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TITLE: Ethnic Disparity of Mitochondrial Peptides and Prostate Cancer Risk

PRINCIPAL INVESTIGATOR: Pinchas Cohen, MD

CONTRACTING ORGANIZATION: University of Southern California
Los Angeles, CA 90089

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Institute for Medical Research,
Durham VA, NC 27701.

Cedars-Sinai Medical Center
LA, CA 90048.

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13. SUPPLEMENTARY NOTES

14. ABSTRACT (200words)

Mitochondrial-dysfunction is associated with prostate-cancer (PC). We hypothesized that differences in MDP-expression levels contributes to the elevated-risk of PC in Black men. Our project is testing the potential of MDPs as biomarkers for PC risk, both as circulating pre-diagnostic biomarkers and as surrogate mitochondrial genetic risk indicators in a group of Black and White men with and without PC. Our Specific Aims include 1) To confirm the association between SHLP2 levels and prostate cancer risk; 2) to determine the association between additional plasma MDPs and prostate cancer risk among Black and White men in a newly enrolled population; and 3) to determine the contribution of mitochondrial versus nuclear genetic-race, relative to self-reported-race, to the MDP-PC-risk connection. We have succeeded in enrolling substantial numbers of patients of both ethnicities. Our data from year-1 of the project confirms that MDP levels are generally lower in plasma of PC patients. This study is the first to study racial differences in MDP levels and their effect on PC. MDP levels and mitochondrial genetic origin may serve as diagnostic biomarkers of PC. Moreover, this study may set the stage for future mitochondrial-related interventions in minority populations.

15. SUBJECT TERMS
Mitochondria, mtDNA, mitochondrial derived peptides, prostate cancer risks, biomarkers

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Introduction

Incidence and death by cancer is highly disparate between racial and ethnic groups, with several possible causes such as differences in socioeconomic status, behavioral risk factors, and underlying genetics all playing a role. In particular, Black Americans are at higher risk of developing and dying from a number of cancers such as colorectal, lung, and prostate cancer. Prostate cancer is the second leading cause of cancer death in men in America and is one of the most racially disparate cancers. Blacks are 1.6 times more likely to be diagnosed with prostate cancer than Whites and 2.4 times more likely to die of the disease.

OUR AIMS for this project included:

Aim 1: Confirm the association between high serum SHLP2 levels and decreased prostate cancer risk and lower SHLP2 levels. Our preliminary data with a small cohort of Black and White men undergoing prostate biopsy at the Durham VA found that SHLP2 levels were significantly lower in men with PC vs. men without PC and that Black men had significantly lower levels than White men. Indeed, a SHLP2 level >350 pg/ml had a negative predictive value of ≥95% regardless of race, and thus may be an excellent biomarker to avoid biopsy. While these findings are very encouraging, a larger dataset is needed to validate our results.

Aim-2: Determine the association between plasma MDPs and prostate cancer risk among Black and White men in a newly enrolled population. Because both humanin and a recently discovered MDP from the 12S rRNA region, MOTS-c, can only be detected in plasma and not serum, we are analyzing plasma from Whites and Blacks with and without PC newly recruited from the Durham VA. We measured circulating levels of humanin, MOTS-c, SHLP-6, and SHLP2 and determine the association between these MDPs, race, and cancer status. In this report, we identified lower levels of MDPs in Black men. We also report lower MDP levels in PC patients.

Aim-3: Aim 3: Determine the contribution of mitochondrial versus nuclear genetic-race, relative to self-reported-race, to the MDP-PC-risk connection. Using the Illumina Expanded Multi-Ethnic Genotyping Array, we will analyze DNA from the subjects studied in Aims 1-2. We will determine the percent genetic (both mitochondrial and nuclear) African ancestry relative to self-reported race and determine their contributions to MDP levels and PC risk in a multivariable analysis examining additional SES and lifestyle factors.

Keywords: PROSTATE CANCER, HEALTH DISPARITIES, MITOCHONDRIA, PEPTIDES, DIAGNOSTICS, HUMANIN MOTS-C, SHLP2.

Accomplishments

Please note that pages 7-8 of the report contain unpublished data that should be protected.

Major goals of the project

1: Confirm the association between high serum SHLP2 levels and decreased prostate cancer risk.
2: Determine the association between plasma MDPs and prostate cancer risk among Black and White men in a newly enrolled population.
3: Determine the contribution of mitochondrial versus nuclear genetic-race, relative to self-reported-race, to the MDP-PC-risk connection.

What was accomplished under these goals?

1) **Major Activities**

a. Patient and Sample Recruitment

Plasma samples have been collected from 372 participants. Participants are of Black, White, and Asian/Pacific Islander origin. Black men comprise 215/372 (58%) of samples, and White men comprise 156/372 (42%) of the plasma samples. 71 (33%) of Black men had negative biopsy results and 140 (65%) had positive biopsy results. 64 (41%) White men had negative biopsy results and 88 (56%) had positive biopsy results.

Race	Biopsy Result	Count
Asian or Pacific Islander	Positive	1
Black	*NULL*	4
Black	Negative	71
Black	Positive	140
White	*NULL*	4
White	Negative	64
White	Positive	88
	Total	**372**

b. Conduct Humanin, MOTS-c, SHLP2, and SHLP6 assays on plasma samples

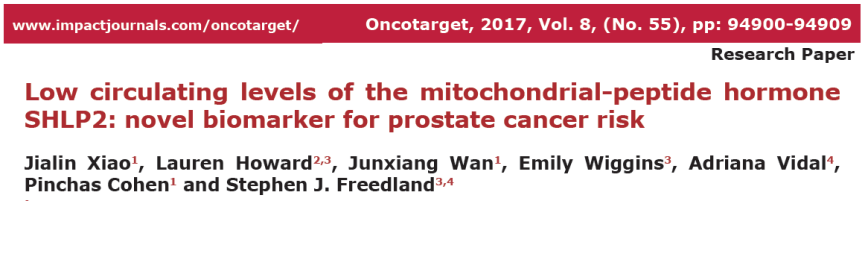
Our assay team ran 800 assays on 200 patients (100 Black men and 100 White men; 50 each with and without positive biopsies for prostate cancer). Data is shown below.

2) **Specific objectives that have been achieved**: We demonstrated as shown below (fulfilling the major goals of aim-2) that:
   a. MDP levels are lower in Black men compared to White men
   b. MDP levels are lower in biopsy positive cancer patients compared to negative ones.

3) **Significant Results**

a. Two key papers have been published as a result of our work so far:

i. Xiao J, Howard L, Wan J, Wiggins E, Vidal A, Cohen P, Freedland SJ. Low circulating levels of the mitochondrial-peptide hormone SHLP2: novel biomarker for prostate cancer risk. *Oncotarget*. 2017; 8:94900-94909.

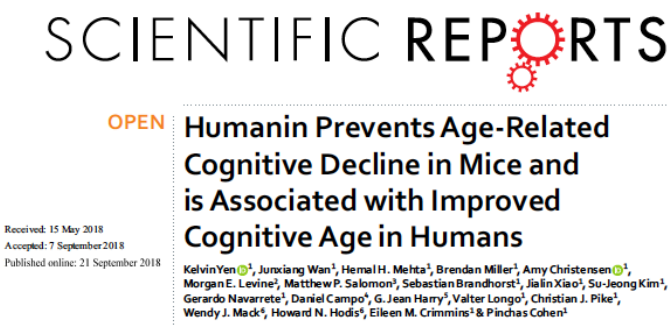

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 55), pp: 94900-94909

Research Paper

Low circulating levels of the mitochondrial-peptide hormone SHLP2: novel biomarker for prostate cancer risk

Jialin Xiao[1], Lauren Howard[2,3], Junxiang Wan[1], Emily Wiggins[3], Adriana Vidal[4], Pinchas Cohen[1] and Stephen J. Freedland[3,4]

ii. Yen K, Wan J, Mehta HH, Miller B, Christensen A, Levine ME, Salomon MP, Brandhorst S, Xiao J, Kim SJ, Navarrete G, Campo D, Harry GJ, Longo V, Pike CJ, Mack WJ, Hodis HN, Crimmins EM, Cohen P. Humanin Prevents Age-Related Cognitive Decline in Mice and is Associated with Improved Cognitive Age in Humans. *Nature Scientific Reports*. 2018; 8:14212. doi: 10.1038/s41598-018-32616-7.


SCIENTIFIC REPORTS

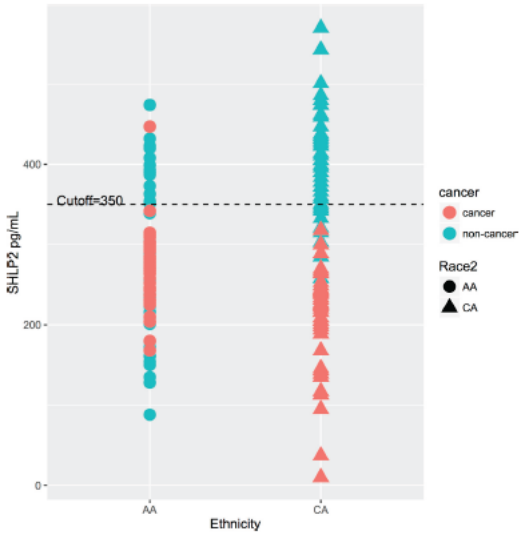
OPEN

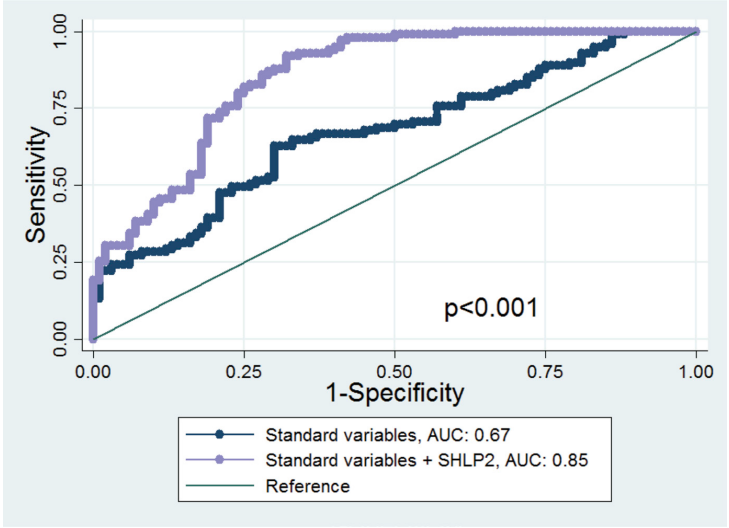
Humanin Prevents Age-Related Cognitive Decline in Mice and is Associated with Improved Cognitive Age in Humans

Received: 15 May 2018
Accepted: 7 September 2018
Published online: 21 September 2018

Kelvin Yen[1], Junxiang Wan[1], Hemal H. Mehta[1], Brendan Miller[1], Amy Christensen[1], Morgan E. Levine[2], Matthew P. Salomon[3], Sebastian Brandhorst[1], Jialin Xiao[1], Su-Jeong Kim[1], Gerardo Navarrete[1], Daniel Campo[4], G. Jean Harry[5], Valter Longo[1], Christian J. Pike[1], Wendy J. Mack[4], Howard N. Hodis[4], Eileen M. Crimmins[1] & Pinchas Cohen[1]

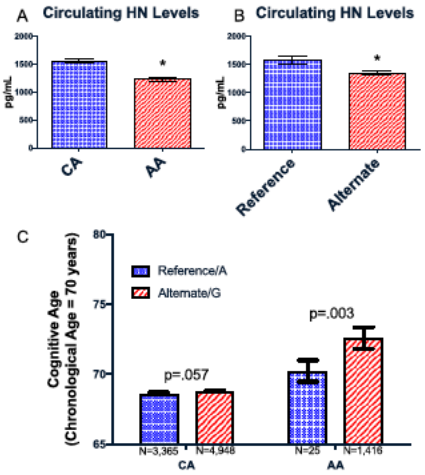
b. Some of the key findings in these papers include:





Demonstrating the potential clinical utility of serum SHLP2: the distribution of SHLP2 levels with a cut-off at 350-pg/ml (Left panel). A cut-off of 350-pg/ml SHLP2 differentiate between controls and PCa cases in both black and white men. Among men with SHLP2 > 350-pg/ml, 0/37 white (100% NPV) and only 1/20 black men had PCa (95% NPV). ROC curve and AUC statistics before and after adding SHLP2 in the model (Right panel). The true positive rate (sensitivity) is plotted in function of the false positive rate (1–specificity) for the model excluding or including SHLP2 levels. The AUC is a measure of how well a quantitative test can distinguish between subjects with and without prostate cancer. The AUC of the model including only age, DRE, race and PSA to predict PCa risk was 0.67. This improved to 0.85 when SHLP2 was added to the model ($p < 0.001$). Thus, lower SHLP2 was linked with increased PCa risk in white men, but no significant association was observed in black men. While SHLP2 > 350-pg/ml ruled out PCa in both races with high accuracy, SHLP2 was unrelated to PCa grade. These data suggest the circulating mitochondrial-derived peptide hormone, SHLP2 plays a key role in the development and racial disparity of prostate cancer.

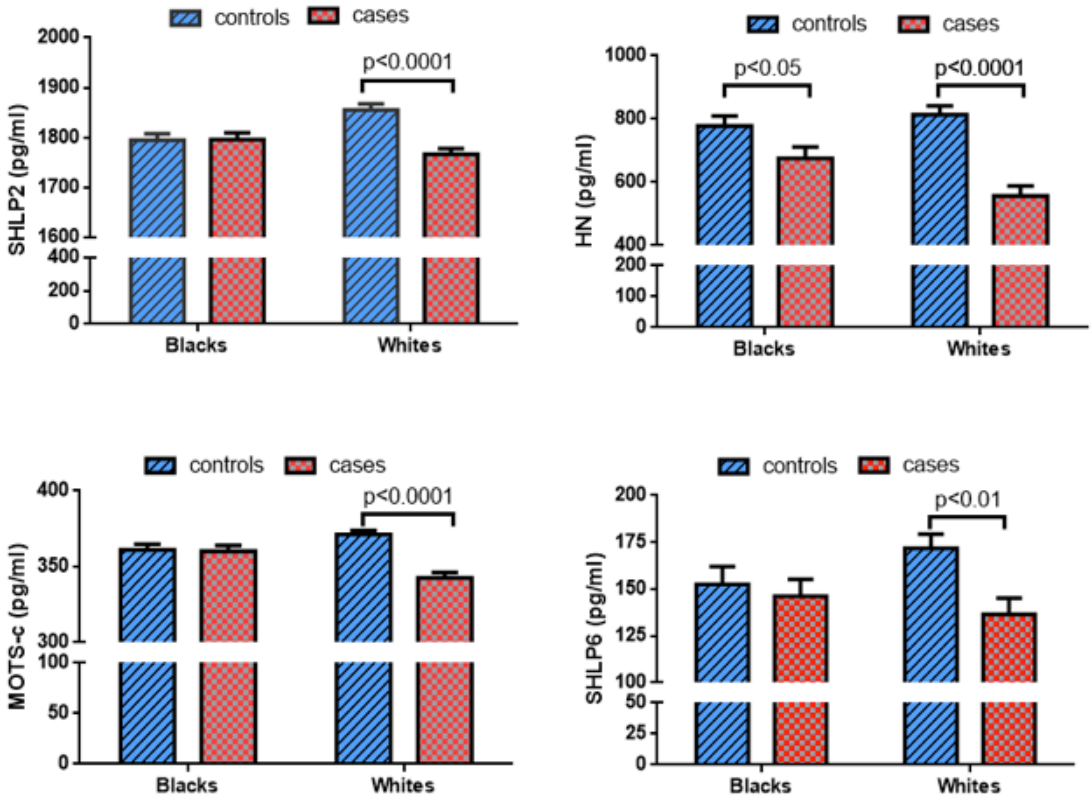
In the second paper listed above, using a population of 200 Black and White patients without cancer, we showed that Blacks have a lower level of humanin compared to matched White subjects.



Furthermore, we identified a specific SNP (rs2854128) in the humanin-coding region of the mitochondrial genome that is associated with a decrease in circulating humanin levels. In a large, independent cohort, consisting of a nationally representative sample of older adults, we found that this SNP is associated with accelerated cognitive aging, supporting the concept that humanin is an important factor in aging-related diseases in an ethnic-specific fashion. On the left, it is shown that humanin levels and cognitive age is associated with SNP rs2854128. (A) African-Americans have lower circulating humanin levels than Caucasian-Americans. (B) Individuals with the mitochondrial SNP rs2854128 have a significant decrease in circulating humanin levels. (C) RS2854128 is associated with an increased cognitive age that is more pronounced in African-Americans. *Signifies $p < 0.05$.

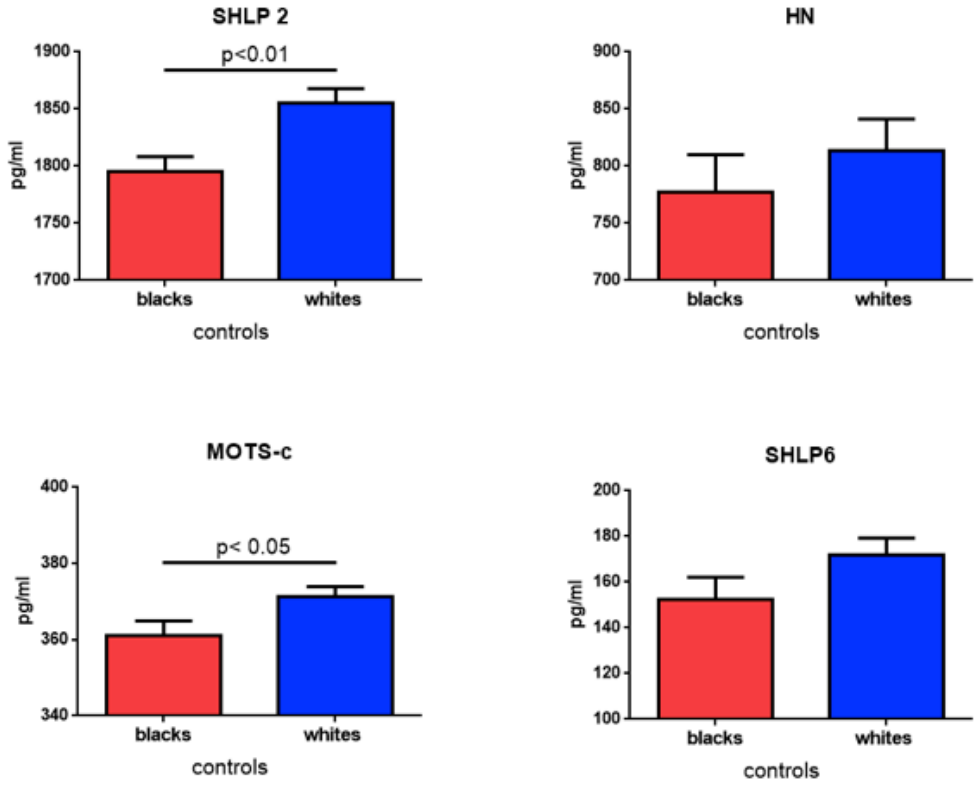
Recent unpublished results obtained during the first year of funding

As noted above, we ran our four novel MDP assays (for humanin, SHLP2, SHLP6, and MOTS-c) on 200 plasma samples collected over the last year (100 Blacks, 100 Whites, each set with 50 Cancers and 50 controls). The results are shown below:



The levels of all 4 MDPs are significantly lower in White patients with Cancer compared with White subjects with negative biopsies. In comparison, only plasma humanin levels are lower in Black patients with Cancer compared with Black subjects with negative biopsies. Of note is that the degree of reduction (while very highly significant) varies from 10-30%. In comparison, the reductions in serum SHLP2 were more prominent (close to 50%). The reasons for this are unclear and we will evaluate this in the next year. These results also suggest a more general issue related to OVERALL mitochondrial function in patients that may predispose them to cancer. We will also evaluate the potential demographic and lifestyle aspects of this observation.

Furthermore, when we compared the levels of these MDPs in biopsy negative men between Black and Whites, the following picture emerged:



Plasma SHLP2 levels were lower in Black men compared to White men, however, the degree of difference was smaller than we observed for serum SHLP2. Interestingly, MOTS-c levels were also lower in Blacks, while humanin and SHLP6 levels were not significantly different.

4) Stated goals not yet met.

In the coming two years we plan to:

a) Measure serum levels of SHLP2 in a large cohort of Black and White patients with or without positive biopsies for prostate cancer (fulfilling aim-1)
b) Correlate all MDPs with lifestyle factors and other demographic, physical and biochemical markers
c) Analyze DNA from these patients to fulfil aim-3.

Opportunities for training and professional development

Dr. Cohen has a track record in training underrepresented minority students and is involved in a program with Howard University that sends two undergraduate students to his lab for three months every summer since 2017 that has resulted in a highly successful research experience for these URM students.

Dissemination of the results to communities of interest

Dr. Cohen has presented this work in the American Aging Association meetings in 2018. Some data has also been published by Drs. Cohen and Freedland (see list of publications below)

Plans for the next reporting period

As noted above, we are planning to continue to 1) recruit patients; 2) measure serum SHLP2 as well as 3) measure all MDPs in a larger cohort, 4) sequence mtDNA on the subjects, and 5) conduct analysis of the data.

Impact

Ethnic disparity in health outcomes is one of the greatest challenges facing our nation. Black men are at greater risk for aggressive Prostate Cancer, and recent data from our laboratory has identified that black men have lower levels of the mitochondrial peptide humanin and the small humanin-like peptide SHLP2. These peptides are encoded within the mitochondrial DNA (mtDNA) and gene variants of these novel mitochondrial small open reading frames (sORF) are maternally inherited and are strongly associated with ethnicity. Our recent data shows that low levels of SHLP2 are a potent predictor of prostate cancer risk, with higher levels offering apparent protection from the disease, in an ethno-dependent fashion. Thus, we believe that black men may be at greater risk for the clinical consequences of mitochondrial altered function and peptide secretion, leading to various diseases including prostate cancer.

Short-Term Impact - Biomarker Discovery: We are testing the novel hypothesis that the levels of the four recently described mitochondrial peptides (humanin, MOTS-c, SHLP-2 and SHLP-6) could serve as biomarkers for PC risk. If successful, these findings will establish the mitochondria as a key player in understanding prostate cancer pathogenesis and will initiate further studies to test the possible clinical utility of using mitochondrial derived peptide (MDP) levels in the screening and prognostication of patients with high risk for developing prostate cancer, particularly African Americans. Most men diagnosed with PC will not die from their cancer, and there is need for new methods to differentiate those men who need treatment from those with a non-lethal phenotype. Future clinical trials will confirm the extent to which MDP levels alters tumor characteristics or provides an independent biomarker for PC prognosis in black and white men.

Mid-Term Impact - Target Populations for Intervention: With evidence that MDPs affects PC risk or prognosis in high-risk populations through an inflammatory pathway, we lay the groundwork for interventions to increase the levels of these peptide hormones in black and white men who are at risk for PC progression or recurrence. As our recent data indicates that dietary interventions modify mitochondrial peptide levels, we see a possible direction in providing lifestyle advice based on these diagnostic markers that could improve with appropriate recommendations.

Long-term Impact - Feasible Change in Clinical Care: Mitochondrial-based therapeutics of various kinds are being tested for a number of diseases (including some MDPs). It is entirely possible that such therapies will directly or indirectly raise MDP levels and thus treat the primary disease or delay the development of recurrence or metastasis (which we have already shown in xenograft studies in mice treated with MDPs. Our study will provide important information toward establishing effective blood MDP levels to target in order to improve PC outcomes.

Changes/Problems:

At this point we do not see any problems with the execution of our proposed aims and do not anticipate any changes in the plans that were submitted in the original grant.

Products

Database and Biospecimen collection: Described under accomplishments.

List of publications since DOD grant funding

1. Xiao J, Howard L, Wan J, Wiggins E, Vidal A, Cohen P, Freedland SJ. Low circulating levels of the mitochondrial-peptide hormone SHLP2: novel biomarker for prostate cancer risk. *Oncotarget*. 2017; 8:94900-94909.
2. Liang P, Henning SM, Guan J, Grogan T, Elashoff D, Olefsky JM, Cohen P, Aronson WJ. Role of Host GPR120 in Mediating Dietary Omega-3 Fatty Acid Inhibition of Prostate Cancer, *JNCI: Journal of the National Cancer Institute*. September 6th, 2018, doi.org/10.1093/jnci/djy125 [Epub ahead of print].
3. Yen K, Wan J, Mehta HH, Miller B, Christensen A, Levine ME, Salomon MP, Brandhorst S, Xiao J, Kim SJ, Navarrete G, Campo D, Harry GJ, Longo V, Pike CJ, Mack WJ, Hodis HN, Crimmins EM, Cohen P. Humanin Prevents Age-Related Cognitive Decline in Mice and is Associated with Improved Cognitive Age in Humans. *Nature Scientific Reports*. 2018; 8:14212. doi: 10.1038/s41598-018-32616-7.
4. Vidal AC, Howard LE, de Hoedt A, Cooperberg MR, Kane CJ, Aronson WJ, Terris MK, Amling CL, Taioli E, Fowke JH, Freedland SJ. Neutrophil, lymphocyte and platelet counts, and risk of prostate cancer outcomes in white and black men: results from the SEARCH database. Cancer Causes Control. 2018; 6:581-588. doi: 10.1007/s10552-018-1031-2.
5. Freedland SJ, Vidal AC, Howard LE, Terris MK, Cooperberg MR, Amling CL, Kane CJ, Aronson WJ; Shared Equal Access Regional Cancer Hospital (SEARCH) Database Study Group. Race and risk of metastases and survival after radical prostatectomy: Results from the SEARCH database. Cancer. 2017 Nov 1;123(21):4199-4206. doi: 10.1002/cncr.30834.

Participants and Other Collaborating Organizations
Site-1: University of Southern California
Individuals have worked on the project:

Name:	Pinchas Cohen
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-0035-8366
Nearest person month worked:	1.2
Contribution to Project:	He was responsible for coordinating all aspects of the project including experimental design and data analysis. Dr. Cohen supervised all personnel at USC and insure communications with Dr. Freedland.
Funding Support:	USC school account

Name:	Junxiang Wan
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	She performed Humanin, SHLP2 SHLP6, MOTS-c ELISA assays which she has developed on 200 subjects.
Funding Support:	NIA 1P01AG034906; BIG AFAR award; USC school account

Name:	Hemal Mehta
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	She helped Dr. Wan in MDP extraction procedure from the plasma.
Funding Support:	NIA 1P01AG034906; BIG AFAR award; USC school account

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Dr. Cohen has been awarded the following new grants:
R01AG061834 (Cohen PI) 09/01/2018 - 08/31/2023 1.2 calendar months
Humanin is an AD resilience factor through its interaction with APOE4
U54CA233465 (Carpten PI) 11/01/2018 – 10/30/2021 1.2 calendar months
FLORIDA-CALIFORNIA CANCER RESEARCH, EDUCATION & ENGAGEMENT HEALTH EQUITY CENTER

Dr. Freedland has been awarded the following new grants:
R01CA220327 (Freedland PI) 04/01/2018 - 03/31/2023 1.8 calendar months
Racial Differences in Prostate Cancer Molecular Subtyping
DOD (Freedland PI) 9/30/2017 – 9/29/2020 1.2 calendar months
Exploiting Dysregulation of Cholesterol Homeostasis as a Novel Therapy for Prostate Cancer

Site 2: Cedars Sinai Medical Center, Los Angeles, CA
& Institute for Medical Research/Durham Veteran's Administration, Durham, NC

Name:	Stephen Freedland
Project Role:	Collaborating-Investigator and Subaward Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-8104-6419
Nearest person month worked:	1.0
Contribution to Project:	Freedland oversaw all personnel at CSMC and participated in regular communication with Dr. Cohen on all study-related aspects. Dr. Freedland oversaw patient recruitment and plasma collection at Cedars and Durham VA Dr.
Funding Support:	Cedars accounts and various NIH and DOD grants

Name:	Adriana Vidal
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.35 Calendar Months
Contribution to Project:	Dr. Vidal provided intellectual input into the coordination and selection of samples to be used by the Prime Awardee in the execution of the study. Dr. Vidal along collected and archived plasma samples and shipped those to USC (Cohen Lab) for MDP analysis.
Funding Support:	Cedars accounts

Partner's Contribution to the Project: Dr. Freedland was in charge of patient recruitment and sample collection. He sent Dr. Cohen 200 plasma samples from controls and cases to analyze the mitochondrial derived peptides (MDP) levels by ELISA.

- **Financial Support –** Nothing to report
- **In-Kind Support –** Nothing to report
- **Facilities –** Nothing to report
- **Collaboration –** Nothing to report
- **Personnel exchanges** – Nothing to report
- **Others –** Nothing to report

Special Reporting Requirements

We are not aware of any special reporting requirements for this project.

Appendices

None