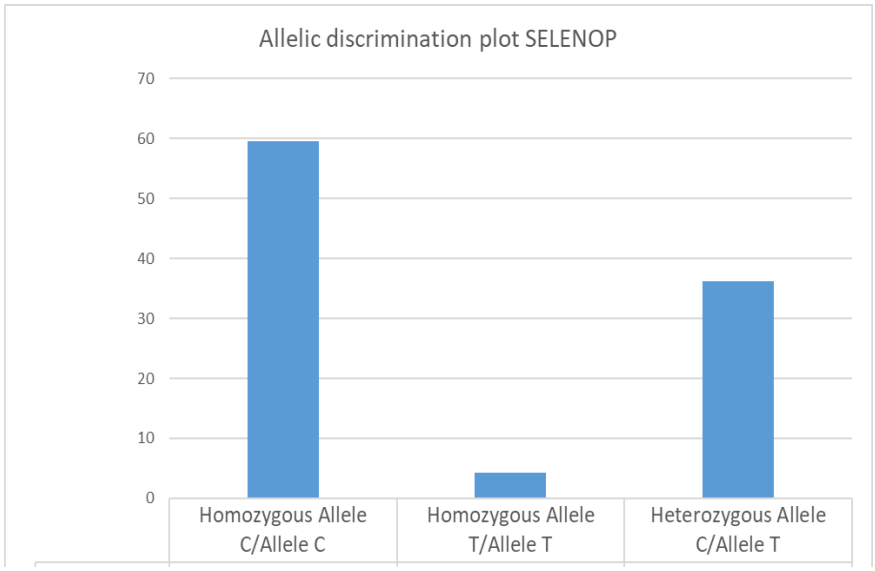


Figure 4. SELENOP allele distribution

Major Task 4. Statistical Analysis of the data.

Subtask 1: Collect and organize data from Major Tasks 1-3
Subtask 2: Meet statistician from UIC Analysis Core
Subtask 3: Data analysis.

The statistical evaluation of the data performed by Dr. Liu is presented in the context of the obtained results for all tasks and the manuscript submitted in the appendix. Additional statistical evaluation of the data obtained from the new TMA was delayed due to the time required by our statistician to recover from from a surgical procedure and is now planned for the beginning of August.

Aim 2: Determine whether the absence of SELENOF in the prostate reduces the time to the appearance of prostate cancers, the incidence of these tumors, and their severity in mouse models genetically engineered to develop prostate cancer.

Major Task 1. To determine whether the lack of SELENOF accelerates the development and severity of prostate cancer in a PTEN$^{+/-}$ background.
Due to time restrictions due to the pandemic, it was decided not to pursue this animal model and instead focus on the model described below in Major Task 2.

Major Task 2: To determine whether the lack of SELENOF accelerates the development and severity of prostate cancer in a Hi-myc background. These planned animal studies involved the mating of *selenof* knockout mice to mice that get prostate cancer due to the over-expression of the c-myc gene (Hi-Myc).

Subtask 1: Obtain Hi-myc mice. These mice were obtained and used for subsequent studies. Done
Subtask 2: Cross SELENOF$^{-/-}$ and Hi-myc mice. Following multiple breeding schedules, mice with the required genotype (*selenof*$^{-/-}$//*Hi-Myc* vs. *selenof*$^{+/+}$//*Hi-Myc*) have been generated. Done.
Subtask 3: Harvest tissue for analysis Done,..see Table.

Summary of Results of the Effect of SELENOF Knockout on Hi-Myc-Induced Prostate Lesions

	Hi-Myc	SELENOF KO	Double Homozygous		
N	17	6	5 (4)[1]	4 (3)[1]	9 (7)[1]
Age at termination (weeks)	32	32	32	36	32+36
All ACAR in lateral prostate	17 (100%)[2,3]	0	3(60)[3]	3(75)	6 (67%)[2]
Multiple ACAR	14 (82)[4]	0	2	1	3 (33)[4]
Small only[5]	0	0	2	0	2 (22)
≥ 1 medium size[5]	12 (71)	0	0	1	1 (11)
≥ 1 large size[5]	2 (12)	0	0	0	0
One ACAR[5]	3 (18)	0	1	2	3 (33)
Small[5]	2 (12)	0	0	2	2 (22)
Medium size[5]	1 (6)	0	1	0	1 (11)
mPIN in lateral prostate only[5]	0	0	2	0	2 (22)
AH in anterior prostate	9 (53)	0	3	4	7 (78)
Few	6 (35)	0	3	1	4 (44)
Many	3 (18)	0	0	3	3 (33)
ACAR in anterior prostate	02[2,6]	0	0	3[6]	3 (33)[2]
Dilated anterior prostate[5]	0	0	0	2	2 (22)
Dilated seminal vesicle[5]	0	2 (33)	0	0	0

Abbreviations: ACAR = adenocarcinoma; mPIN = prostatic intraepithelial neoplasia

[1] In parentheses is the number of animals for whom no additional sections are needed

[2] P = 0.032 for difference between Hi-Myc and Double Homozygous group (2-sided Fisher exact test)

[3] P = 0.043 for difference between Hi-Myc and Double Homozygous group (2-sided Fisher exact test)

[4] P = 0.028 for difference between Hi-Myc and Double Homozygous group (2-sided Fisher exact test)

[5] Statistics not done

[6] P = 0.003 for difference between Hi-Myc and Double Homozygous group (2-sided Fisher exact test)

SPECIFIC AIM 3: To reduce the levels of SELENOF in immortalized and primary human prostate epithelial cells as well as increase SELENOF in human tumor cell lines cell lines. These derivative cells will be examined for features associated with the transformed phenotype.

Aim 3: The goal of this aim was to alter the levels of SEL*E*NOF in tissue culture cells to determine the consequences on a host of transformation-related parameters. Such functional studies are critical in distinguishing a contributing role for (the loss of) SELENOF in cancer progression from a bystander effect. The goals set forth in the original SOW for this aim have essentially been satisfied and the results have been published (Hong et al. IJMS 2021 for details.) and presented at an international meeting in February 2021.

Subtask 1: Reduce the levels of SELENOF in RWPE-1 and primary prostate epithelial cells.
Subtask 2: Direct SELENOF to the RWPE-1 and primary prostate epithelial cells.
Subtask 3: Determine whether the manipulations described in Subtasks 1 and 2 enhance the transformed phenotype.

A targetable inhibitor of SELENOF translation. Identifying upstream negative regulators of SELENOF in prostate cancer might be exploited to restore SELENOF expression and mitigate the detrimental effects of SELENOF loss in prostate tumorigenesis. This approach is supported by preliminary data demonstrating that knocking down SELENOF in cells with high endogenous SELENOF levels is sufficient to enhance transformation-associated phenotypes. Our focus on SELENOF regulation at the level of protein synthesis was initiated because of the dedicated factors required for selenoprotein translation. Translational regulation of SELENOF in cancer cells was supported by our observation that SELENOF protein levels are 15-fold lower in PC-3 prostate cancer-derived cells compared to immortalized RWPE-1 cells, although SELENOF mRNA levels were marginally higher (< 2 fold) in PC3 cells.

A possible regulator of SELENOF protein synthesis is eukaryotic initiation factor 4a3 (eIF4a3), a DEAD-box family helicase involved in the hierarchy of translational control for a subset of selenoproteins. Under low selenium availability, eIF4a3 levels are higher and eIF4a3 binds to the SECIS element of the mRNA encoding another selenocysteine-containing protein, glutathione peroxidase 1 (GPX1), preventing the binding of necessary translation factors and inhibiting GPX1. There is a strong structural similarity between the SECIS elements of GPX1 and SELENOF mRNAs (Figure 5), suggesting that eIF4a3 may also bind the SECIS element of SELENOF mRNA, negatively regulating SELENOF translation. Furthermore, eIF4a3 is predicted to bind precisely at the location of the SELENOF 811 polymorphism (Fig. 5) that determines the read-through of UGA as selenocysteine and whose frequency is significantly higher (5-10 fold) among the genomes of cancers obtained from African American men compared to Caucasian men, a group with higher prostate cancer mortality than Caucasians (see below). We previously reported that this polymorphism is functional and contributes to determining the amount of SELENOF made as a direct function of selenium availability.

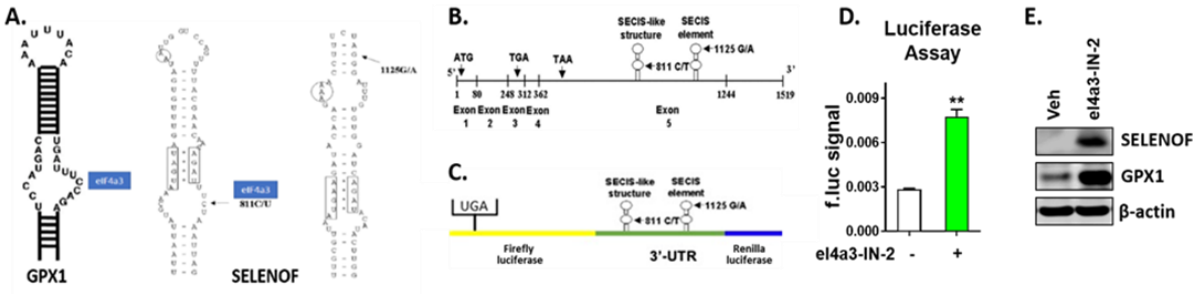


Figure 5. eIF4a3 controls translation of SELENOF. (A) The SECIS element in the 3'-UTR of the GPX1 selenoprotien with the eIF4a3 binding site indicated (left) and the SECIS element of SELENOF indicating the presumptive binding site to eIF4a3 mRNA (right). (B) Structure of the SELENOF cDNA and the sites of SNPs prevalent among African Americans. (C) Structure of the luciferase reporter construct engineered to include an in-frame UGA codon in the firefly luciferase RNA and the 3'-UTR of SELENOF mRNA. (D-E) Treatment of PC3 cells with eIF4a3 inhibitor (eIF4a3-IN-2, 10 uM for 72 h) increased the readthrough of the UGA codon as indicated by the enhancement of the normalized firefly luciferase signal and protein levels of SELENOF in PC3 cells. GPX1 levels were assessed as a positive control. **p<0.01.

The Cancer Genome Atlas – The Prostate Adenocarcinoma (PRAD) database through the UALCAN web portal was used to investigate if there is a difference in expression of eIF4a3 between prostate cancer and benign prostate tissue. eIF4a3 expression was significantly higher in prostate cancers with Gleason scores 8 through 10 compared to benign samples (Figure 6). Cancers with a Gleason score of 10 had the highest amount of eIF4a3, but this observation did not reach statistical significance due to the low number of samples available.

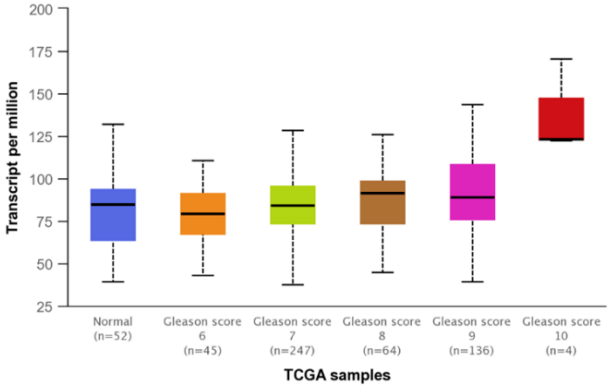


Figure 6. eIF4a3 mRNA levels in PRAD based on patient Gleason scores. TCGA-PRAD data analysis indicates significantly higher eIF4a3 expression in Gleason scores 8-10 compared to benign tissue. The figure was generated using the UALCAN web-portal tool, Minimal and maximal transcripts per million are shown in the graph.

A highly selective pharmacological inhibitor of eIF4a3, eIF4a3-IN-2, allosterically binds eIF4a3 and non-competitively inhibits its ATPase activity. PC3 cells with low SELENOF levels were exposed to 10 uM eIF4a3-IN-2 for 72 hours and western blotting of protein extracts indicated that treatment with the inhibitor resulted in significantly increased SELENOF and GPX1 (positive control) levels. The eIF4a3 inhibitor was also tested using a modified luciferase reporter construct designed to quantify UGA recoding ability directed by the SELENOF SECIS. A pmirGLO Dual-Luciferase miRNA target expression vector was altered to include an in-frame UGA codon in the firefly luciferase gene, introduced by changing a cysteine to a selenocysteine by site-directed mutagenesis. The entire SELENOF 3'-UTR after the stop codon, including the SECIS, was cloned downstream of the firefly luciferase gene. Modification of the UGC (Cys) to a UGA introduces a termination signal in the firefly luciferase gene in the absence of the 3'-UTR. If a functional SECIS element is present, the UGA codon will be recognized as selenocysteine, the firefly luciferase gene can be translated, and luciferase activity can be detected. Transfectants of the construct were selected, individual clones were isolated, expanded and protein extracts were obtained from stable transfectants. Firefly luciferase activity reflects the stem-loop structure's ability to recode the UGA codon as selenocysteine and was detected using a Promega GloMax 20/20 Luminometer. Retaining the original cysteine codon in the luciferase results in maximal luciferase activity that is completely eliminated when the cysteine codon is converted to a UGA codon unless the construct also contains the SELENOF 3'-UTR, which increases luciferase activity by 70-fold. After treatment of PC3 cells expressing the reporter construct with eIF4a3-IN-2 for 48 hours, the relative luciferase units were 3-fold higher compared to cells transfected with the vehicle, DMSO (Figure 6). These data indicate that eIF4a3 regulates SELENOF translation, presumably by binding to the SECIS element of SELENOF mRNA and eIF4a3 over-expression in prostate cancer could contribute to the observed lower levels of SELENOF.

Tissue samples from patients who had undergone radical prostatectomies were obtained from the UIC Biorepository and were stained by IHC with a validated eIF4a3 antibody. The preliminary results from a small number of benign and tumor prostate cores (n = 8) indicated that the levels of eIF4a3 in tumor tissue were greater than benign tissue (Figure 7). Although eIF4a3 is primarily known to be a nuclear protein functioning in RNA splicing, eIF4a3 appeared nuclear in benign prostate tissues with low levels in the cytoplasm of luminal cells. In the prostate cancer cores, the levels of eIF4a3 were dramatically elevated compared to benign tissue and high levels were found in the cytoplasm. The localization of eIF4a3 in the cytoplasm is significant due to selenoprotein translation occurring in the cytoplasm, indicating that increased cytoplasmic localization of eIF4a3 could contribute to the downregulation of SELENOF translation in prostate cancer.

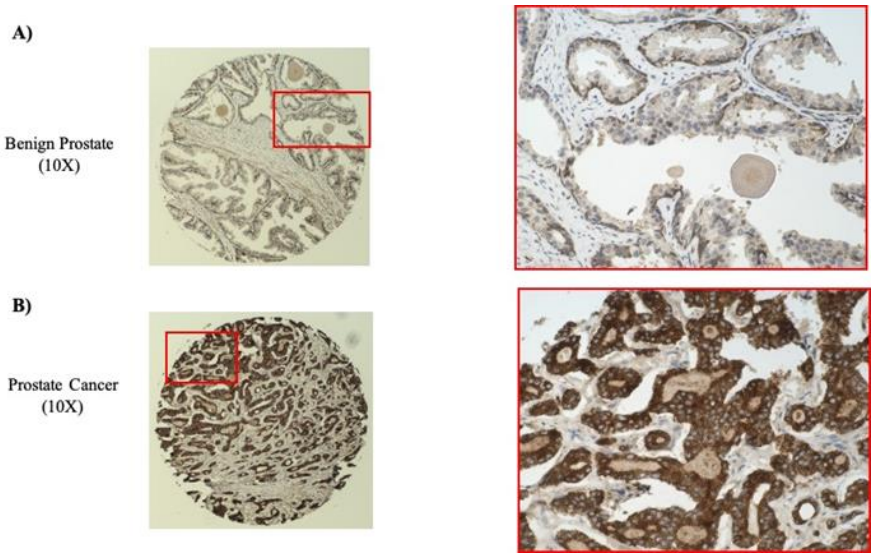


Figure 7. eIF4a3 levels are increased in prostate cancer compared to benign prostatic tissue. Representative image of A) benign and B) tumor prostatic tissue stained for eIF4a3 by immunohistochemistry (magnification is 20X). In benign tissue, some nuclear staining was seen in luminal cells. In prostate cancer, there was an increase in cytoplasmic eIF4a3 staining.

- **What opportunities for training and professional development has the project provided?**

  Dr. Elhodaky was an M.D. and Ph.D. student supported by the DOD award. He successfully defended his thesis and is currently a resident in Pathology at the Northwestern School of Medicine.
  In addition to Dr. Elhodaky, an undergraduate student (Shrinidhi Kadkol) has participated in this project, becoming adept at molecular cloning and analysis, as well as receiving two monetary awards for his efforts on the project, an Honors Council Award and Liberal Arts and Sciences Undergraduate Research Initiative (LASURI). He applied and was accepted to the M.D./Ph.D. Program at the University of Illinois at Chicago. In addition, funds from this DOD award were used to support Soumen Bera, a visiting scholar.

- **How were the results disseminated to communities of interest?**

  Our progress was presented virtually twice to the UIC Cancer Center. All other speaking opportunities were suspended due to the pandemic. Much of these results were recently published:

  Hong, L.K., Kadkol, S., Sverdlov, M., Kastrati, I., Ehhodaky, M., Deaton, R., Sfanos, K.S., Wang, H., Liu, L., **Diamond, A.M.** Loss of SELENOF induces the transformed phenotype in human immortalized prostate epithelial cells. Int. J. Mol. Sci., doi: 10.3390/ijms222112040, PMID: 34769469 222:12040, 2021.

  Zigrossi, A., Hong, L.K., Ekyalongo, R.C.,Cruz-Alverez, C., Gomick, E., **Diamond, A.M.** and Kastrati, I. SELENOF is a new tumor suppressor in breast cancer. Oncogene 41, 1263-1268, doi: 10.1038/s41388-021-02158-wPMID: 35082382, 2022

  Our DOD-supported work was presented at the 12 International Symposium on Selenium in Health and Disease in Honolulu, Hawaii in February 2022.

- **What do you plan to do during the next reporting period to accomplish the goals?**

  N/A

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

  Prostate cancer is a very common source of morbidity and mortality among men in the United States and across the world and there is disparity in the disease impact on African Americans. The data obtained during the first year of DOD funding for the first time established that the loss of SELENOF is a likely contributor to prostate cancer progression and not a mere bystander where its loss is a consequence, not a cause of the disease. This was established using tissue culture cells where SELENOF levels were reduced using a shRNA and consequentially the cells gained the ability to grow in soft agar and migrate in culture, two parameters associated with aggressive cancer. Collectively, these accomplishments reveal a new and significant aspect of prostate cancer and establish SELENOF as a likely prostate cancer tumor suppressor. The results with the health disparity TMA already have provided data using human clinical samples indicating that SELENOF genotype/levels is a likely contributor to the disparity in prostate cancer experienced by African American men.

- **What was the impact on other disciplines?**

  Based on the data obtained from our investigation into the mechanism by which SELENOF levels are reduced in prostate cancer indicating that post-transcriptional mechanisms are likely involved, we have identified a likely cancer-related aberration in the translational control of SELENOF that may involve the

translation factor EIF4a3. Among its other functions, EIF4a3 is induced in times of low selenium availability and suppresses the translation of several selenium containing proteins known to be sensitive to selenium status by binding to the SECIS element of the RNAs encoding those selenoproteins. There is a structurally similar binding site in the SECIS element of SELENOF, which include the polymorphism associated with prostate cancer mortality and differently represented among Africa Americans.

- **What was the impact on technology transfer?**
  Nothing to report

- **What was the impact on society beyond science and technology?**

  Health disparity is a significant issue facing society with many factors contributing to the increased risk of aggressive prostate cancer and dying from the disease affecting African American men. Current evidence supports the conclusion that there are genetic factors that account, at least in part, to these circumstances. Our efforts supported by the DOD are contributing to the discovery of one such genetic factor: a functional polymorphism in the SELENOF gene that is approximately 10 times more prevalent in African Americans and is associated with the risk of dying from prostate cancer. Understanding the mechanism by which this naturally occurring genetic variation increases the risk of suffering from prostate cancer is hoped to help to identify those at greatest risk so that increased surveillance and better care can be provided, as well as potentially identify new targets for therapy, to help reduce the burden of prostate cancer on the African American population. Initial studies have indicated that inhibiting eIF4a3 with a commercially available compound can restore SELEOF levels. This data indicates that restoring SELENOF levels is feasible and is a strategy that can be further pursued towards developing a novel therapeutic to treat advanced prostate cancer, particularly among African American patients.

5. Challenges/Problems

- **Changes in approach and reasons for change.**

  Changes in the original goals were addressed in the last technical report and the only other change is continuing to address the potential of inhibiting eIF4a3 as a potential new therapy.

- **Actual or anticipated problems or delays and actions or plans to resolve them.**

  The major source of delays in the completion of this project was the shutting of the University with me and the other team members being required to isolate at home during the bulk of the pandemic. The work has been reinitiated with safety precautions implemented and additional progress made during the no-cost extension.

- **Changes that had a significant impact on expenditures.**

  Expenditures were significantly reduced last year to the pandemic. A “no cost extension” was requested and approved to provide the funds required to complete the project.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards and/or select agents.**

  Nothing to report

- **Significant changes in use or care of human subjects.**

  Nothing to report

- **Significant changes in use or care of vertebrate animals.**

  Nothing to report

6. Products

- **Publications, conference papers and presentations.**
  - **Journal Publications.**
    - Hong, L.K., Kadkol, S., Sverdlov, M., Kastrati, I., Ehhodaky, M., Deaton, R., Sfanos, K.S., Wang, H., Liu, L., **Diamond, A.M.** Loss of SELENOF induces the transformed phenotype in human immortalized prostate epithelial cells. Int. J. Mol. Sci., doi: 10.3390/ijms222112040, PMID: 34769469 222:12040, 2021.
    - Zigrossi, A., Hong, L.K., Ekyalongo, R.C.,Cruz-Alverez, C., Gomick, E., **Diamond, A.M.** and Kastrati, I.SELENOF is a new tumor suppressor in breast cancer. Oncogene In Press, 2021.
  - **Books or other non-periodical, one time publications.**
    Nothing to report
  - **Other publications, conference papers, and presentations.**

  The data obtained with funds from this grant has been presented twice to the UIC Cancer Center this year.

  Dr. Diamond was a conference organizer and presented his data on the role of SELENOF loss on the health disparity experienced by African American men at the 12th International Symposium on Selenium and Medicine that was held in February 2022 in Hawaii.

  - **Website(s) or other internet sites(s)**
    Nothing to Report

  - **Technologies or techniques**
    Nothing to report

  - **Inventions, patent applications, and/or other licenses**
    Nothing to report

  - **Other products**
    Nothing to report

7. Participants & Other Collaborating Organizations

- **What individuals have worked on the project?**

  Dr. Maarten Bosland, qualified collaborator: No change
  Dr. Mostafa Elhodaky, Ph.D. Candidate: Has graduated with his degree and currently a Pathology Resident.
  Michael Schlicht, technician: No longer working on the project
  Yves Helou: Research technician. No change
  Soumen Bera. Visiting sscholar.

- **Has there been a change in the active or other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Dr. Diamond was awarded the following small grant ($8,000):
  Activation of androgen receptor by tyrosine kinases in prostate cancer
  University of Illinois Cancer Center
  03/01/2022 – 12/31/2022 Diamond (P.I.)

- **What other organizations were involved as partners?**

  Nothing to report

8. Special Reporting Requirements

- **Collaborative Awards:**
- **Quad Charts:**

9. Appendices

Hong, L.K., Kadkol, S., Sverdlov, M., Kastrati, I., Ehhodaky, M., Deaton, R., Sfanos, K.S., Wang, H., Liu, L., Diamond, A.M**.** Loss of SELENOF induces the transformed phenotype in human immortalized prostate epithelial cells. Int. J. Mol. Sci., doi: 10.3390/ijms222112040, PMID: 34769469 222:12040, 2021.

Zigrossi, A., Hong, L.K., Ekyalongo, R.C.,Cruz-Alverez, C., Gomick, E., Diamond, A.M. and Kastrati, I. SELENOF is a new tumor suppressor in breast cancer. Oncogene 41, 1263-1268, doi: 10.1038/s41388-021-02158-wPMID: 35082382, 2022