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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.

We obtained the "120 Case High Grade Race Disparity TMA" from the Prostate Cancer Biorepository Network (PCBN). These TMA is specifically designed for comparing biomarkers in African American and Caucasian patients. Being enriched for higher grade cases, the TMA includes 60 cases of tumor and normal tissue from each group matched on age, grade and key clinical and demographic data across 3 blocks.

Subtask 2: Obtain ethnicity array from CPCTR.

This TMA was no longer available and a Consecutive TMA from CPTCR was instead obtained. This TMA consisted of 561 cores arranged in the order the samples were collected from prostate cancers obtained following prostatectomy.

Subtask 3: Optimize staining.

Staining optimization was performed using "practice slides" obtained from the Pathology Department for both SELENOF and E-cadherin, the latter being a marker for staining of the outer membrane. Following optimization, the slides were stained with the designated antibodies, yielding high quality images. An example for the multiplex staining of SELENOF and eIF4a3, a potentially targetable inhibitor of SELENOF translation over-expressed in prostate cancer, are presented in Figure 1.

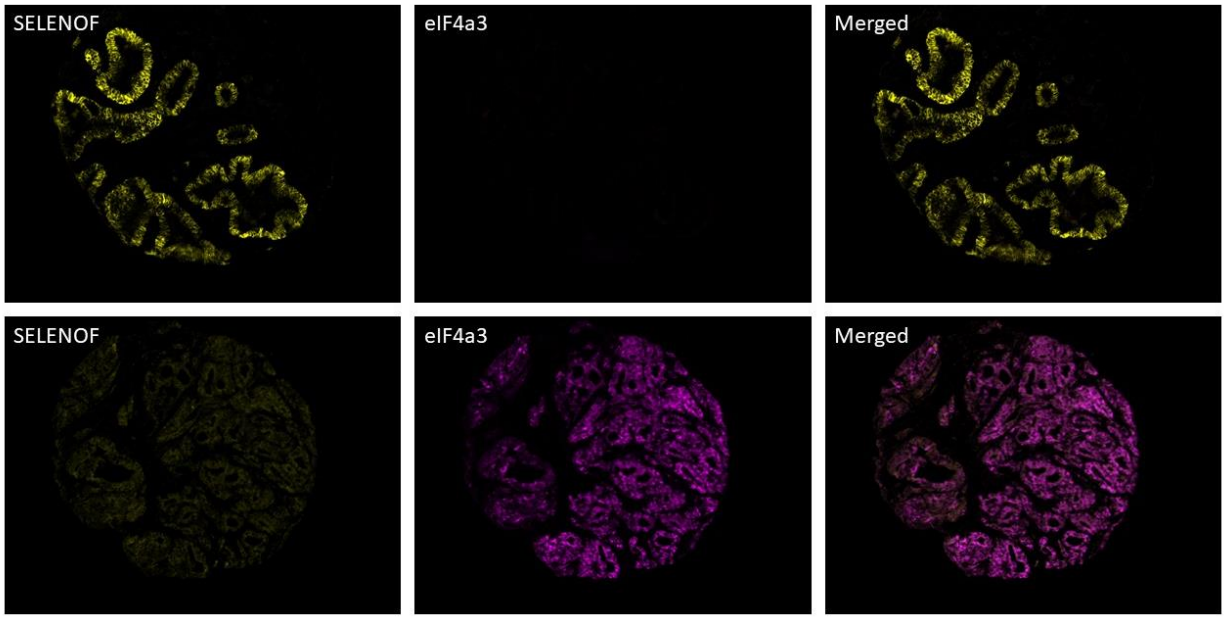


Figure 1. Example of benign glands expressing high levels of SELENOF and undetectable levels of eIF4a3 (Top panels) and tumor tissue displaying low levels of SELENOF and high levels of eIF4a3 (Bottom panels).

Subtask 4: Quantify SELENOF levels

The comparisons of SELENOF intensities between benign and tumor-appearing regions, and between races are listed in Supplemental Table 1. We analyzed averaged SELENOF levels, and the relative amount or percentages of membrane associated SELENOF, calculated as the amount of SELENOF that co-localized with E-cadherin, compared to that amount determined for the entire cell. Paired t-tests revealed that SELENOF levels and distribution were dramatically lower in cancer cells when compared to benign tissues, consistent with our previous study. Differences in SELENOF levels that co-localized with E-cadherin, presumably at the outer cellular membrane, were analyzed by paired t-test for significance. SELENOF levels that did not co-localize with E-cadherin were considered cytoplasmic. Regardless as to whether signals obtained were from the membrane or cytoplasmic regions, epithelial cells from prostate cancer cores had significantly lower levels and percentages of SELENOF in the membrane than benign prostate cores (Table 1, top).

To investigate if there was a difference in SELENOF levels between African Americans and Caucasians, race comparisons on log transformed SELENOF levels, the relative cellular distribution were performed for each tissue and for the benign versus cancer differences (Table 1, bottom). There were no significant differences in SELENOF levels or membrane percentages in benign prostate tissues between Caucasians and African Americans, regardless of whether the entire cell, cytoplasmic, or membrane regions were considered. Contrary to the previous study indicating that prostate tissue from African

Americans had lower levels of SELENOF compared to that of Caucasians, tissue from African Americans had significantly higher levels of SELENOF in prostate cancer cores compared to Caucasians. Possible reasons for the differences in these results include the different designs of the TMAs, the much smaller sample size for the TMA presented here and much fewer low-grade cancers in this analysis compared to the previous one. The higher levels of SELENOF in African American cancer cells were statistically significant whether the entire cell (p=0.036), cytoplasmic (p=0.036), or membrane (p=0.024) regions were compared. Similar race differences were also found in the cytoplasmic (p=0.047), membrane (p=0.039) regions, and the entire cell (p=0.036). The difference in SELENOF levels and distribution between benign and cancer cells were slightly higher in African Americans than Caucasian samples, although these differences were not statistically significant.

To assess if membrane located SELENOF levels were associated with prostate cancer stage, and if race modified the association, multivariate ordinal logistic regression models for Gleason score were employed. In these models, the effects of SELENOF that co-localized with E-cadherin in terms of percentage, race, and their interactions were tested. Gleason grading is a scoring system used by pathologists based on histology. Each prostate core is assigned a score ranging from 1 to 5, with 1 representing benign, well-differentiated glands, 3 representing moderately differentiated glands, and 5 representing poorly differentiated glands. Two scores are reported with the primary score being the most prevalent Gleason grade in the biopsied core and a secondary score representing the second most prevalent Gleason grade. The primary and secondary scores are added together to calculate the Gleason score. Out of 60 African American and 59 Caucasian patients, 20 (16.8%) patients had Gleason grade of 3+3, 26 (21.9%) had 3+4 or 3+5, 19 (16.0%) had 4+3, and 54 (45.4%) had 4+4, 4+5, or 5+4. In the multivariate ordinal logistic regression models, the Gleason grades 4 or higher were treated as an ordered categorical outcome SELENOF distribution (percentages) and interactions with race were examined separately: the SELENOF percent distribution in cancer and benign cells Table 2), and benign minus cancer percentage differences (Table 3).

The model selection process of SELENOF distributions in benign and cancer cells, and interactions between race and cancer cell membrane distribution indicated significant associations with Gleason grades. Higher cytoplasmic levels decreased the risk of higher Gleason grades (OR = 0.92, p-value = 0.0003). Among Caucasian patients, higher membrane levels strongly reduced the risk of higher Gleason grades (Estimate = -0.34, p-value = 0.0006; OR of Caucasian membrane association is 0.71). Such protective effects of membrane localization were significantly reduced in African American patients (estimate of interaction = 0.26, p-value = 0.0132). The effect of SELENOF in the membrane of cancer cells in African American patients had an OR closer to 1 compared to that in Caucasians (OR=0.92, p-value=0.015).

The model selection process for the ordinal logistic regression model using benign-cancer SELENOF percent differences, race, and their interactions as predictors for Gleason grades revealed race-differential effects of the benign-cancer difference in SELENOF percentages in the entire cell and in the cytoplasm (Supplemental Table 2). Benign-cancer cell percent differences did not influence Gleason grade in Caucasians (OR=0.98, p-value = 0.80). However, an increase in benign-cancer cell SELENOF percent difference in the entire cell significantly increased the risk of having higher Gleason grade for African Americans (OR = 1.57, 95% CI = 1.2, 2.04). These results indicated that African Americans were almost 1.6 times more likely to have a higher Gleason score with each percent increase in the difference in SELENOF levels between benign and prostate cancer. Similarly, benign-cancer cytoplasmic percent difference did not influence Gleason grades among Caucasians (OR=0.999, p-value = 0.99). However, an increase in the benign-cancer cell SELENOF cytoplasmic percentage difference significantly reduced the risk of having a higher Gleason grade for African Americans (OR = 0.59, 95% CI = 0.43, 0.80). In other words, among African Americans, each percent increase in the benign-cancer cytoplasmic difference resulted in and odds of 1/0.59 = 1.70 times in having a lower Gleason grade.

Table 1. Mean (SD) of SELENOF Intensities by Tissue Type and Race.

	Cancer Tissue			Benign Tissue			Benign - Cancer Difference		
	Mean (SD)			Mean (SD)			Mean (SD)		p-value
Overall Sample N=119									
Nuc Mean	0.12 (0.22)			0.84 (0.99)			0.72 (0.95)		<0.0001
Cyt Mean	0.1 (0.19)			0.76 (1.03)			0.67 (0.99)		<0.0001
Mem Mean	0.18 (0.3)			1.18 (1.39)			1.01 (1.33)		<0.0001
Cell Mean	0.13 (0.23)			0.87 (1.06)			0.76 (1.02)		<0.0001
Prct Nuc Pos	1.84 (4.61)			15.89 (18.97)			14.17 (18.26)		<0.0001
Prct Cyt Pos	1.41 (3.67)			12.98 (16.67)			11.67 (16.2)		<0.0001
Prct Mem Pos	2.67 (6.81)			22 (22.59)			19.49 (22.05)		<0.0001
Prct Cells Pos	1.81 (4.71)			16.19 (19.68)			14.5 (19.08)		<0.0001
By Race African American N=60; Caucasian N=59;									
	African American	Caucasian	p-value	African American	Caucasian	p-value	African American	Caucasian	p-value
Nuc Mean	0.16 (0.26)	0.08 (0.15)	0.045	0.96 (1.05)	0.73 (0.93)	0.18	0.8 (0.99)	0.65 (0.91)	0.40
Cyt Mean	0.14 (0.24)	0.06 (0.12)	0.036	0.86 (1.09)	0.67 (0.97)	0.24	0.72 (1.03)	0.61 (0.96)	0.52
Mem Mean	0.24 (0.38)	0.11 (0.18)	0.024	1.35 (1.49)	1.02 (1.27)	0.17	1.12 (1.42)	0.91 (1.24)	0.40
Cell Mean	0.17 (0.28)	0.08 (0.15)	0.036	1 (1.12)	0.76 (0.99)	0.19	0.83 (1.06)	0.68 (0.97)	0.43
Prct Nuc Pos	2.59 (5.55)	1.08 (3.29)	0.038	18.26 (20.0)	13.72 (17.75)	0.22	15.67 (19.48)	12.62 (16.94)	0.37
Prct Cyt Pos	2.07 (4.67)	0.73 (2.07)	0.047	14.97 (17.7)	11.14 (15.51)	0.22	12.9 (17.06)	10.39 (15.3)	0.40
Prct Mem Pos	3.87 (8.64)	1.45 (3.93)	0.039	25.2 (23.86)	19.06 (20.95)	0.23	21.34 (23.72)	17.58 (20.21)	0.36
Prct Cells Pos	2.62 (5.83)	0.99 (3.02)	0.036	18.62 (20.9)	13.95 (18.26)	0.23	16 (20.41)	12.94 (17.64)	0.39

Note: All p-values were from Log-transformed data due to skewness in the observed distribution.

Table 2. SELENOF Percent in Benign and Cancer Cells as Covariates

	Estimate (SE)	p-value	Odds Ratio (95% CI)
Benign Cyt Prct	-0.26 (0.07)	0.0003	0.92 (0.86, 0.98)
Race: Cauc as reference African Americans	-0.12 (0.39)	0.75	OR for Cancer Mem Prct in Caucasians: 0.71 (0.59, 0.87) OR for Cancer Mem Prct in African Americans: 0.92 (0.86, 0.98)
Cancer Mem Prct	-0.34 (0.10)	0.0006	OR for Cancer Mem Prct in Caucasians: 0.71 (0.59, 0.87) OR for Cancer Mem Prct in African Americans 0.92 (0.86, 0.98)
Cancer Mem Prct * African Americans	0.26 (0.10)	0.0132	

Table 3. Benign-Cancer SELENOF Percent Differences as Covariates.

	Estimate (SE)	p-value	Odds Ratio (95% CI)
Race: Cauc as reference African Americans	-0.48 (0.44)	0.28	1.61 (0.26, 1.47)
Benign – Cancer diff in Cell Prct	-0.02 (0.08)	0.80	OR for Diff in Cell Prct in Caucasians: 0.98 (0.85, 1.14)
Benign – Cancer diff in Cell Prct * African Americans	0.47 (0.15)	0.0024	OR for Diff in Cell Prct in African Americans 1.57 (1.20, 2.04)
Benign – Cancer diff in Cyt Prct	-0.001 (0.09)	0.99	OR for Diff in Cyt Prct in Caucasians: 0.999 (0.84, 1.19)
Benign – Cancer diff in Cyt Prct * African Americans	-0.52 (0.18)	0.0033	OR for Diff in Cyt Prct in African Americans 0.59 (0.43, 0.80)

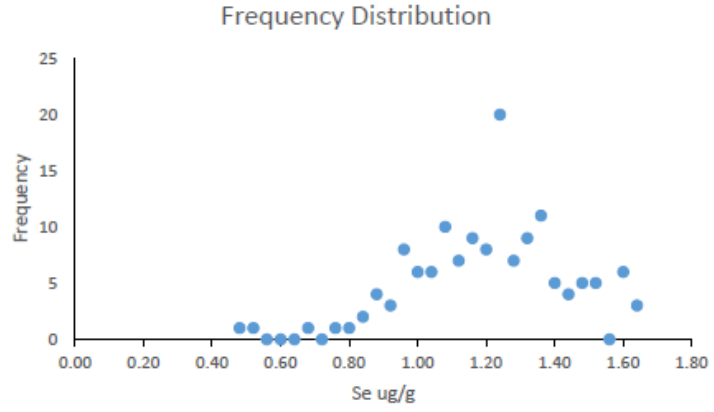
In addition, the Consecutive Prostate Cancer TMA obtained from the CPTCR has been multiplex stained for SELENOF and the signals on a tissue and individual cell basis have been obtained for nuclear, cytoplasmic and outer membrane localization and these data are currently being assessed by the statistician.

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 2.



Mean	Median	Mode	Range
1.19	1.21	1.24	1.16

Figure 2. Selenium levels in obtained prostate cancer tissues.

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

DNA was isolated from mostof the frozen prostate samples and genotyped to identify SELENOF allele identity and frequency. The data is presented below in Figure 3. SELENOP genotyping is scheduled to occur during the no-cost extension.

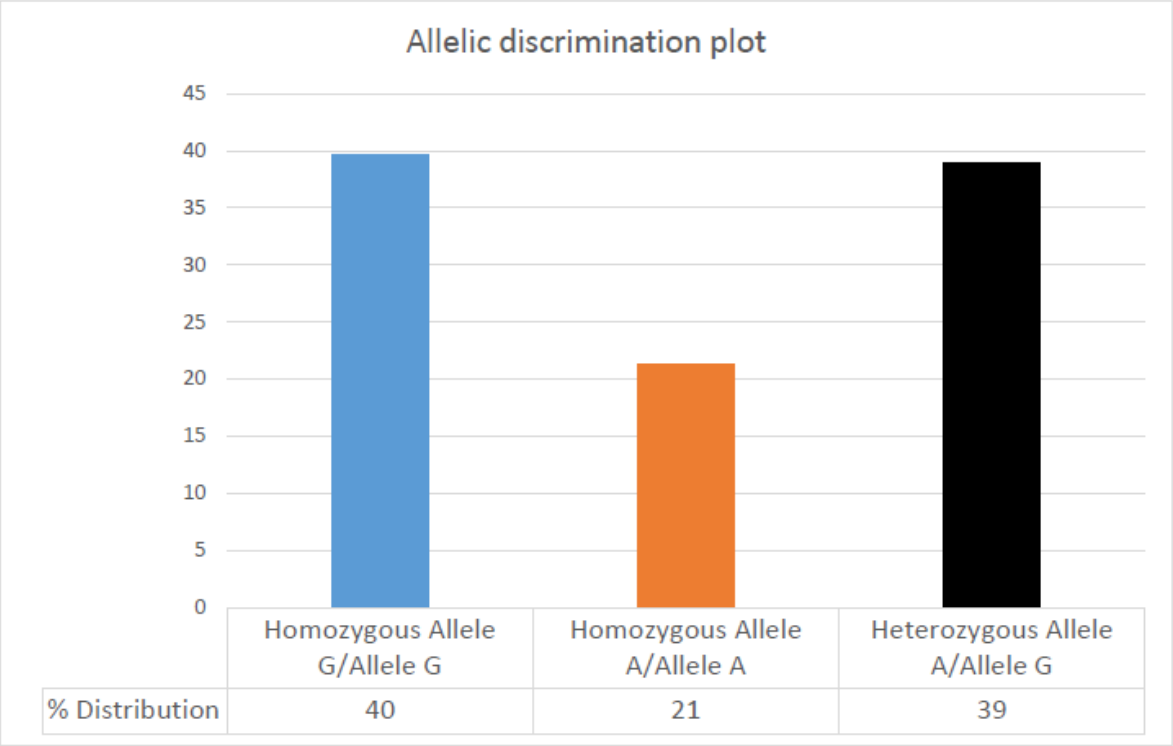


Figure 3. *SELENOF* Allele distribution among the obtained clinical samples.

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.

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.

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.

Subtask 3: Harvest tissue for analysis

We have examined prostates obtained from the mice that were null for *selenof* and over-express Myc and have decided to harvest the mice at 26 weeks to characterize the prostates, unless there are signs of stress or illness. These studies are ongoing and a sufficient number of mice of the desired genotypes have been born, negating the need for additional breeding.

Major Task 3: Analyze data obtained from Major Tasks 1 and 2.

This will be accomplished at the end of subtask 2.

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 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 and scheduled for presentation at an international meeting in February. During these studies, a negative regulator of SELENOF protein synthesis, eukaryotic translation initiation factor 3 (EIF4a3), has been identified which is over-expressed in cancer. The predicted binding site for EIF4a3 in the untranslated region of SELENOF mRNA corresponds to the functional at-risk polymorphism that is 5-10 more frequent in the genomes of African Americans. EIF4a3 is therefore a likely oncogene that is potentially targetable with existing inhibitors. This observation was used as a foundation for a research proposal that has been submitted to the DOD to translate the results of this current grant to a project that is currently under review with significant translational potential.

Subtask 1: Reduce the levels of SELENOF in RWPE-1 and primary prostate epithelial cells.

RWPE-1 clones expressing reduced levels of SELENOF due to the stable introduction of shRNA constructs were generated and the reduction of SELENOF was verified by both western blots and confocal microscopy (Figure 4). Difficulties in obtaining stable transfectants of primary cells has impeded the progress using those cells.

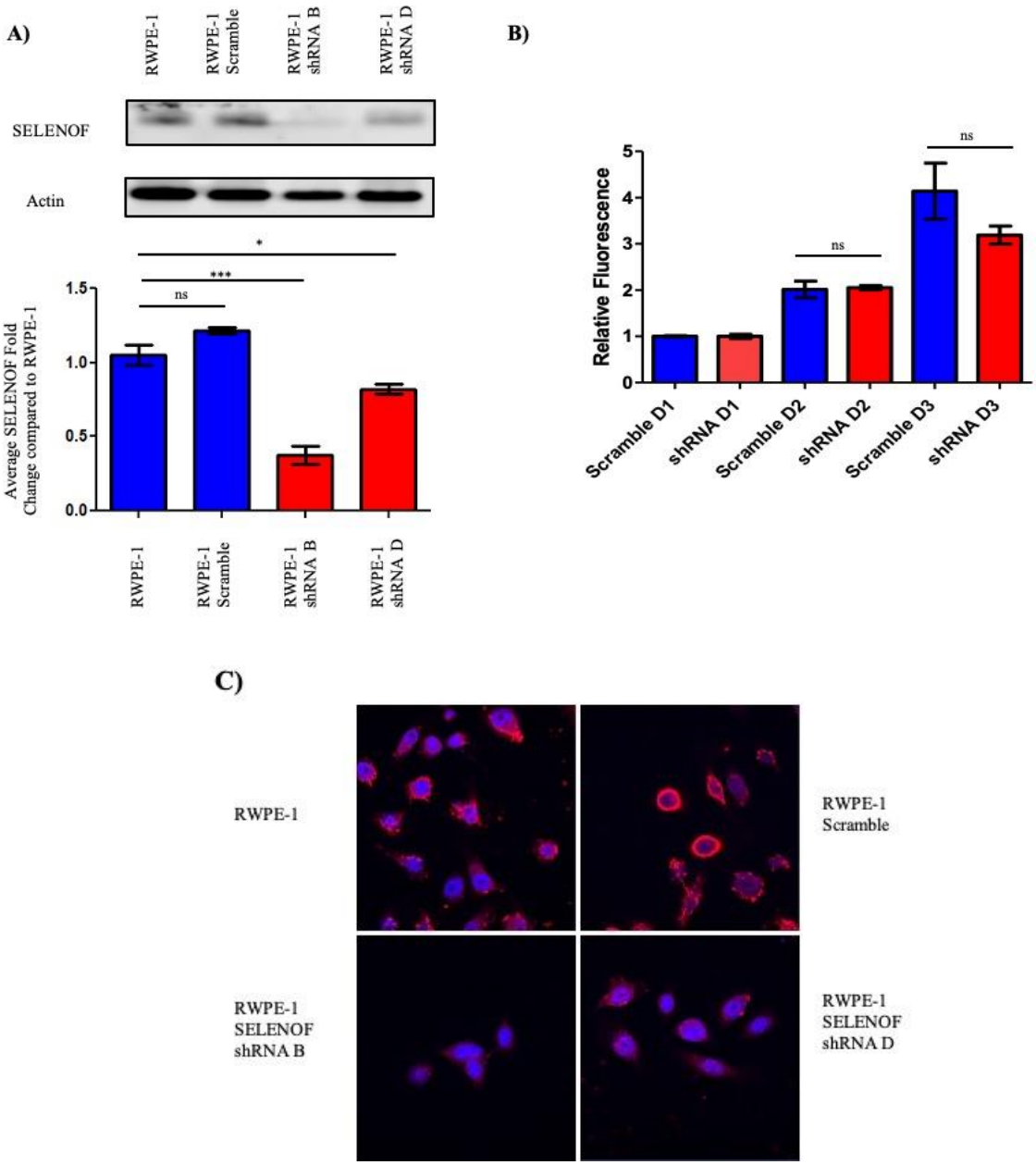


Figure 4. Reduction of SELENOF levels in RWPE-1 cells. A) SELENOF levels were successfully reduced with a shRNA vector. Western blots using anti-SELENOF antibodies were quantified and a two-tailed t-test was performed for significant differences parental RWPE-1 cells and transfected RWPE-1 cells. n= 3, * $p<0.05$, *** $p<0.001$. B) Fluorometric dsDNA quantification was performed after 3 days. Data are represented as the mean $\pm$ SEM, ns, not significant, n =3. C) Parental RWPE-1 and RWPE-1 scramble cells exhibit SELENOF membrane-associated localization shown in red, similar to what is seen in human benign tissues. Nuclei are stained blue with DAPI. Both shRNA RWPE-1 transfected clones have diffuse SELENOF staining in the cytoplasm similar to what is seen in prostate cancer tissue and prostate cancer cell lines (magnification is 63X).

Subtask 2: Direct SELENOF to the RWPE-1 and primary prostate epithelial cells.

This subtask was changed to focus on the loss of SELENOF on RWPE-1 cells as this mimics the circumstances of prostate cancer in humans where SELENOF levels are dramatically reduced. In addition, we and other groups have observed restrictions to ectopically expressing SELENOF that have not been resolved.

Subtask 3: Determine whether the manipulations described in Subtasks 1 and 2 enhance the transformed phenotype.

In order to address the issue of whether SELENOF during prostate cancer progression was contributing to the malignant phenotype or was a bystander observation, RWPE-1 cells with reduced SELENOF levels were examined for several changes associated with the transformed phenotype. Of note, The reduction of SELENOF levels did not alter the proliferation of shRNA RWPE-1 cells relative to RWPE-1 scramble cells after 24, 48, and 72 hours. The ability of cells to grow in semi-solid media was assessed as anchorage-independent growth, a common phenotype of transformation. As seen in Figure 5, reduction of SELENOF in non-transformed RWPE-1 cells dramatically increased the ability of these cells to grow in the semi-solid media.

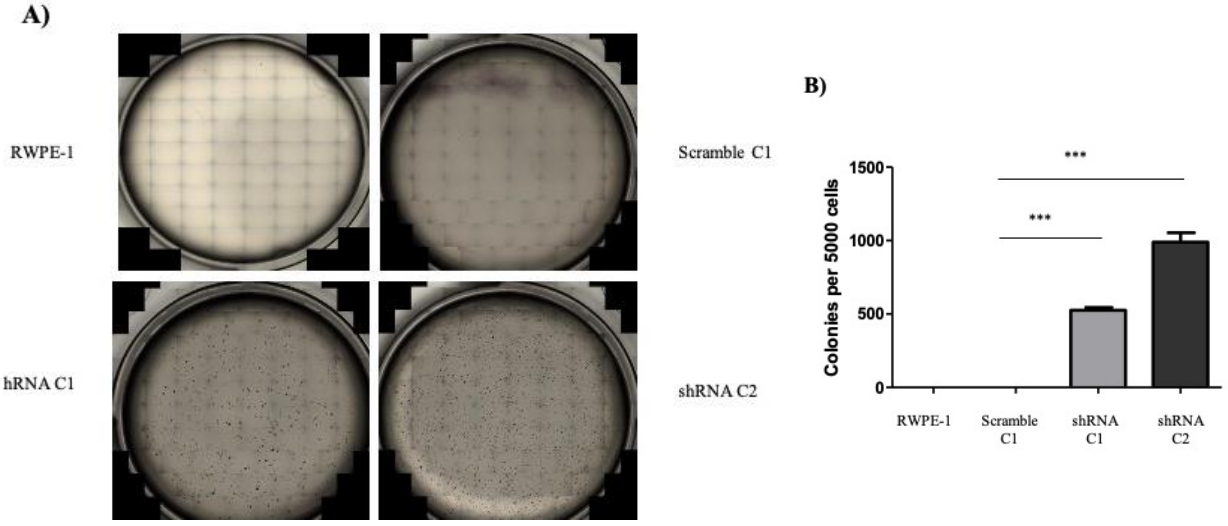
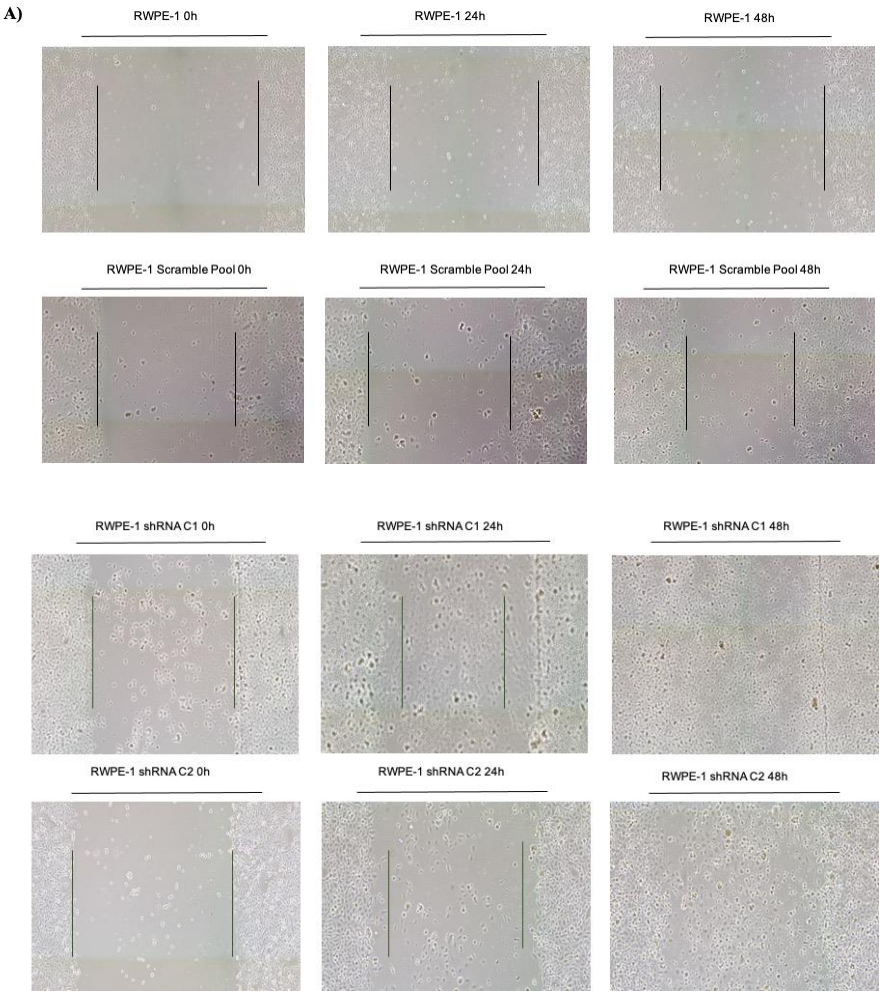


Figure 5. Reduction of SELENOF in RWPE-1 cells results in anchorage-independent growth. A) Representative images of cells cultured in soft agar used to quantify the number of colonies formed. RWPE-1 and RWPE-1 Scramble C1 did not form any colonies. B) Quantification of the average number of colonies formed per 5000 cells plated. Data is represented in mean $\pm$ SEM, n=3, and a two-tailed paired t-test was used to determine statistical differences ***$p<0.001$.

A scratch or wound healing assay was next used to determine the ability of cells to migrate on a tissue culture dish, a surrogate for aggressive or advanced cancer cells. Parental RWPE-1, RWPE-1 scramble, and two clones of shRNA RWPE-1 cells were plated to form a fully confluent cell monolayer. A scratch was created using a pipette tip and the width of the scratch was measured. Although no differences in proliferation were observed when SELENOF levels were reduced, aphidicolin was used as an anti-proliferative agent to ensure differences in proliferation would not affect the results. Forty-eight hours after the initial scratch, both siRNA RWPE-1 clones nearly closed the wound completely while the parental RWPE-1 and RWPE-1 scramble-transfected cells only decreased the width of the wound slightly (Figure 6). The widths of the scratch at 24 and 48 hours were compared to the initial scratch width made at 0 hour as a fold change (Figure 6). No significant difference was observed when the RWPE-1 scramble pool cells when compared to the parental RWPE-1 cells. Both siRNA RWPE-1 clones

significantly migrated faster when compared to the RWPE-1 scramble cells at 48 hours ($p<0.0001$, Figure 6).



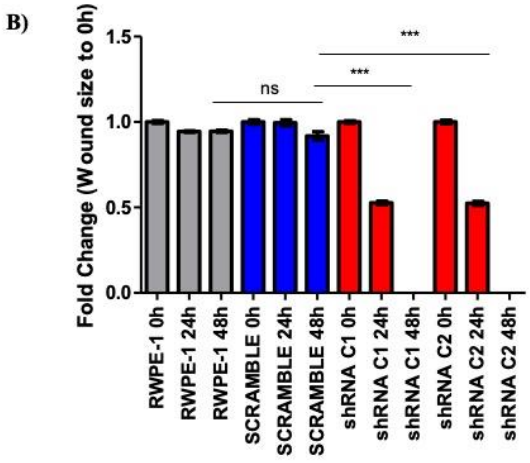


Figure 6: Reduction of SELENOF increases the migration of RWPE-1 cells in a scratch assay. A) Representative images were captured at 0h, 24h, and 48h for RWPE-1, RWPE-1 scramble, and 2 clones of RWPE-1 shRNA SELENOF cells. B) Quantification of the scratch from three independent experiments are shown. The images were captured using the EVOS FL Auto Imaging system (ThermoFisher) and the width was measured using ImageJ. Data is represented as the mean $\pm$ SEM, n=3 and a two-tailed paired t-test was used to determine statistical significance, *** $p < 0.001$.

The metabolism of the benign prostate includes a truncated TCA cycle with glycolysis predominating to accumulate high levels of citrate required for sperm health. Prostate carcinogenesis often involves a shift in energy metabolism from glycolysis to oxidative phosphorylation. To investigate the effects of SELENOF on mitochondrial respiration in prostate cells, the oxygen consumption rate (OCR) of RWPE-1 cells with reduced SELENOF and control cells were measured in real time using the Seahorse XFe24 platform. The OCR is presented as the fold change of shRNA RWPE-1 cells compared RWPE-1 scramble cells in Figure 7. Reducing the levels of SELENOF significantly increased basal oxidative

phosphorylation by approximately 3-fold (Figure 7) compared to control RWPE-1 Scramble cells. Maximal respiration is calculated by measuring the OCR after ATP synthase is inhibited by oligomycin and an uncoupler, FCCP, is injected into the assay. The spare respiratory capacity is the difference between the peak of OCR after FCCP injection and the initial basal OCR. Reducing the levels of SELENOF in RWPE-1 cells resulted in increased maximal respiration and spare respiratory capacity by 3.5-fold and 4-fold respectively. ATP production (Figure 7), which is determined as the difference in OCR before and after oligomycin injection that inhibits ATP synthase also increased by 3-fold. Together, these results indicate that the reducing SELENOF levels in RWPE-1 cells increase mitochondrial respiration and presumably ATP synthesis.

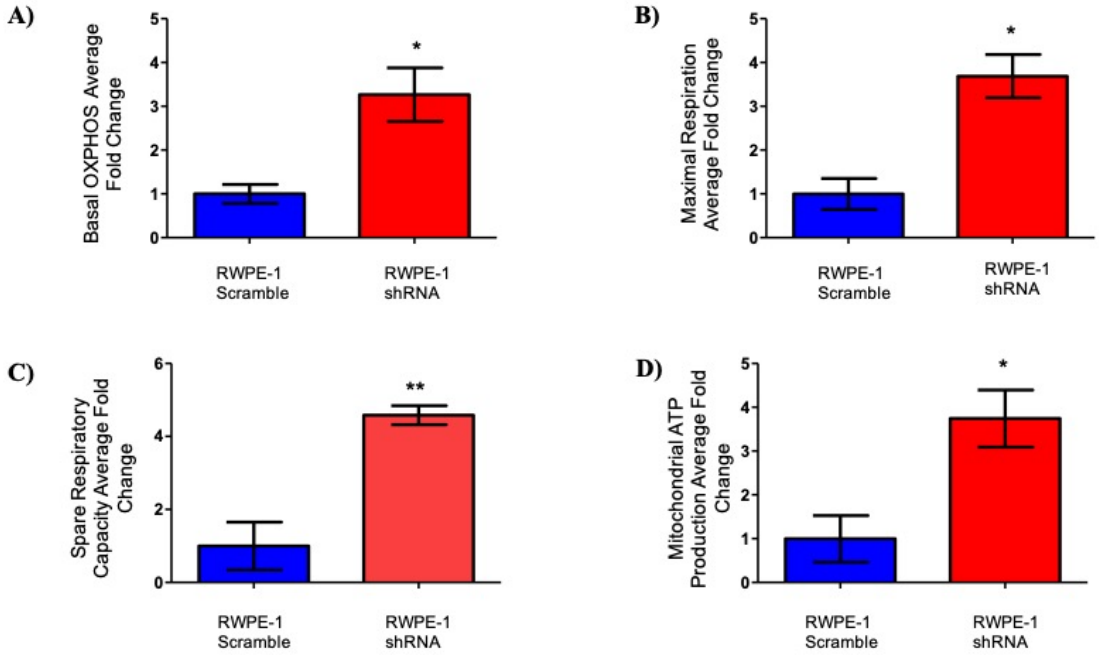


Figure 7. Reduction of SELENOF increases oxygen consumption in RWPE-1 cells. Average fold changes are represented for A) basal, B) maximal respiration after FCCP injection, C) spare respiratory capacity (difference in peak OCR and basal measurements), and D) mitochondrial ATP production (difference before and after oligomycin injection). Data is presented as the mean ± SEM, n =3, two-tailed paired t-test, * $p < 0.05$, ** $p<0.01$, n=3.

- **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. This year 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.

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

This year, 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.

A manuscript that mirrors our results on the impact of *SELENOF* in health disparities in prostate cancer to breast cancer has been accepted to *Oncogene.*

Our DOD-supported work will be 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?**

Specific Aim 1: The proposed work on the health disparity TMA from PCBN was completed and is being prepared for publication. All the tissues for the other planned TMA have been collected, DNA recovered, and selenium levels determined. In this coming year, the completion of the TMA will be accomplished, and the TMA will be stained not only for SELENOF as initially proposed, but for eIF4a3 as well. The technology to multi-plex stain the slides has already been optimized. Signals will be analyzed as we have done in the past and associations with selenium levels and genotypes will be investigated.

Specific Aim 2: Existing animals will be euthanized at 26-27 weeks, prostates will be removed and histologically analyzed.

Specific Aim 3: As the goals of this aim have been achieved, we will continue to characterize a commercially available inhibitor of the eIF4a3 protein that inhibits SELENOF translation as a potential therapeutic.

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 and 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.

- **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 is a conference organizer and scheduled to present his data on SELENOF on the health disparity experienced by African American men at the 12th International Symposium on Selenium and Medicine scheduled for February 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.

- **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?**

Nothing to report

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

Nothing to report

8. Special Reporting Requirements

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

9. Appendices