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AWARD NUMBER: W81XWH-17-1-0274

TITLE: Continuous AhR Activity Accelerates Prostate Cancer Progression in African-American Men

PRINCIPAL INVESTIGATOR: Joann Powell

RECIPIENT: Clark Atlanta University
Atlanta, GA 30314

REPORT DATE: July 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
Joann Powell

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5f. WORK UNIT NUMBER

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Clark Atlanta University
Atlanta, GA 30314

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14. ABSTRACT

Recent studies demonstrate that for men with clinically localized, non-metastatic high-risk prostate cancer (PCa) receiving long-term androgen deprivation therapy (ADT) and dose-escalated radiotherapy (RT), a pre-RT PSA value greater than 0.5 ng/ml after ADT predicts for decreased time to distant metastases. African-American (AA) men were significantly associated with failure to achieve a pre-RT PSA value less than 0.5 ng/ml. These elevated PSA levels are a direct result of sustained androgen receptor signaling despite ADT. AA men would benefit greatly from more potent anti-androgenic therapies in combination with radiation. Several independent studies have shown that the aryl hydrocarbon receptor (AhR) can regulate androgen receptor signaling. AhR has been widely studied for mediating the carcinogenic responses to environmental toxins and its transcriptional regulation of drug metabolizing enzymes such as cytochrome P450-1A1 and 1B1 (CYP1A1 and CYP1B1) following ligand activation. However, evidence is emerging that AhR may have intrinsic functions that promote prostate cancer progression. Published results from our laboratory recently revealed that AhR is constitutively active in advanced prostate cancer cell lines and no longer requires ligand activation for activity. Chemical and shRNA mediated ablation of AhR signaling decreases expression of AhR responsive genes and androgen responsive genes, including PSA. In addition, treatment with an AhR antagonist reduced the growth rate of castration-resistant prostate cancer cells and restored cell responsiveness to the anti-androgen bicalutamide. The ability of AhR to regulate androgen receptor signaling in advanced prostate cancer cells identifies it as a prime target to ablate androgen receptor signaling in AA men.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Our preliminary data on the expression of AhR protein in a limited number of human prostate tumors supported our parallel findings in human prostate carcinoma cell lines: that AhR protein is elevated in direct correlation with the malignancy of the disease (Fig 6). In this specific aim, we will examine the AhR expression in a larger sample size of human prostate tumors to test the hypothesis that AhR overexpression is universally associated with prostate cancer progression and thus is a possible prognostic biomarker. We will also compare AhR expression in samples from AA patients to CA patients matched for stage, grade and time to PSA progression to determine if AAs have enhanced AhR activity compared to CA. For this purpose, we proposed to use the tissue micro-arrays (TMA) from the Prostate Cancer Biorepository Network (PCBN). All PCBN specimens contain standard pathology data and a large subset of specimens are linked to clinical, pathology and outcome data. We have not received approval from PCBN and therefore proceeded with tissue samples for our in house biorepository.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Aryl Hydrocarbon Receptor, Androgen receptor, Prostate cancer, Castration resistant prostate cancer, African-American, Prostate cancer health disparity.

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task: Compare AhR activity in prostate cancer and matched normal tissue from AA and CA men with low, moderate and high Gleason scores as well as clinical PSA levels using AhR antibodies.

Milestone: Obtain HRPO approval (12 months)

Milestone: Correlate AhR expression and nuclear localization to Gleason score and PSA progression in AA and CA prostate cancer tissue samples. (20 months)

Milestone: Obtain ACURO approval (20 months)

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift reporting activities to reporting accomplishments.

Major Task: Compare AhR activity in prostate cancer and matched normal tissue from African-Amreican (AA) and Caucasian American (CA) men with low, moderate and high Gleason scores as well as clinical PSA levels using AhR antibodies.

Subtask 1: Compare AhR activity in prostate cancer and matched normal tissue from AAs and CAs to determine if AA cancer tissue has a higher AhR expression.

Results: 200 prostate tissue samples were stained with AhR antibody. An H-score for AhR cytoplasmic and AhR nuclear intensity was determined by screening with digital pathology using Leica Biosystems imaging and individual sample H-scores were also verified by an independent pathologist. There was a measurable increase in cytoplasmic AhR staining in AA tumor compared to matched normal. However, there was a decrease in nuclear AhR staining in the AA tumor samples. This decrease could be a consequence of active AhR signaling which results in proteomic degradation of the AhR protein. Overall, there was not a significant increase in AhR expression, cytoplasmic or nuclear, in AA samples compared to CA tissue samples.

Methods: Analyzed 50 tumor and 50 matched normal prostate cancer tissue slides from African-American (AA) men as well as 50 tumor and 50 matched normal prostate cancer tissue slides from Caucasian-American (CA) men by immunohistochemistry (IHC) for expression of AhR. Briefly, tissue arrays will be dewaxed with xylene, rehydrated in graded concentrations of alcohol and treated with hydrogen peroxide prior to blocking with normal goat serum. The slide will be incubated with AhR antibody. The antibody titer, concentration and incubation time will be determined using corresponding optimization slides. Antibody binding will be detected using a labeled streptavidin-biotin kit with 3'3-diaminobenzidine as the chromogen (DAKO). Hematoxylin will be used as a counterstain. Results of staining will be scored by two independent observers by rating staining intensity from 0 for below the level of detection to 3 for strongest expression.

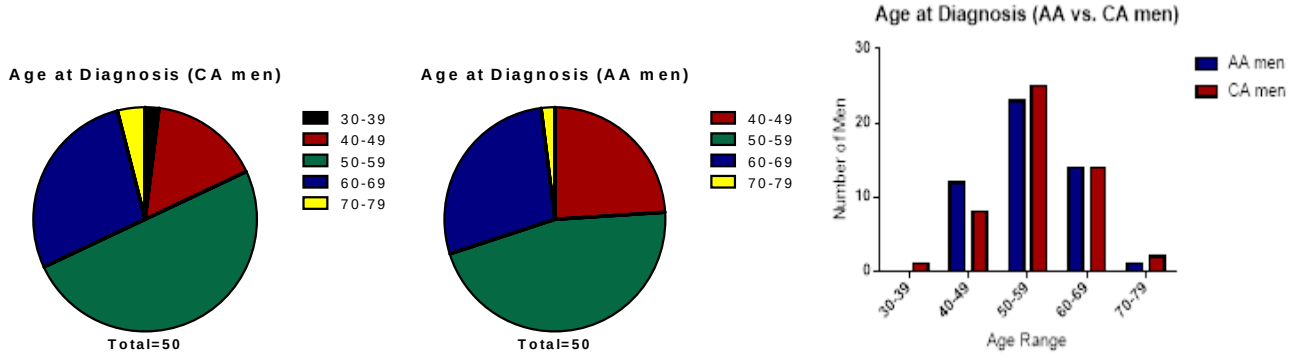
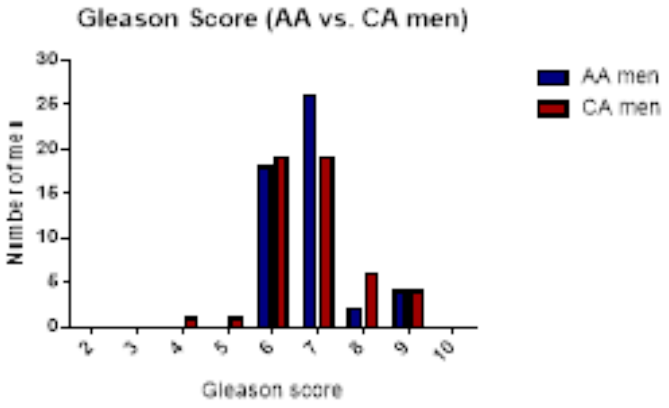


Figure 1: The AA samples ranged from age 43 to 72 with a median age of diagnosis of 55. The CA samples range from 39 to 70 with a median age of diagnosis of 56. The majority of AA (23) and CA (25) samples were from men who were diagnosed between age 50 and 59.



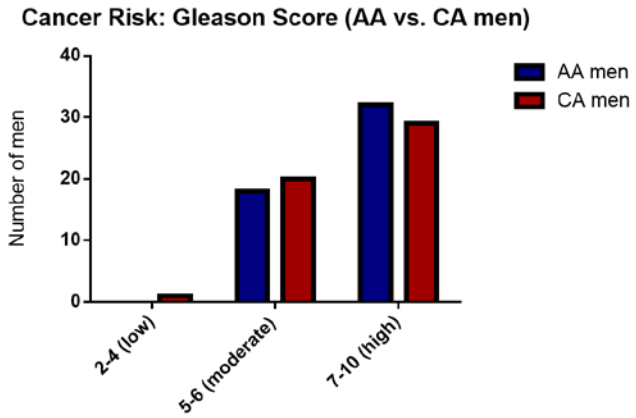
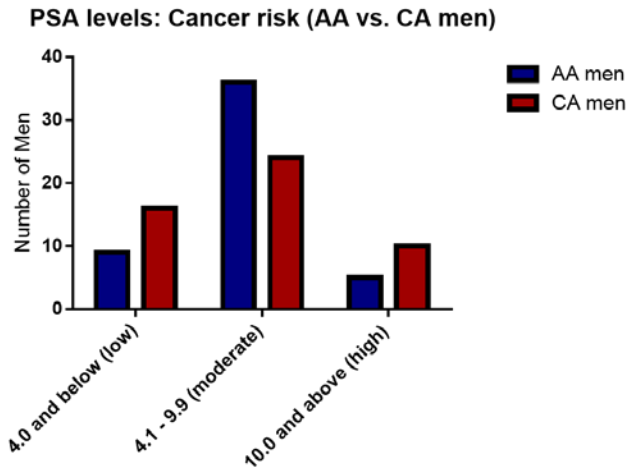


Figure 2: CA samples range from Gleason score 4 to 9. AA samples range from Gleason score of 6 to 9. The average Gleason score for both groups is 6.8 with a median of 7. 58% of CA samples are in the high Gleason range compared to 64% of AA samples.



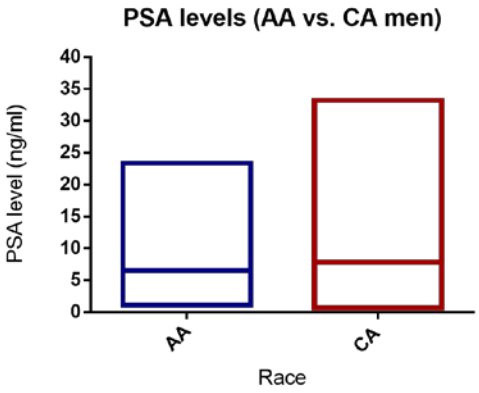
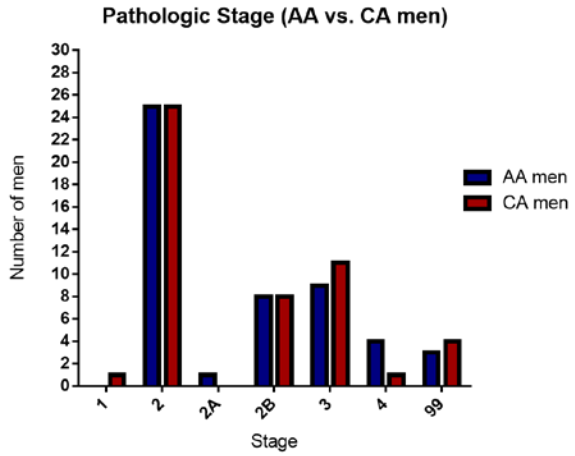


Figure 3: 82% of the AA tissue samples have a PSA of 4.0 or higher compared to 68% of the CA samples. The average PSA of AA samples is xx. The average PSA for CA samples is 7.8 vs 6.5 for AA samples.



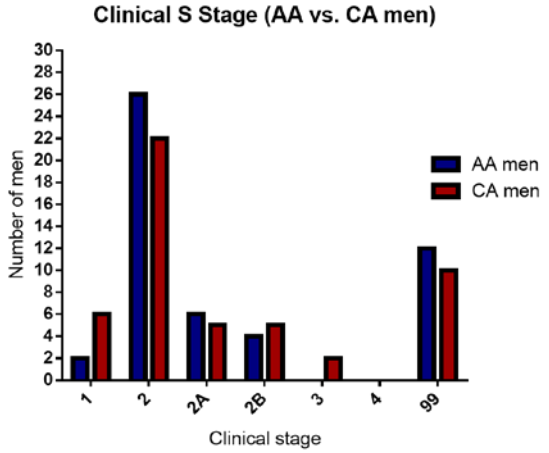
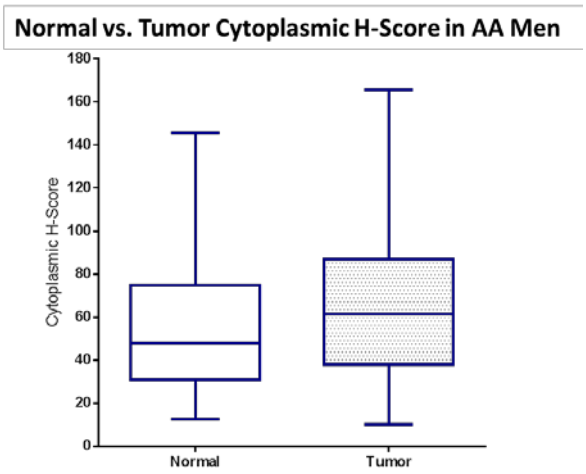
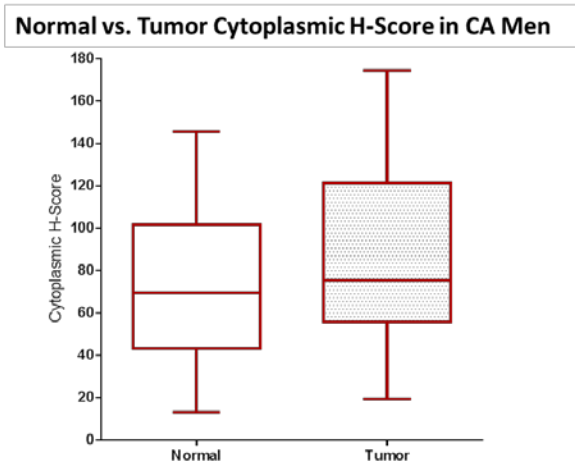
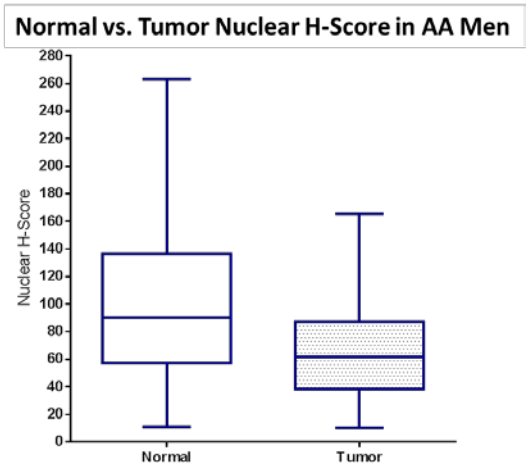


Figure 4: The majority, approximately 50% of the tissue samples were classified in clinical and pathological stage 2. A clinical classification was not assigned to 20% of the samples.







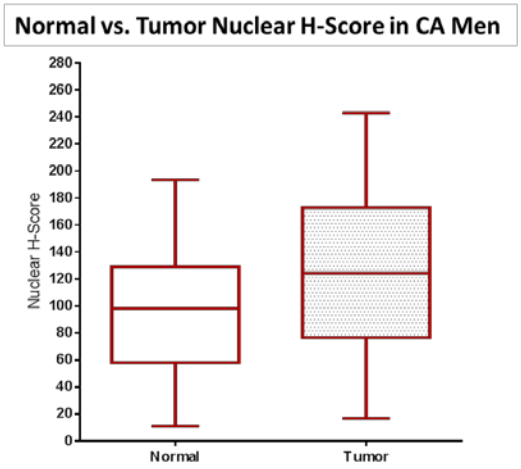
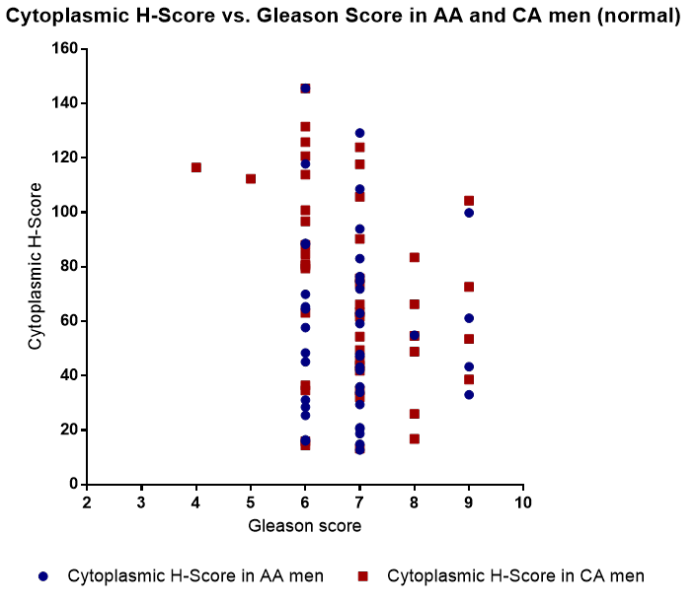
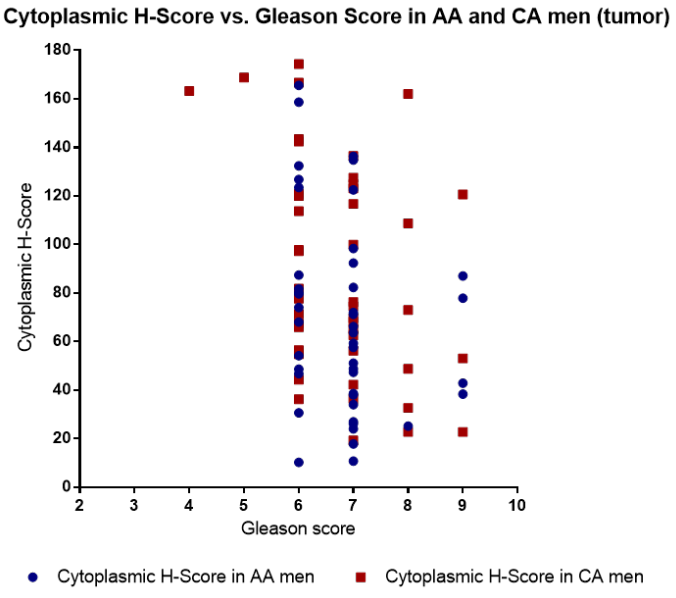


Figure 5: The cytoplasmic H-score for AhR staining increased in tumor (AA68/CA72) compared to matched normal samples (AA56/CA88). However, the nuclear H-score in AA normal (97) exceed the tumor H-score of 68).

Subtask 2: Compare AhR activity in low, moderate and high Gleason score prostate cancer tissue samples.





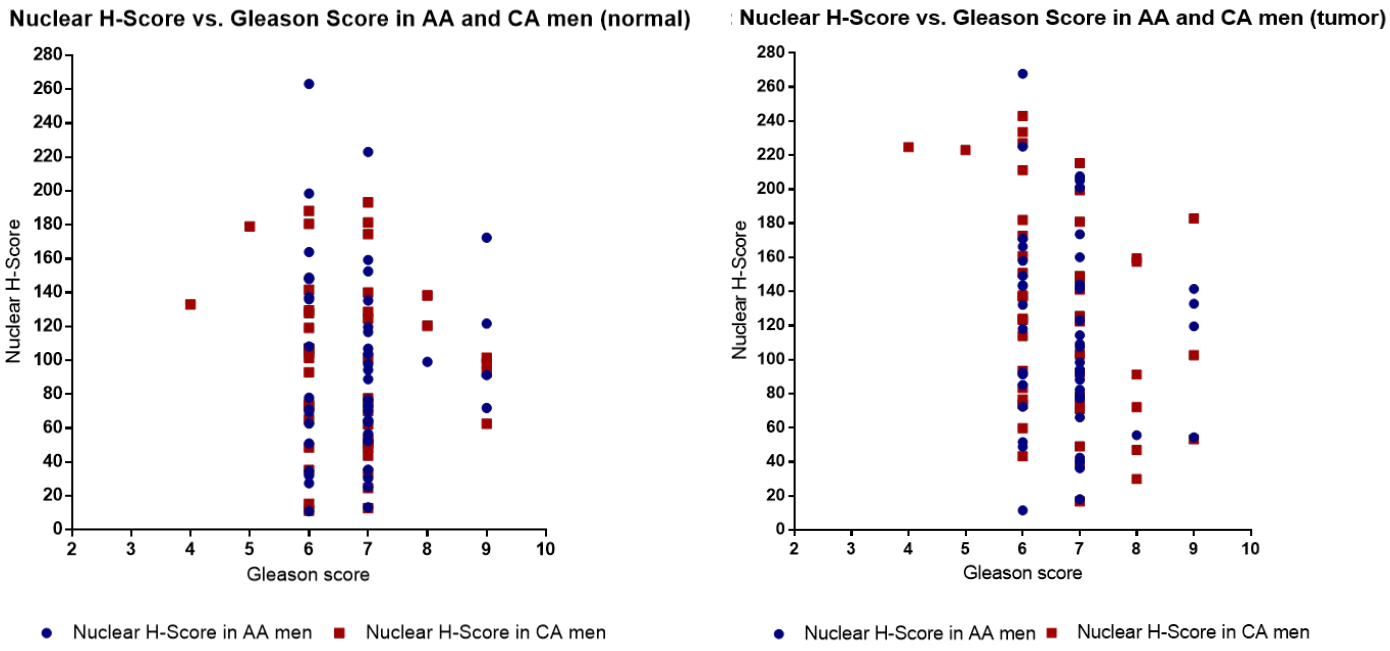
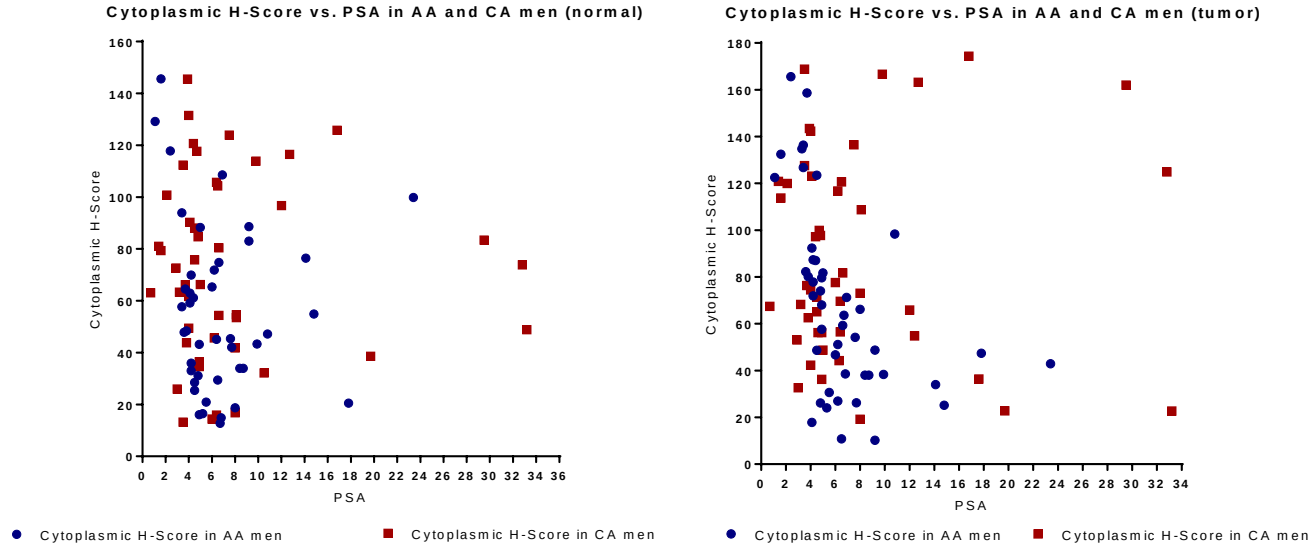


Figure 6: There was no observed correlation between the cytoplasmic or nuclear H-score and the Gleason Score in AA or CA tissues.

Subtask 3: Correlate AhR activity in clinical prostate cancer tissue to clinical indicators of progression (PSA).



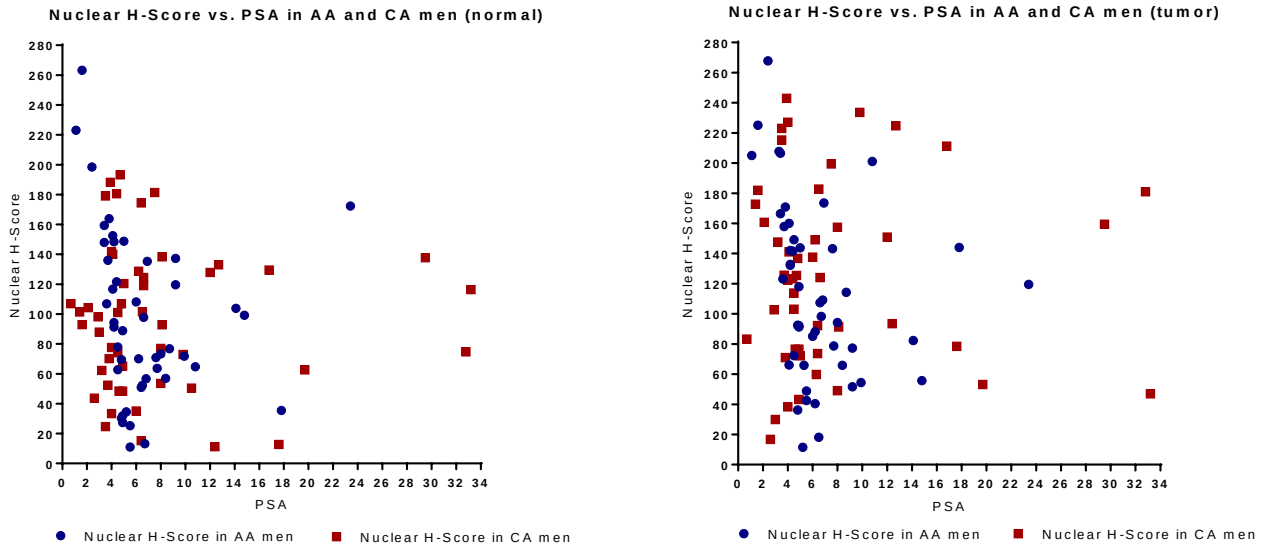


Figure 7: There was no observed correlation between the cytoplasmic or nuclear H-score and the PSA in AA or CA tissues.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

The PI (Joann Powell) and research technician attended the AACR Annual Meeting in April 2019. The PI also attended the 33rd BEYA STEM Global Competitiveness Conference in February 2019 and the AhR 2018 Symposium in August 2018.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

A research poster was presented at the AACR Annual meeting and oral presentations were given at the 33rd BEYA STEM Conference as well as the AhR symposium. A review article was also published: Ghotbaddini M, Moultrie V, and Powell JB. Constitutive Aryl Hydrocarbon Receptor Signaling in Prostate Cancer Progression. Journal of Cancer Treatment and Diagnosis. Volume 2 Issue 5 (2018). http://www.cancertreatmentjournal.com/articles/constitutive-aryl-hydrocarbon-receptor-signaling-in-prostate-cancer-progression.html

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

In order to obtain the additional tissue arrays we originally planned to use in the immunohistochemical studies, Dr. Angel DeMarco of the PCBN has requested that we complete an antibody validation based on a paper he has written. Although we have provided ample data validating the specificity of our antibody, he is requesting this additional experiment before approving our request for the tissue microarrays (see appendices for further information.)

We will also complete Specific Aim 3 which is to evaluate the effects of modified AhR expression on in vivo tumor progression. Please see the appendices and "*actual or anticipated problems or delays and actions or plans*" for further information.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies),*

Nothing to report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

To date, we have NOT received approval of our application for access to PCBN tissue microarrays. Despite submitting the application and ample data to validate antibody specificity, the director is requesting further experiments to validate the specificity of the antibody. Please see appendices for the application and data provided to PCBN. While awaiting approval and optimizing the protocol for making formalin-fixed paraffin-embedded (FFPE) cell plugs, we moved forward with staining of tissue samples from our in house biorepository and collaborators from Fox Chase Cancer Center. Although cell line controls are NOT accepted as an useful experimental approach to analytically validate immunohistochemical staining, the director of PCBN will not approve our application without this data. We will continue our efforts to produce this data and obtain the microarray slides.

We also have had continued problems recreating the E006HT clones which are needed to move forward with in vivo studies. We have eradicated the mycoplasma contamination by having the biological safety cabinet (Labconco Purifier Logic Biological Safety Cabinet) recertified. We are currently expanding the clones and hope to have completed validation within the next 3-6 months. Once the newly created clones are fully validated, we will submit IACUC documents, obtain ACURO approval and move forward with the planned *in vivo* studies. Please see action plan below and appendices for plans to resolve the delay in commencing *in vivo* studies.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

In order to complete the proposed *in vivo* studies within the timeframe of the grant, we propose to complete the studies within the Center for Laboratory Animal Resources (CLAR) Satellite facility located in the Research Center for Science and Technology (RCST) on the campus of Clark Atlanta University. Considering the PI now has access to an animal facility located in the same research building as the PIs research laboratory, we are proposing to amend the *in vivo* training to occur in conjunction with implantation of the clones. Because the PI and research technician will not receive prior training, Dr. Bekir Cinar (Associate Professor in the Center for Cancer Research and Therapeutic Development-CCRTD at Clark Atlanta University) has agreed to provide training and technical support throughout the duration of the *in vivo* studies. Dr. Cinar has vast experience in intracardiac injection of prostate cancer cells. Dr. Cinar and the PI are members of the same research group and can collaborate on a daily basis if necessary. Please see the letter of support and biosketch for Dr. Cinar in appendices.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Ghotbaddini M, Moultrie V, and Powell JB. Constitutive Aryl Hydrocarbon Receptor Signaling in Prostate Cancer Progression. Journal of Cancer Treatment and Diagnosis. Volume 2 Issue 5 (2018). http://www.cancertreatmentjournal.com/articles/constitutive-aryl-hydrocarbon-receptor-signaling-in-prostate-cancer-progression.html

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Maryam Ghotbaddini, Sakura McLaughlin and Joann B. Powell. Constitutive AhR signaling enhances androgen receptor signaling and growth in prostate cancer cells. AACR 2019 Annual Meeting. April 2019. Poster Presentation.

Powell, J. Role of Biomedical Researchers in Cancer Health Disparities Research. 33rd BEYA STEM Global Competitiveness Conference. February 2019. Oral Presentation.

Powell, J. Ectopic Over-Expression of AhR Induces Androgen Independent Signaling In LNCaP Prostate Cancer Cells. AhR 2018 Meeting. August 2018. Oral Presentation.

- **Website(s) or other Internet site(s)**
List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Sakura McLaughlin
Project Role: Research Technician
Nearest person month: 11 months
Contribution of Project: assisted with all aspects of the project. The research associate helped with maintenance of cell lines, performed in vitro studies and assisted the PI with keeping records and notebooks of all experimental data.
Funding Support: W81XWH-17-1-0174

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

PROSTATE CANCER **BIOREPOSITORY NETWORK**

prostate cancer biospecimens for your research

Application for Access to PCBN Tissue Microarrays (TMAs)

Please note that the TMA application will not be accepted without accompanying antibody and IHC assay validation data. This should include full length westerns with positive and negative controls showing one band at correct size and likewise IHC on tissues or cells that are known positive and negative controls. We cannot accept company antibody spec sheets as validation data. All antibody and IHC assay validation must be done "in house."

Instructions to Applicants

This form has five sections. It is compulsory that sections A-C and E are completed in full to avoid delays in review and disbursement of material. Section D (TMA Information) may be completed depending on your application needs. Please elaborate as fully as you can your requirements.

Signing the form indicates that you have familiarized yourself with the PCBN Tissue & Data Access Policy and terms of agreement, and that you agree to be bound by them.

Upon completion, please submit the completed form, along with all necessary attachments (such as a clear and concise analytical validation data for antibodies for IHC assays) to the Prostate Cancer Biorepository Network at query@prostatebiorepository.org. Please include a short summary of your request in the email body text.

SECTION A: Applicant Details		
Principal Investigator (PI) Name Joann B. Powell	PI Telephone 404-880-6794	PI Email jpowell@cau.edu
Institution Clark Atlanta University		Department Center for Cancer Research & Therapeutic Development
Institution Address 223 James P. Brawley Drive Atlanta, GA 30314		Institution Type ☑ Academic/Government ☐ Commercial ☐ Non-profit
Contact Person Sakura McLaughlin	Contact Telephone 404-880-6386	Contact Email smclaughlin@cau.edu
Legal Contact Person (MTA purposes) Travis Patton	Legal Contact Telephone (MTA purposes) 404-880-6985	Legal Contact Email (MTA purposes) tpatton2@cau.edu

SECTION B: Billing Information		
Billing Contact Person Tamika Austin	Billing Contact Telephone 404-880-8414	Billing Contact Email taustin@cau.edu
Billing Address 223 James P. Brawley Drive Atlanta, GA 30314	Same as Institution Address	Payment Details ☑ Purchase ☐ Order Credit ☐ Card Other
Funding Source DOD-PCRP	Grant ID# W81XWH-17-1-0274	Grant End Date 06/30/2020

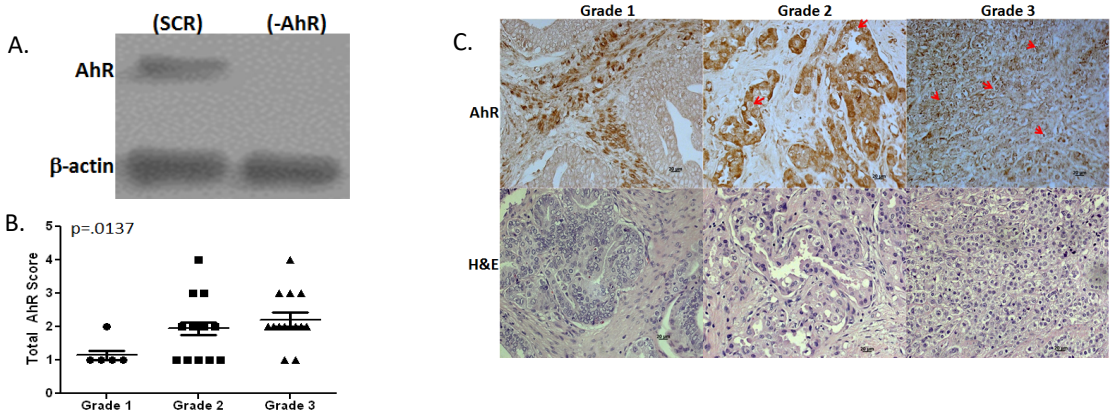
SECTION C: Project Information	
Project Title	Continuous AhR Activity Accelerates Prostate Cancer Progression in African American Men
Hypothesis	The objective of this proposal is to compare the level and effect of AhR activity in African-American and Caucasian-American prostate cancer cells and tissues. We hypothesize that constitutive AhR signaling is responsible for the sustained androgen receptor signaling seen in castration resistant prostate cancer (CRPC) and that AA men have elevated AhR activity compared to Caucasian men.

Specific Aims

Specific Aims: The following specific aims are designed to assess AhR signaling in prostate cancer cells and tissue from AA men and to determine if ablation of AhR signaling is possible therapeutic option.
Specific Aim 1: Compare effect of AhR activity in AA and Caucasian-American (CA) prostate cancer cell lines.
Specific Aim 2: Determine if AhR is universally upregulated in AA prostate cancer tissue
Specific Aim 3: Evaluate the effects of modified AhR activity on in vivo tumor progression.

Protocol / Method to be used (for IHC assays, this section MUST include antibody validation data for specificity including by IHC). Append additional files as needed.

Detailed Methods: Prostate carcinoma tissue microarray will be analyzed by immunohistochemistry (IHC) for expression of AhR. Briefly, tissue arrays will be dewaxed with xylene, rehydrated in graded concentrations of alcohol and treated with hydrogen peroxide prior to blocking with normal goat serum. The slide will be incubated with AhR antibody. The antibody titer, concentration and incubation time will be determined using corresponding optimization slides. Antibody binding will be detected using a labeled streptabidin-biotin kit with 3'3-diaminobenzidine as the chromogen (DAKO). Hematoxylin will be used as a counterstain. These experiments will be performed in the histology core at Clark Atlanta University which is equipped with an automated slide staining and hybridization station and led by Dr. Peri Nagappan, a research associate with over 20 years' experience in staining methods. The statistical significance will be calculated by our core statistician, Dr. Fisseha Abebe, using non-parametric Kruskal-Wallis one-way ANOVA for multiple comparisons followed by post-hoc Dunns's test for paired comparisons (core facilities support letter from Dr. Jin Zou).



Name of Pathologist associated with the study (If TMA or tissue slides require microscopic examination)

Results of staining will be scored by two independent observations rating staining intensity and percentage. The first observation will be completed using the Aperio ImageScope analysis software from Leica Biosystems and the second observation will be completed by Dr. Gabriella Oprea, pathologist and associate professor at Emory University School of Medicine.

IRB Approval Type	IRB Approval Number	IRB Approval Dates
☐ Full ☑ Expedited ☐ Exempt	HR2017-7-742-1/AR2	10/08/18-07/02/19

Section D: TMA Information		
TMA	# Sections	Justification for # Sections per Array Requested
8 Case Test ☐		
40 Case Screening ☐		
80 Case Grade/Stage ☐		
200 Case Grade/Stage ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.
320 Case Enrichment ☐		
235 Case Natural History of Prostate Cancer * ☐		
10 Case Test PSA Progression* ☐		
726 Case PSA Progression* ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.
150 Case Race Disparity* ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.
114 Case Race Disparity ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.
120 Case Race Disparity* ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.
56 Case Hormone Sensitivity ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.

217 Case Biochemical Recurrance ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.
50 Case Benign Prostatic Hyperplasia ☐		
119 Case High-Grade PIN ☐		
Fixation ☐		
Ischemia/Fixation Delay ☐		
27 Case Lymph Node Mets* ☐		
52 Case Lymph Node Mets* ☐		
900 Case Radical Prostatectomy* ☐		
136 Case Zone of Origin* ☐		
140 Case Genomic* ☐		
45 Case Bone and Visceral Metastasis from Rapid Autopsy* ☑	2	H&E and IHC with AhR
20 Case Bone and Visceral Metastasis from Rapid Autopsy* ☑	2	We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody.

42 LuCaP PDX Models* ☐		
15 Case Metastasis from Rapid Autopsy * ☐		
135 Case Grade/Stage Radical Prostatectomy ☑		We require a section for H&E histology and a section for immunohistochemical staining with AhR antibody

*Due to the level of effort and source of funding, access to these materials may require collaboration

Section E: Shipping Information		
Shipping Address	☑ Same as Institution Address	**Submit**

We/I have read, understood and agree with the Tissue Access Policy and Conditions of Use for Tissue and Data Bank Resources. We/I agree that the samples provided by PCBN will be used for the research work detailed in the attached proposal. The material will not be used for other studies, or distributed to third parties. Tissue and their products will not be used for commercial purposes. We/I realize that there is the potential that this human biological material may contain infectious agents and, therefore, will handle it appropriately.

Principal Investigator Signature	Date
	10/15/2018

Lane	Cell line
1	PREC
2	LNCaP
3	C4-2
4	C4-2 (-AhR Clone 1)
5	C4-2 (-AhR Clone 2)
6	DU145
7	DU145 (-AhR Clone 1)
8	DU145 (-AhR Clone 2)
9	DU145 (-AhR Clone 3)

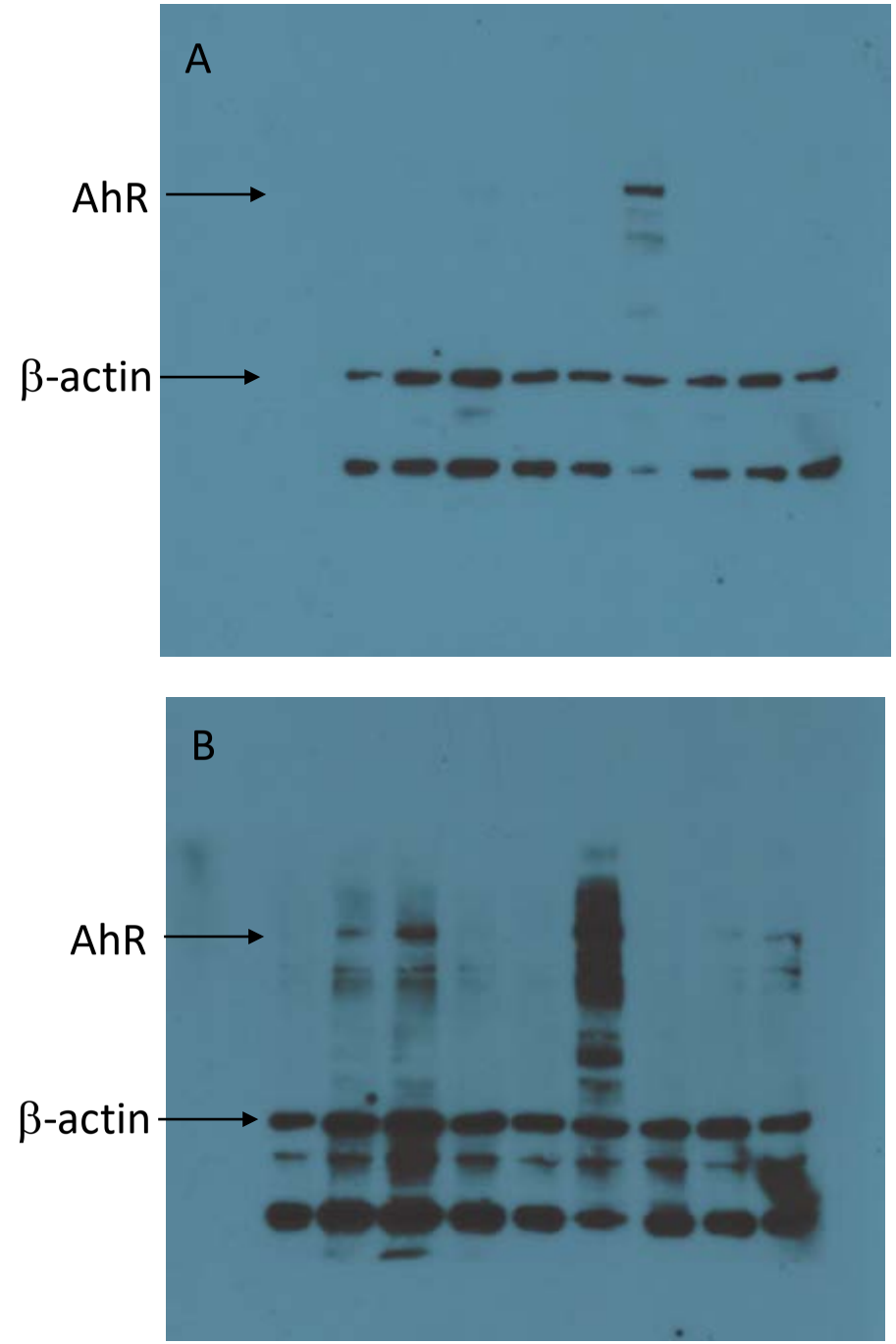
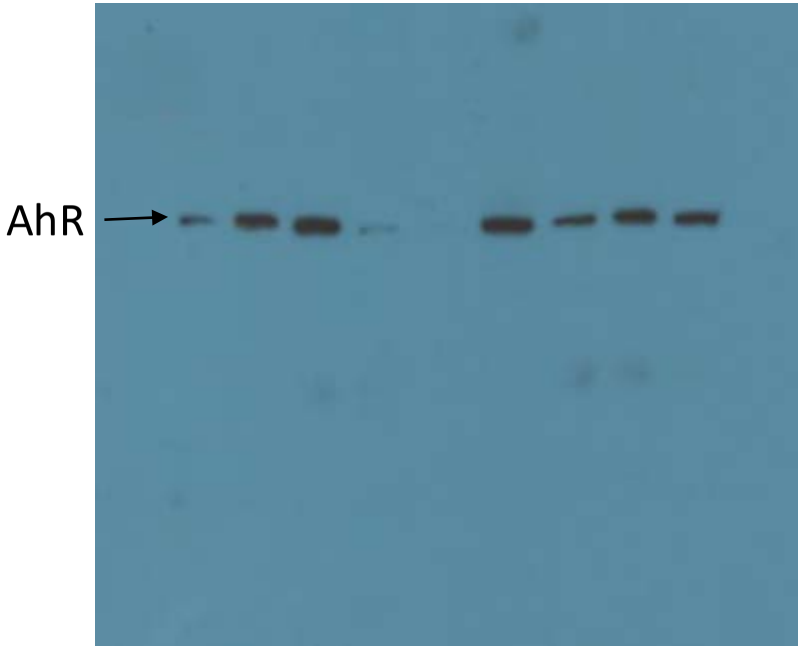


Figure legend: Protein samples were isolated using the Thermo Scientific NEPER Extraction kit for cellular fractions or commercially available cell lysis buffer (Cell Signaling) for total protein. Protein samples were resolved by SDS-PAGE and transferred to a PVDF membrane. Immunoblotting was carried out with 1 mg/ml mouse AhR monoclonal antibody at 1:1000 dilution in 5% milk in combination with β-action monocolonal antibody at 1:2500. Blots were washed three times (15 min each) with TBST. The blots were then incubated in 1:2500 dilution of secondary antibody and washed three times (15 min each) with TBST, three times (10 min each) with TBS and once with ddH20 (10 min). Bands were visualized with the enhanced chemiluminescence (ECL) kit as specified by the manufacturer. Multiple exposures of each set of samples were produced. Figure “A” represents a 5 minutes exposure and Fig “B” represents a 30 minute exposure.

Due to the non-specific binding that occurs with the β-actin antibody (seen on previous blot), we are also including a blot probed only with the AhR monoclonal antibody to demonstrate the specificity of the antibody in clones that over-express AhR. As you can see our antibody results in one single band for AhR.

Lane	Cell line
1	LNCaP
2	LNCaP Over-expression Clone 1
3	LNCaP Over-expression Clone 2
4	LNCaP Over-expression Clone 3
5	LNCaP Over-expression Clone 4
6	LNCaP Over-expression Clone 5
7	LNCaP Over-expression Clone 6
8	LNCaP Over-expression Clone 7
9	LNCaP Over-expression Clone 8



AhR was stably overexpressed in LNCaP cells using AhR retroviral vector. We used G418 sulfate for selection of AhR transfected cells. Protein samples were resolved by SDS-PAGE and transferred to a PVDF membrane. Immunoblotting was carried out with 1 mg/ml mouse AhR monoclonal antibody at 1:1000 dilution in 5% milk.

Immunocytochemistry confirmed the presence of AhR in the nucleus of DU145, PC3 and PC3M cells. AhR was also detected in the nucleus by western blotting following cellular fractionation. The absence of any staining overlay in the merged image of the DAPI stained nuclei and FITC-stained AhR further demonstrated that LNCaP cells do not possess nuclear AhR. This further confirms that specificity of the AhR primary antibody.

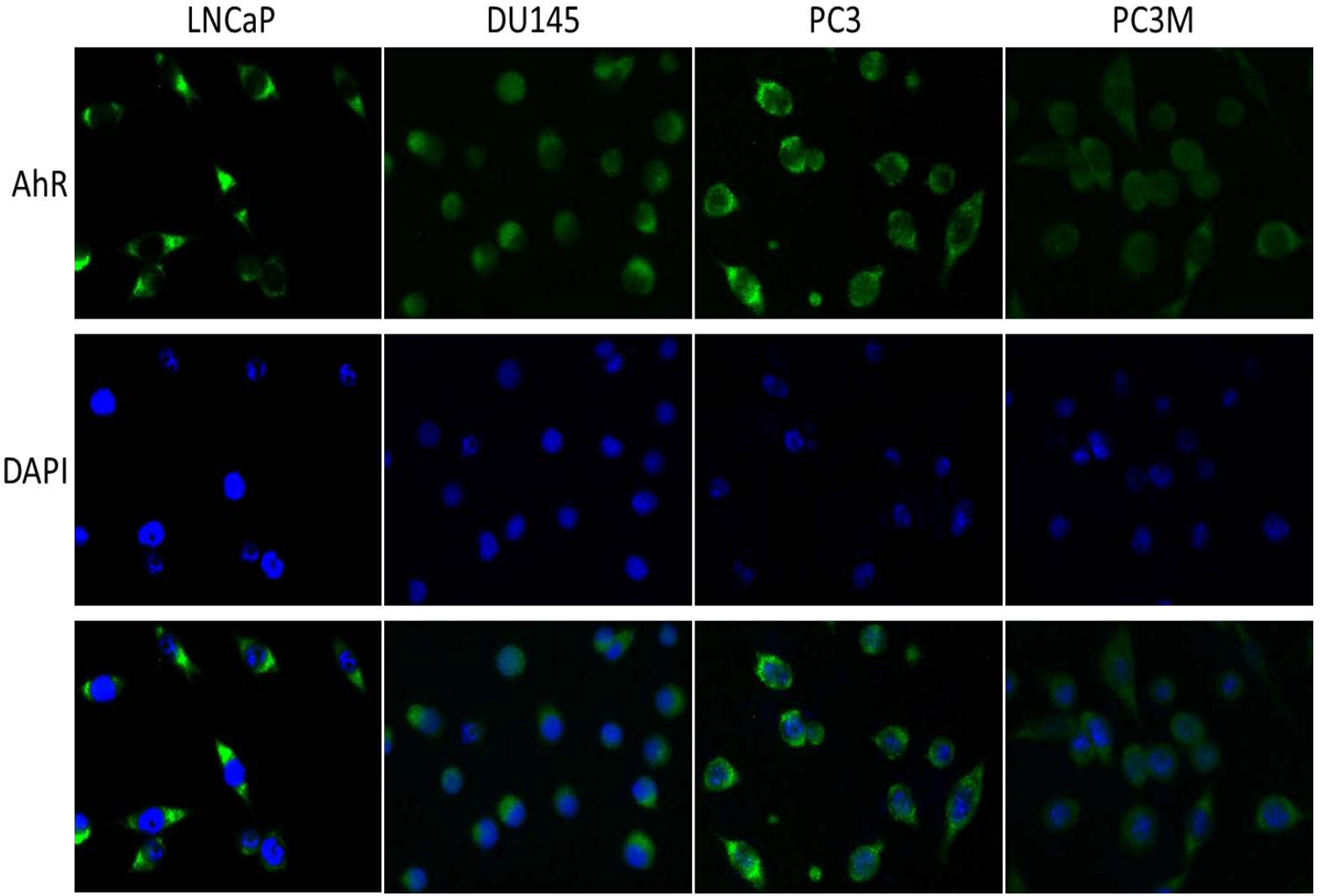


Figure Legend: Subcellular localization of AhR in prostate cancer cell lines by immunocytochemical staining: Cells were grown on coverslips and fixed with methanol:acetone. AhR was visualized by staining with rabbit anti-AhR polyclonal antibodies followed by FITC-conjugated goat anti-rabbit antibody. The nuclei were counter stained with DAPI fluorescence dye. Images from FITC and DAPI fluorescence channels were merged. Images were captured on an Olympus wide fluorescence microscope (400x magnification).

Immunohistochemical (IHC) staining confirmed previous reports that both AhR and AR are located in the cytoplasm and nucleus of C4-2 cells. TCDD induced AhR nuclear localization in LNCaP cells and further enhanced AhR nuclear localization in C4-2 cells. Cells were treated with 1 µM TCDD to preclude protein degradation that was previously induced with 10 µM treatment.

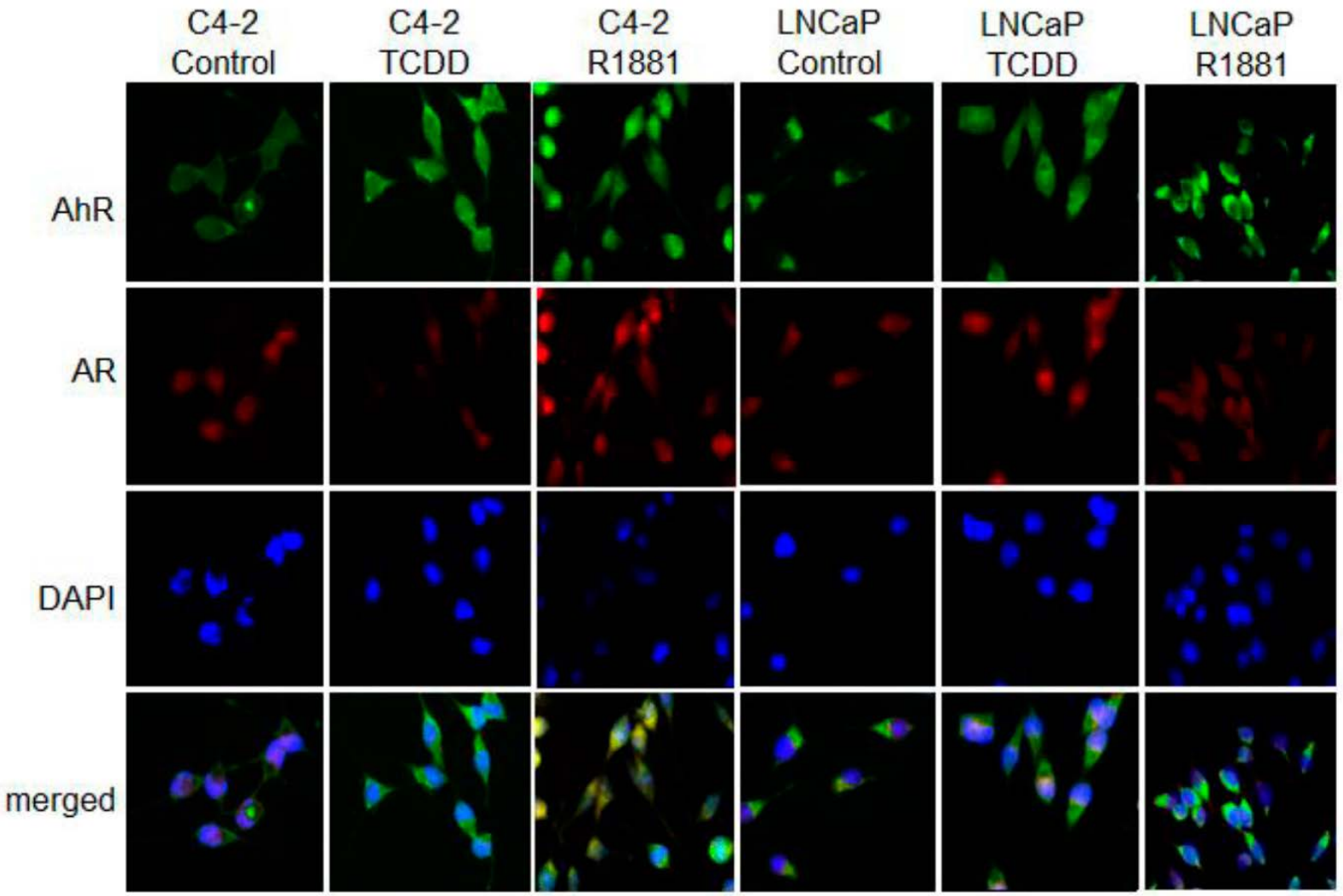


Figure Legend: IHC staining of LNCaP and C4-2 prostate cancer cells. Cells were exposed to 1 µM TCDD or 10 nM R1881 for 24 h. AhR was visualized by staining with FITC-conjugated goat anti-rabbit antibody and AR with Rhodamine-conjugated rabbit anti-mouse antibody. The nuclei were counter-stained with DAPI fluorescence dye. Images from FITC, Rhodamine and DAPI-fluorescence channels were merged

Clark Atlanta University

Department of Biological Sciences
Center for Cancer Research and Therapeutic Development
Bekir Cinar, Ph.D.
Tel: (404) 880-8438 | Email: bcinar@cau.edu

Joann Powell, PhD
Associate Professor
The Center for Cancer Research and Therapeutic Development
Clark Atlanta University
Atlanta, GA 30314

May 1, 2019

RE: DoD-PCRP (Continuous AhR Activity Accelerates Prostate Cancer Progression in African American Men)

Dear Joann,

I would like to express my enthusiastic support of your research project entitled, ***"Continuous AhR Activity Accelerates Prostate Cancer Progression in African American Men"*** from the Department of Defense Prostate Cancer Program. As you know, my laboratory studies the mechanism of prostate cancer progression and metastasis as well as therapeutic relapse in cell and animal models. My laboratory routinely performs tumorigenesis and metastasis assays using imaging in mouse model *(Kuser-Abali et al, Nat Commun. 2015 Sep 1;6:8126. doi: 10.1038/ncomms9126; Cinar et al. July 2015 Cancer Research 75(15 Supplement):1956-1956; DOI:10.1158/1538-7445.AM2015-1956).*

Based on review of your protocol, I believe that this research project is both significant and feasible. I am happy to provide research support and technical expertise for the successful completion of your *in vivo* experiments to evaluate the effects of modified AhR expression on in vivo tumor progression and metastasis. Your work evaluating the role of AhR in prostate cancer health disparities is an important topic in prostate cancer biology. Importantly, the results of this study may increase our knowledge on how aggressive prostate cancer evolves in men of African genetic ancestry and contribute to reduction of disparities in prostate cancer mortality. I look forward to working with you on this collaboration.

Sincerely yours

Bekir Cinar, Ph.D.
Associate Professor
Graduate Program Coordinator
Department of Biological Sciences
Clark Atlanta University

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: CINAR, BEKIR

eRA COMMONS USER NAME (credential, e.g., agency login): CINARB

POSITION TITLE: ASSOCIATE PROFESSOR

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Ankara University, Ankara	DVM	06/1992	Veterinary Medicine
The University of Virginia School of Medicine, Charlottesville, VA	PhD	08/2002	Biochemistry and Molecular Genetics
Boston Children's Hospital, Harvard Medical School, Boston, MA	Postdoctoral Fellow	04/2006	Cancer Biology and Urologic Oncology

A. Personal Statement

I lend this proposal to study evaluating the role of AhR in prostate cancer health disparities preclinical animal models. I **have** the expertise, leadership, motivation, and necessary skills to successfully carry out the proposed research. I am an Associate Professor (tenure tract) in Biological Sciences (DBS) and a core member of the Center for Cancer Research and Therapeutic Development (CCRTD) at Clark Atlanta University (CAU). Also, I am an associate member of Emory Winship Cancer Institute (EWCI) that is 20-30 min away from CAU campus. I have beel stitying the mechanism of prostate cancer progression and metastasis as well as therapeutic relapse in cell and precilinical animal models. I **studied** crosstalk between androgen receptor (AR) and NFκB signaling (**1**) as well as **studied** the molecular and functional interactions between tumor suppressor STK4/MST1/Hippo and oncogenic AKT signaling in prostate cancer (**2**). I have **demonstrated** that the STK4/Hippo-YAP pathway plays a critical role in metastatic castration-resistant prostate cancer (CRPC). I have **identified** STK4/MST1 as a potent negative regulator of androgenic signaling and suppressor of prostate cancer (**3**). I have **demonstrated** that the interaction of nuclear YAP with AR contributes to metastatic CRPC (**4**). Our published study has provided a rationale for to investigate the novel role of dopamine signaling in the emergence of aggressive prostate cancer (**5**). In addition, as a principal investigator (PI), I have **obtained** fellowship grants from private foundations and have recently **received** Excellence in Research Award from National Science Foundation. Also, I have submitted investigator initiated several R01 proposals to the NIH/NCI and Idea Development Award proposals to the DoD PCRP. As a collaboraotor, I will play a critical role in this project and will provide research support and technical expertise for the successful completion of proosed *in vivo* experiments in mouse model.

1. **Cinar B**, Yeung F, Mayo MW, Freeman MR, Zhau HYE, Chung LWK. Identification of a negative regulatory cis-element in the enhancer core region of the prostate specific antigen (PSA) promoter: Implications for intersection of androgen receptor and nuclear factor-κB signaling in prostate cancer cells, **Biochem J.** 2004, 379:421-31. PMID: 14715080 PMCID: PMC1224078
2. **Cinar B,** Fang PK, Lutchman M, Di Vizio D, Adam RM, Pavlova N, Rubin MA, Yelick PC, Freeman MR. The pro-apoptotic kinase Mst1 and its caspase cleavage products are direct inhibitors of Akt1. **EMBO J**. 2007, 26(21):4523-34. PMID: 17932490 PMCID: PMC2063482.
3. **Cinar B,** Collak FK, Lopez D, Akgul S, Mukhopadhyay NK, Kilicarslan M, Gioeli DG, Freeman MR. MST1 is a multifunctional caspase-independent inhibitor of androgenic signaling. **Cancer Res,** 2011, 71(12):4303-13. PMID: 21512132 PMCID: PMC3117069.
4. Kuser-Abali G, Alptekin A, Lewis M, Garraway I, **Cinar B.** YAP and AR interactions contribute to the switch from androgen-dependent to castration-resistant growth in prostate cancer, **Nat Commun**, doi:10.1038/ncomms9126, Sep 1, 2015. PMID: 28230103 PMCID: PMC5327734.

5. Mohanty, SK., Yagiz, K., Pradhan, D., Luthringer, DJ., Amin, MB., Alkan, A. and **Cinar, B***. STAT3 and STAT5A are potential therapeutic targets in castration-resistant prostate cancer. **Oncotarget,** 2017 8(49):85997-86010. PMID: 29156772 PMCID: PMC5689662

B. Positions and Honors

Positions and Employment

1995-2002	Research Assistant, University of Virginia, School of Medicine, Charlottesville, VA
2002-2006	Postdoctoral Fellow, Boston Children's Hospital, Harvard Medical School, Boston, MA
2006-2009	Instructor-Faculty, Department of Surgery, Harvard Medical School, Boston, MA
2009-2015	Research Scientist I-Faculty, Samuel Ochin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA
2010-2015	Assistant Professor, Medicine and Biomedical Sciences, Cedars-Sinai Medical Center, CA
2015-present	Associate Professor of Biological Sciences, Clark Atlanta University, Atlanta, GA

Honors

1993-1999	Graduate Scholar Award Abroad, The Higher Educational Council, Ankara, Turkey
1999-2002	Graduate Fellowship, University of Virginia school of Medicine
2006	Postdoctoral Fellowship Recognition Award, AUA Foundation
2006	AACR Scholar-in-Training Award, AACR Foundation
2006	Outstanding Graduate Scholar Award, AUA Foundation
2006	Travel Award, Society for Basic Urologic Research (SBUR)
2006	Travel Grant, ENDO 2006 Meeting, The Endocrine Society
2008	Employee Service Award, Boston Children's Hospital
2008-present	Associate Professor in Biochemistry, The Higher Education Council of Turkey
2017-present	Honorary Member, UVA Medical Alumni Association

Other Experiences and Professional Memberships

Manuscript reviewer-

The British Journal of Pharmacology, The International Journal of Biochemistry and Cell Biology, PLoS One, Frontiers in Oncology, Molecular Cancer Therapeutics, International Journal of Medical Science, Current Cancer Drug Targets, Drug Discovery Letter, The Journal of Biological Chemistry, Nature Communications, Epigenetics, Genome Medicine, and Oncotarget, Cancer Biomarkers, Cellular Physiology and Biochemistry Future Medicine, Biopharma and Biochemistry, Toxicological Sciences, and The Journal of Clinical Investiation.

Grant Reviewer-

2011-2012	*Review Panel Member, DoD-FY11-PCRP*
2012	*External Reviewer, DoD-FY12-PCRP*
2013	*External Reviewer, DoD-FY13-PCRP*
2015	*External Reviewer, Horizon 2020 European Union Research Consortium*
2015	*External Reviewer, Prostate Cancer United Kingdom (PCUK) Research Program*
2017-2018	*Review Panel Member, DoD-FY17-PCRP*
2017	*External Reviewer, FY15-PCUK Research Program*
2018	*External Reviewer, UT San Antonio Cancer Research Program*
2018	*External Reviewer, Austrian Science Foundation*
2018-2019	*Review Panel Member, National Science Foundation-MCB*

Membership-

2003-present	*Active Member, American Association for Cancer Research (AACR)*
2003-present	*Active Member, Society for Basic Urologic Research (SBUR)*
2012-2015	*Active Member, American Society for Biochemistry and Molecular Biology (ASBMB)*
2003-2009	*Student Member, The Endocrine Society*
2006-present	*Active Member, Turkish American Scientist and Scholars Association (TASSA)*
2014-2015	*Active Member, American Society of Clinical Oncology (ASCO)*

2015-present Active Member, The Center for Cancer Research and Therapeutic Development (CCRTD)
2016-present Active Member, Winship Cancer Institute of Emory University
2018-present Active Member, American Society of Cell Biology (ASCB)

C. Contributions to Science

The Hippo pathway controls organ size and tumorigenesis by restricting cell proliferation and contributing cell death. The STK4-encoded MST1 protein kinase is a key component of the Hippo pathway in mammalian. Using proteomics approach, I originally identified STK4/MST1 as a binding partner of AKT protein complexes isolated from lipid rafts of PC cells. I demonstrated that MST1 functioned as a potent inhibitor of AKT signaling in vitro and in vivo. Also, I showed that MST1 protein levels were progressively declined during prostate tumor progression to the castration-resistant state, which coincided with increases in AKT activity (**a**). As an independent investigator, I identified MST1 as a potent negative regulator of androgenic signaling and suppressor of PC cell growth (**b**). In addition, I demonstrated that interaction of YAP with AR might play a critical role in CRPC and identified the FDA approved drug, Verteporfin, as a potent inhibitor of YAP-AR interaction and suppressor of CRPC growth (**c**). Furthermore, a collaborative study from my laboratory has revealed that STK4/MST1 may restrict aggressive tumor growth by modulating the activity of several molecular pathways that control development, oncogenesis and cellular metabolisms (**d**).

a. **Cinar B**, Fang PK, Lutchman M, Di Vizio D, Adam RM, Pavlova N, Rubin MA, Yelick PC, Freeman MR. The pro-apoptotic kinase Mst1 and its caspase cleavage products are direct inhibitors of Akt1. **EMBO J**, 2007, 26(21):4523-34. PMID: 17932490 PMCID: PMC2063482.
b. **Cinar B**, Collak FK, Lopez D, Akgul S, Mukhopadhyay NK, Kilicarslan M, Gioeli DG, Freeman MR. MST1 is a multifunctional caspase-independent inhibitor of androgenic signaling. **Cancer Res**, 2011, 71(12):4303-13. PMID: 21512132 PMCID: PMC3117069.
c. Kuser-Abali G, Alptekin A, Lewis M, Garraway I, **Cinar B.** YAP and AR interactions contribute to the switch from androgen-dependent to castration-resistant growth in prostate cancer. **Nature Commun**, doi:10.1038/ncomms9126, Sep 1, 2015. PMID: 28230103 PMCID: PMC5327734
d. Ready D, Yagiz K, Amin P, Yildiz Y, Funari V, Bozdag S, **Cinar B**. Mapping the STK4/Hippo signaling network in prostate cancer cell. **PLoS One,** 2017, 12(9):e0184590. PMID: 28880957 PMCID: PMC5589252

The growth factor PI3K-AKT-mTOR signaling pathway plays a critical role in cancer cell survival and is associated with poor cancer prognosis including PC. My laboratory showed that AKT interacted with and phosphorylated MST1 on Thr120 residue, thereby attenuating the growth suppressive functions of STK4/MST1 (**a**). In addition, my laboratory demonstrated that MYC in concert with EZH2 silenced MST1 through DNA methylation and histone modification during PC progression to the metastatic, castration-resistant state (**b**). Moreover, in a collaborative study, we reported that scaffold attachment factor B1 regulates the AR in concert with the growth inhibitory STK4/MST1 kinase and the methyltransferase EZH2 (**c**).

a. Collak FK, Yagiz K, Luthringer DJ, Erkaya B, **Cinar B**. Threonine-120 phosphorylation regulated by phosphoinositide-3-kinase/Akt and mammalian target of rapamycin pathway signaling limits the antitumor activity of mammalian sterile 20-like kinase 1. **J Biol Chem**, 2012, 287(28):23698-709. PMID: 22619175 PMCID: PMC3390644.
b. Kuser-Abali G, Alptekin A, **Cinar B**. Overexpression of MYC and EZH2 cooperates to epigenetically silence MST1 expression. **Epigenetics,** 2014, 9(4):634-43. PMID: 24499724; PMCID: PMC4121373.
c. Mukhopadhyay, N.K., Kim, J., You, S., Morello, M., Hager, M.H., Huang, W.C., Ramachandran, A., Yang, J., **Cinar, B**., Adam, R.A., Oesterreich, S., Di Vizio, D., Freeman, F.R. Scaffold Attachment Factor B1 Regulates the Androgen Receptor in Concert with the Growth Inhibitory Kinase MST1 and the Methyltransferase EZH2. **Oncogene**, 2015, 33(25):3235-45. PMID: 23893242 PMCID: PMC3934948

Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is an ErbB1 ligand and prostate stromal growth factor and AR protein expression. I discovered that AR protein levels were highly sensitive to regulation by cap-dependent mRNA translation in the downstream of HB-EGF-activated PI3K-AKT-mTOR signaling (**a**). In addition, my collaborative study identified heterogeneous nuclear ribonucleoprotein K (hRNPK) as a novel regulator of AR mRNA translation in PC cells (**b**). Moreover, TOK-001 and abiraterone are potent 17-heteroarylsteroid (17-HAS) inhibitors of Cyp17, one of the rate-limiting enzymes in the biosynthesis of

testosterone from cholesterol in prostate cancer cells. We found that TOK-001 suppressed AR protein levels by possibly modulating RNA translation without effecting its mRNA expression (**c**).

a. **Cinar B**, de Benedetti A, Freeman MR. Post-translational regulation of the androgen receptor by the mammalian target of rapamycin. **Cancer Res**. 2005, 65:2547-53. PMID: 15805247
b. Mukhopadhyay, N.K., Kim, J., **Cinar, B**., Ramachandran, A., Hager, M, Adam, R.M., Raychaudhuri, P., De Benedetti, A, Freeman, M.R., Heterogeneous nuclear ribonucleoprotein K is a novel regulator of androgen receptor translation. **Cancer Res**, 2009, 69:2210-18. PMID: 19258514 PMCID: PMC2659763
c. Soifer H, Souleimanian N, Wang, S, Voskresenskiy, A, Collak FK, **Cinar B**, and Stein CA**.** Direct regulation of androgen receptor activity by potent CYP17 inhibitors in prostate cancer cells. **J Biol Chem**, 2012, 287:3777-87. PMID: 22174412 PMCID: PMC3281677

Evidence suggests that cellular signaling transiting through cholesterol-rich, lipid raft microdomains provides survival. AKT1 is an important mediator of growth, survival, and metabolic signaling (**a**). I studied crosstalk between AR and AKT signaling in lipid raft in PC cells and demonstrated that signals channeled through AR and AKT1 intersect within lipid raft microdomains (**b**). These signal-transduction processes occur within seconds to minutes after initiation with an agonist, suggesting the non-genomic functions of AR. This observation was consistent with the literatures that nuclear receptors can signal by a nongenomic mechanism that operates independently of their transcriptional functions. In addition, we reported that a myristoylated AKT (MyrAKT1), which is the oncogenic AKT1 form, was found to be highly enriched in lipid rafts. Notably, lipid raft-resident MyrAKT1 exhibited a markedly distinct substrate preference compared with MyrAKT immunoprecipitated from cytosol and nonraft membrane fractions (**c**).

a. Freeman MR1, **Cinar B**, Kim J, Mukhopadhyay NK, Di Vizio D, Adam RM, Solomon KR. Transit of hormonal and EGF receptor-dependent signals through cholesterol-rich membranes. **Steroids**, 2007, 72(2):210-7. PMID: 17173942 PMCID: PMC2709209
b. **Cinar B**, Mukhopadhyay NK, Meng G, Freeman MR. Phosphoinositide 3-kinase-independent non-genomic signals transit from the androgen receptor to Akt1 in membrane raft microdomains. **J Biol Chem**, 2007, 282(40):29584-93. PMID: 17635910
c. Adam RM1, Mukhopadhyay NK, Kim J, Di Vizio D, **Cinar B**, Boucher K, Solomon KR, Freeman MR. Cholesterol sensitivity of endogenous and myristoylated Akt. **Cancer Res**, 2007, 67(13):6238-46. PMID: 17616681

The Nuclear factor-kappa B (NFκB) signaling pathway plays a critical role in many aspects of cellular biology including cancer. I investigated crosstalk between AR and NFκB signaling and demonstrated that ectopic expression of AR in metastatic PC cells changed growth behaviors and androgen responsiveness possibly through select gene expression (**a**). In addition, I identified a negative regulatory cis-element (named as XBE) in the enhancer region of the prostate-specific antigen (PSA) gene promoter. I demonstrated that XBE was bound and antagonistically regulated by AR and NFκB transcription factors (**b**). Furthermore, our collaborative study identified NFκB/c-Rel as a negative regulator of AR activity in PC (**c**).

a. **Cinar, B.**, Koeneman KS, Edlund M, Prins GS, Zhau HE, Chung LWK. Androgen receptor mediates the reduced tumor growth, enhanced androgen responsiveness, and selected target gene transactivation in a human prostate cancer cell line. **Cancer Res.** 2001, 61:7310-17. PubMed PMID: 11585771
b. **Cinar B**, Yeung F, Mayo MW, Freeman MR, Zhau HYE, Chung LWK. Identification of a negative regulatory cis-element in the enhancer core region of the prostate specific antigen (PSA) promoter: Implications for intersection of androgen receptor and nuclear factor-κB signaling in prostate cancer cells. **Biochem J,** 2004, 379:421-31. PMID: 14715080 PMCID: PMC1224078
c. Mukhopadhyay NK, Ferdinand AS, Mukhopadhyay L, **Cinar B**, Lutchman M, Richie JP, Freeman MR, Liu BC. Unraveling androgen receptor interactomes by an array-based method: discovery of proto-oncoprotein c-Rel as a negative regulator of androgen receptor. **Exp Cell Res**, 2006, 312(19):3782-95. PMID: 17011549

Complete List of Published Work in MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/45599139/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

I. Current Research Support:

National Science Foundation/MCB

#1832022 Cinar (PI) 09/01/2018-08/31/2021

Title: Excellence in Research Award: Crosstalk between the STK4/Hippo and NF-Kappa B pathway in mammalian cells

Goal: The goal of this project is to uncover the signaling and biochemical mechanisms that mediate STK4/Hippo and NF-Kappa B protein-protein and protein-DNA interactions in mammalian cells.

Overlap: None

II. Pending Application:

2. NSF-OAC #1924063 Wu (PI) 09/01/2019-08/31/2022

Title: CyberTraining: Implementation: Medium: A Collaborative Training Model to Advance CI-competent Research, Education, and Workforce Development

Role: Co-PI, Total cost: $299,991 (Cinar)

Overlap: None

3. National Institute of Health/NIMHD/RCMI (U54)

#408744 Khan (PI) 04/01/2019-03/31/2024

Title: Enhancement of Cancer Research at Clark Atlanta University

Goal: This is a multi-research core proposal and aimed at enhancing cancer research capacity at CAU

Role: Project Lead for the project entitled “The YAP/TAZ-Nuclear Factor Kappa B Axis in Prostate Cancer.”

Total direct cost: $600,000 (Cinar)

Overlap: None

III. Completed Research Support:

Garber Research Scholar Award Cinar (PI) 07/01/2011-12/30/2013

Donna & Jesse Garber Foundation/CSMC

Title: Convergence of MST1 and AR Signaling Regulates Cell Growth in Human Prostate Cancer

Goal: The goal of this project was to investigate the mechanism of how MST1 regulates PC cell growth.

Overlap: None

The New York Academy of Medicine

#R24D00, Cinar (PI) 07/01/2010-06/30/2012

Edwin Beer Fellowship Award

Title: The Intersection of MST1/STK4 and AR Signaling in Prostate Cancer Cells

Goal: The goal was to investigate the antagonism between MST1 and AR signaling in prostate cancer cells.

Overlap: None

The Center for Laboratory Animal Resources (CLAR) manages the animal care and use for all research and teaching projects at Morehouse School of Medicine and all Atlanta University Center (AUC) institutions. The program includes animal health surveillance and veterinary medical care, husbandry of laboratory animals, procurement of all animals, quarantine and stabilization of animals, technical assistance with animal studies, and consultation to the faculty and students. CLAR added a Satellite Animal Facility in the Research Center for Science and Technology (RCST) on the campus of Clark Atlanta University. Therefore, the PI now has access to an animal facility located in the same research building as the research laboratory. The following equipment is available for use in the satellite animal facility:

1) -20°C Freezer (VWR MFV-20)
2) Air Handling Units (2) w/ 2 sets of 60 Individually Ventilated Cages (Tecniplast Smart Flow)
3) Air Handling Units (3) w/ 3 sets of 36 Individually Ventilated Cages (Tecniplast Iso Cage, The Bioexclusive System for Immunocompromised Animals)
4) Biosafety Cabinets (3 Tecniplast Aria)
5) Cage Washer (Tecniplast Oceanus)
6) Carbon Dioxide Euthanization System (Next Advance Quietek)
7) Changing Station (Tecniplast Aria)
8) Deionized Water Purification System (Millipore)