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Abstract:

Recent studies demonstrate that, for men with clinically localized, non-metastatic high-risk prostate cancer receiving long-term androgen deprivation therapy (ADT) and dose-escalated radiotherapy (RT), a pre-RT prostate specific antigen (PSA) value greater than 0.5 ng/ml after ADT predicts for decreased time to distant metastases and a decrease in overall survival. In these studies, AA men were significantly associated with failure to achieve a pre-RT PSA value less than 0.5 ng/ml [2]. 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.

The objective of this proposal is to compare the level and effect of AhR activity in AA and CA prostate cancer cells and tissues. We hypothesize that constitutive AhR signaling is responsible for the sustained androgen receptor signaling seen in CRPC and that AA men have elevated AhR activity compared to CA men. Western blot analysis revealed that AA prostate cancer cell lines have increased AhR expression and nuclear localization compared to the CA counterparts. In addition, inhibition of AhR activity reduces biological properties not affected by inhibition of androgen receptor with antagonist casodex. AhR specific inhibitor decreased the growth rate, migration and invasion of androgen-insensitive prostate cancer cell lines. The level of inhibition achieved correlates to the level of constitutive AhR activity.

Identification of this unknown mechanism for prostate cancer progression will provide a novel therapeutic target to reduce aggressiveness of castration resistant prostate cancer that is not achieved by current therapies and could directly address the health disparity associated with PCa. Previous studies have not considered the existence of constitutive AhR signaling in prostate cancer or its ability to promote prostate cancer progression.

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

The objective of this proposal is to compare the level and effect of AhR activity in AA and CA prostate cancer cells and tissues. We hypothesize that constitutive AhR signaling is responsible for the sustained androgen receptor signaling seen in CRPC and that AA men have elevated AhR activity compared to CA men. Identification of this unknown mechanism of androgen receptor activation will provide a novel therapeutic target to ablate androgen receptor signaling that is not achieved by current therapies and could directly address the health disparity associated with PCa. Previous studies have not considered the existence of constitutive AhR signaling in prostate cancer or its ability to promote prostate cancer progression.

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 1: Compare basal AhR activity in AA E006AA and E006HT cells to CA LNCaP and C4-2 prostate cancer cells.

Major Task 2: Determine the effect of co-inhibition of AhR and androgen receptor on biological properties.

Milestones: Production and characterization of E006HT clones. Identification of AhR as a regulator of androgen receptor signaling in AA prostate cancer cell models. Publication of 1st peer reviewed article

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 from reporting activities to reporting accomplishments.

Major Activities: Compare the effect of AhR activity in African-American (AA) and Caucasian-American (CA) prostate cancer cell lines.

Specific Objective 1 completed: Measured AhR activity in E006AA, E006HT, LNCaP and C4-2 prostate cancer cells by assessing protein levels, cellular localization, DNA interaction and expression of AhR responsive genes.

Results: African-American prostate cancer cell lines have increased AhR protein expression compared to their Caucasian-American (CA) counterparts. African-American Androgen insensitive cell line, E006HT, has the highest AhR expression. The AhR expression in African-American androgen-sensitive cell line, E006AA is also slightly increased compared to the androgen independent Caucasian cell line, C4-2. In comparison, the androgen sensitive LNCaP cells have lowest AhR expression.

Methods: Protein samples were isolated using a 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 AhR antibody (1:500). Blots were washed three times (15 minutes each) with TBST. The blots were then incubated in 1:2500 dilution in secondary antibody and washed three times (15 min each) with TBST, three times (10 min each) with TBS and once with ddH20 (10 mins). Bands were visualized with enhanced chemiluminescence kit as specified by manufacturer.

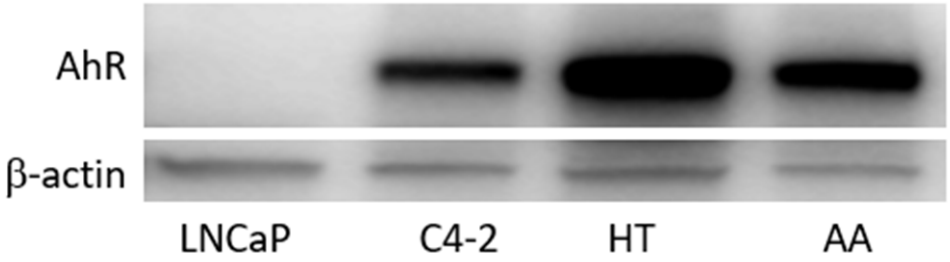


Figure 1: Expression of AhR protein in African-American (HT and AA) and Caucasian-American (LNCaP and C4-2) prostate cancer cell lines. C4-2 and HT are model castration resistant cell lines while LNCaP and AA are models of androgen sensitive prostate cancer cells.

Results: All cell lines contain cytoplasmic AhR protein. Despite AhR being identified as a ligand activated transcription factor, androgen-insensitive prostate cancer cell lines (C4-2 and E006HT) have nuclear AhR expression in the absence of exogenous ligands. African-American androgen sensitive prostate cancer cell line E006AA also has nuclear AhR protein expression.

Methods: To determine AhR cellular localization, cytoplasmic and nuclear protein samples were isolated using the Thermo Scientific NE-PER Extraction kit (Cell Signaling). Immunoblotting was carried out with 1 μg/ml mouse AhR monoclonal antibody (Santa Cruz) at 1:1000 dilution. Following TBST washes, the blots were incubated in 1:2500 dilution of b-tubulin/TOPO3 and washed extensively. Bands were visualized with the enhanced chemiluminescence (ECL) kit as specified by the manufacturer.

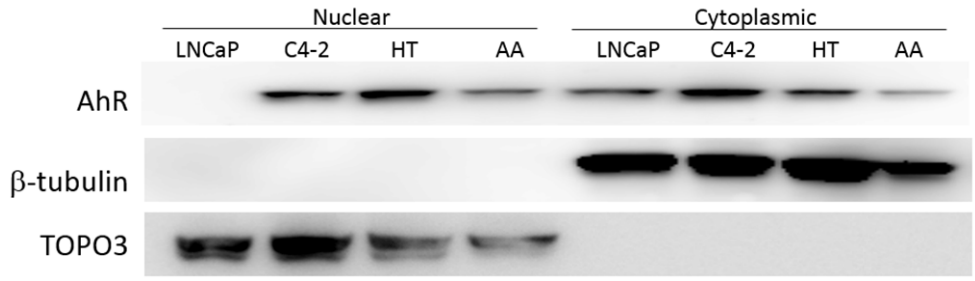


Figure 2: Subcellular localization of AhR in prostate cancer cell lines by nuclear and cytoplasmic staining. The nuclear and cytoplasmic extracts were analyzed by western blotting for AhR protein expression. The relative level of AhR was normalized by the respective B-tubulin or topoisomerase (TOPO3) levels.

Results: Subcellular localization studies show that AhR is localized in the nucleus of C4-2, HT and AA prostate cancer cells but not androgen sensitive LNCaP cells. The promoter binding studies evaluate if nuclear AhR interacts with DNA promoter regions specific to AhR, known as xenobiotic responsive elements (XRE). The results show that LNCaP has modest binding to XREs under normal culturing conditions. However, androgen sensitive African-American prostate cancer cells (AA), have increased binding to XREs compared to LNCaP. Also, HT and C4-2 both have significant promoter binding to XREs. Notably, the African-American cells lines have reduced binding to AREs compared to the Caucasian-American cell lines. These result may indicate that African-American cell lines have increased AhR activity.

Methods: Promoter binding assays were performed to assess constitutive AhR and androgen receptor (AR) binding to xenobiotic responsive elements (XREs) and androgen responsive elements (AREs) respectively. $4x10^4$ cells were plated in a 96-well plate. Cells were transfected with the XRE or ARE reporter construct, as well as with positive and negative control reporter plasmids using attractene. After 18 hours of transfection, media was changed to standard assay media. A dual luciferase assay was performed, and promoter activity values are expressed as arbitrary florescence units (AFU).

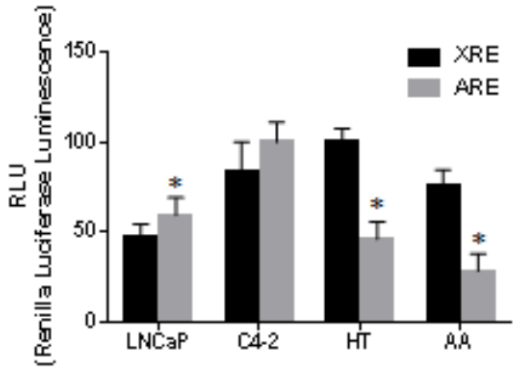


Figure 3: Promoter binding of AhR and AR in prostate cancer cell lines. AhR binding to XRE reporter construct under normal culturing conditions (dark bars). Androgen receptor binding to ARE reporter constructs under normal culturing conditions (light bars). Bar graphs represent mean ± SD of three separate experiments.

Results: AhR is a described as a ligand activated transcription factor. Therefore, AhR is commonly studied for its role as a xenobiotic receptor. However, recent studies indicate that AhR may possess differing intrinsic functions that require no ligand activation. We have already shown that C4-2 cells endogenously overexpress AhR and that the increased expression is accompanied by nuclear localization and constitutive activity. Here, comparison of AhR activity in the AA E006AA/E006HT isogenic pair to the LNCaP/C4-2 cells reveal that African-American cells have enhanced AhR activity over their Caucasian counterparts. Our preliminary data confirms overexpression of AhR protein in the African-American pair compared to the CA cell lines. The results below, in combination with figures 1-3, show that African-American prostate cancer cell lines E00HT and AA have enhanced AhR activity. Both the AhR and CYP1B1 genes are AhR responsive genes.

Methods: qRT-PCR was used to determine expression of AhR responsive genes (AhR and CYP1B1) in both isogenic pairs. We isolated total RNA from cell monolayers using RNeasy Mini Kit (Qiagen) and 2 µg of the total RNA was reverse-transcribed using the Superscript II kit (Invitrogen), according to the manufacturer's recommendations. The cDNA served as a template in a 25 µl reaction mixture and wasvprocessed using the following protocol: an initial denaturation at 95˚C for 3 min, followed by 39 amplification cycles (95˚C for 10s and 55–65˚C for 30s), 95˚C for 10s, 65˚C for 5s and 95˚C for 50s. The 25 µl qPCR reaction mixture was mixed with GoTaq qPCR Master Mix (Promega). Melt curve analyses performed after each run was used to ensure a single product. Relative gene expression was determined using the ΔΔCq calculation method. The primer sequence and specificity of primer sets were validated in previously published work.

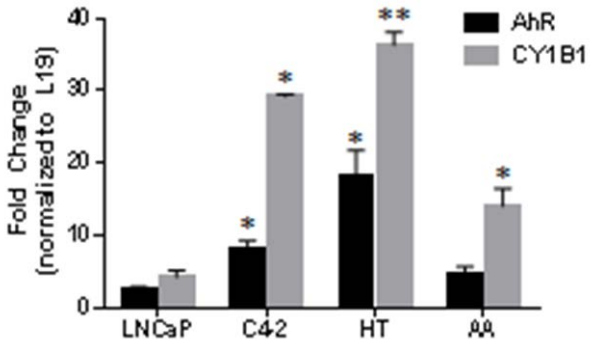
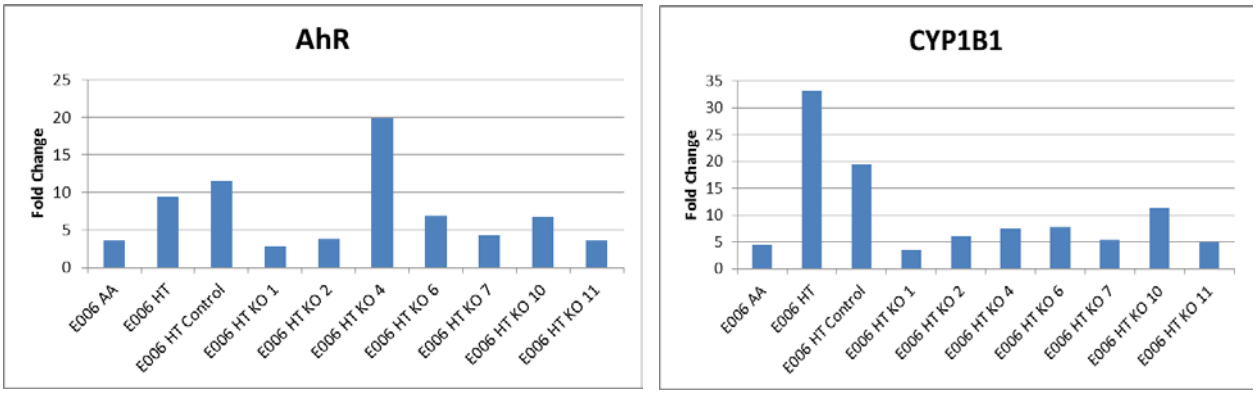


Figure 4: Quantitative real-time PCR was performed to quantify the expression of AhR and CYP1B1. The relative concentration of each PCR product 2aws determined using the ΔΔCq calculation method. L-19 was used as an internal control. Data are expressed as mean +/- SEM (n=3).

Specific Objective 2 in progress: Compare androgen receptor activity in E006HT clones following shRNA mediated depletion of AhR to androgen receptor activity in C4-2 clones with reduced AhR protein by measuring androgen receptor expression, phosphorylation, localization, DNA binding and expression of androgen responsive genes.

Results: We have already shown that depletion of AhR expression results in a decrease of androgen responsive genes KLK2 and KLK3 in C4-2 cells. Furthermore, we have shown that shRNA mediated depletion of AhR decreases AR expression and nuclear localization. This also results in decreased expression of androgen related genes and growth. However, our previous studies did not include AA cell lines, which could have enhanced AhR signaling beyond the level seen in CA cell lines. We created and validated a E006HT cell line with shRNA mediated reduction of AhR protein. The clones will be used to assess the ability of AhR to revert a highly metastatic African-American prostate cancer cell line, which may be responsible for the disparity seen with PCa, to an androgen sensitive phenotype. It is possible that changes in AhR activity can confer a castration resistant phenotype independent of changes to the androgen receptor signaling pathway.

Methods: E006HT cells were incubated with 1 μg of pSuper expression plasmid lentiviral particles containing AhR- shRNA and an empty pSuper vector as a control. At 48 h post-transfection, puromycin was be added to the medium (0.5 mg/mL), and resistant colonies were selected and expanded by limited dilution. We used qRT-PCR (as previously described) to assess for expression AhR responsive genes (AhR and CYP1B1) to select clones for further characterization. Three clones were selected, expanded and assessed for AhR protein expression. The clones that were expanded tested positive for mycoplasma contamination. Therefore, we will repeat these methods to obtain sterile cultures before moving forward with characterization of AhR and AR signaling.



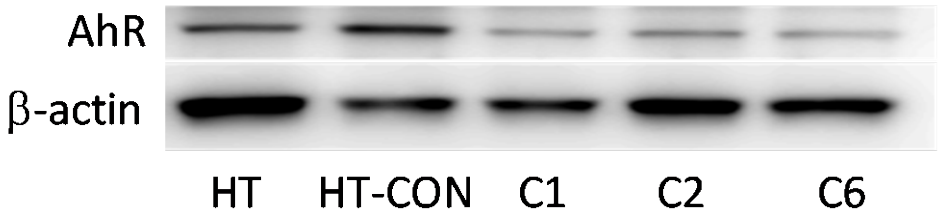


Figure 5: (A-B) Quantitative real-time PCR was performed to determine AhR and CYP1B1 expression in E006HT clones following shRNA mediated knock-down of AhR. The relative concentration of seven clones were assessed by the ΔΔCq calculation method. E006HT control and E006HT parental cells were used as control for basal AhR and CYP1B1 levels. L-19 was used as internal control for normalization of fold change. (C) AhR protein expression in HT parental, HT control and three knock-down clones.

Results: We also assessed expression of AR genes within the E006HT control and Clone 1. Relative to AhR, E006HT cells show modest AR and KLK3 expression. However, preliminary data shows a modest decrease in AR and KLK3. These results will be confirmed in new clones generated following shRNA mediated depletion of AhR.

Methods: Total RNA was isolated from cell monolayers of E006HT control and AhR knock-down using RNeasy Mini Kit (Qiagen) and 2 µg of the total RNA was reverse-transcribed using the Superscript II kit (Invitrogen), according to the manufacturer's recommendations. qRT-PCR was used to determine expression of AhR responsive genes (AhR and CYP1B1) and AR responsive genes (AR and KLK3). Due to mycoplasma contamination, methods will be repeated on new clones.

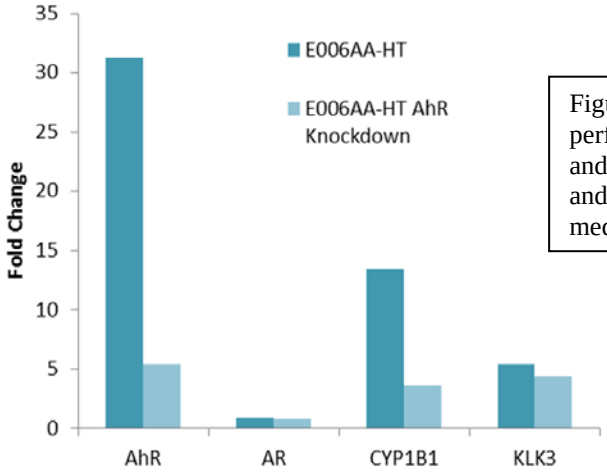


Figure 6: Quantitative real-time PCR was performed to determine AhR, CYP1B1, AR and KLK3 gene expression in E006HT control and knock-down clone 1 following shRNA mediated knock-down of AhR.

Results: We evaluated the growth rate of E006HT control and Clone 1 with decreased AhR expression to determine the effect of constitutive AhR signaling on the growth rate. Decreased AhR expression reduced the growth rate by 20%. However, E006HT (AhR knockdown) cells have a 50% decrease in proliferation in the presence of AhR inhibitor casodex (CDX). The HT control cells do not respond to casodex. Furthermore, AhR inhibitor CH223191 (CH223) decreased the growth rate of both the control and AhR knockdown.

Methods: Growth of E006HT CON and Clone 1 (AhR Knock-down) cells were assayed using the Promega CellTiter 96 Cell Proliferation Assay. Briefly, 50 µl of the 1.0 x 105/mL cell suspension (5,000 cells) were added to each well of the 96-well plate containing 50 µl of media resulting in a total volume of 100 µl. The plates were incubated at 37°C for 72 hours in a humidified, 5% CO2 atmosphere in the presence and absence of 20 µM casodex (CDX) and 50 µM AhR inhibitor CH223191 (CH223). Following incubation, 15 µl of dye solution was added to each well and incubated for 4 hours. Then, 100 µl of stop solution was added to each well and incubated for 1 hour. Absorbances were read at 570 nm using the Synergy H1m multi-mode microplate reader. Due to mycoplasma contamination, methods will be repeated on new clones.

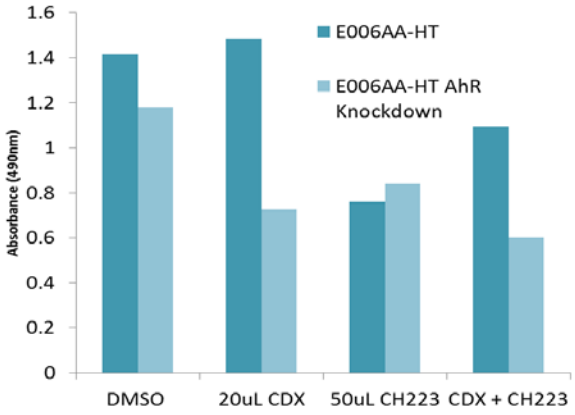


Figure 7: Growth of E006HT control and Clone 1 (AhR Knock-down) in the presence and absence of AR (casodex) and AhR inhibitor (CH223).

Specific Objective 3 completed: Subtask 3: Measure proliferation, migration, invasion and anchorage independent growth of E006HT and C4-2 cells in the presence and absence of AhR inhibitor (CH223191) and androgen receptor inhibitor (casodex).

Results: Inhibition of AhR signaling with specific inhibitor CH223 reduced the growth of both castration resistant prostate cancer cell models (C4-2 and HT). Due to the enhanced AhR signaling in African-American cell line E006AA shown in figures 1-4, CH223 also decreased growth of these androgen sensitive cells. CH223 had no effect on LNCaP cells which showed the lowest level of AhR activity. Androgen receptor inhibitor (CDX) reduced growth of the two androgen sensitive cell lines (LNCaP and AA) but not the androgen-insensitive cell lines (C4-2 and HT).

Methods: Growth of E006HT and C4-2 cells were compared using the Promega CellTiter 96 Cell Proliferation Assay. 50 µl of the 1.0 x 105/mL cell suspension (5,000 cells) were added to each well of the 96-well plate containing 50 µl of media resulting in a total volume of 100 µl. The plates were incubated at 37°C for 72 hours in a humidified, 5% CO2 atmosphere in the presence and absence of 20 µM casodex (CDX) and 50 µM AhR inhibitor CH223191 (CH223). Following incubation, 15 µl of dye solution was added to each well and incubated for 4 hours. Then, 100 µl of stop solution was added to each well and incubated for 1 hour. Absorbances were read at 570 nm using the Synergy H1m multi-mode microplate reader.

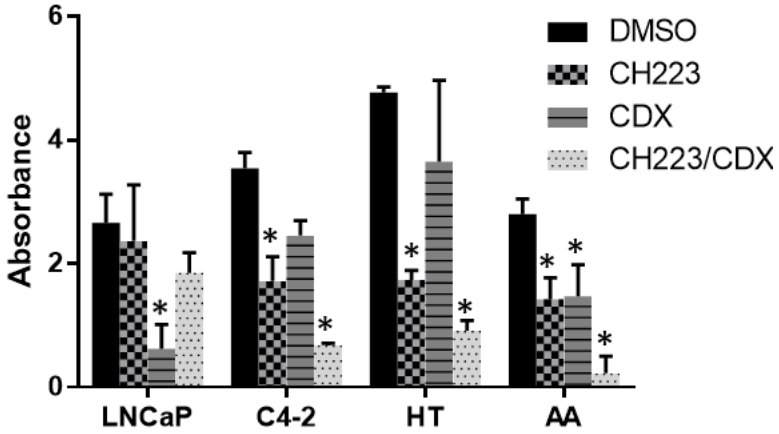


Figure 8: Growth of androgen sensitive (LNCaP and AA) and androgen insensitive (C4-2 and HT) in the presence and absence of AR (casodex) and AhR inhibitor (CH223). LNCaP and C4-2 were derived from Caucasian-American male and AA and HT cells were derived from an African-American male.

Results: Androgen-insensitive cell models C4-2 and E006HT have enhanced constitutive AhR signaling that regulates both cell migration and invasion. Androgen receptor inhibition by casodox (CDX) does not reduce the migration or invasion of either cell line. However, inhibition of AhR signaling by CH223 reduced migration of C4-2 cells by 50% and E006HT cells by over 60%. The level of inhibition correlates to the level of AhR activity revealed in figures 1-4 with E006HT cells having the highest level of AhR expression, nuclear localization, and XRE binding. Although E006AA (AA) does not respond to either CDX or CH223, there is a modest increase (but insignificant) increase compared to LNCaP cells.

Methods: The migratory and invasive potential of E006HT and C4-2 cells in the presence and absence of casodex and AhR antagonist CH223191 will be measured using a fluorescence-based tumor cell invasion assay (FluoroBlok invasion assay kits, BD Biosciences, Franklin Lakes, NJ). The assay has coupled a multi-well insert device containing a micro-porous membrane with a BD Matrigel coating process. The BD Matrigel coat functions as a barrier to the passage of non-invasive cells analogous to the in vivo extracellular basement membrane. Migration studies will be performed in chambers not coated with BD Matrigel. Cell suspensions were prepared by trypsinizing cell monolayers and resuspending the cells in serum-free medium at $5x10^4$ cells/ml with and without bicalutamide. Media (750 µl) containing 5% fetal calf serum will be added to the bottom of each well as a chemo-attractant. A 500 µl aliquot of the cell suspension (2.5x104 cells) was added to the top chamber. The cells were incubated at 37ºC for 24 hours. Following incubation, the medium from the top chamber was carefully removed by aspiration and the insert transferred to a second plate containing 0.5 ml/well of 4 µg/ml DAPI (Molecular Probes, Eugene, OR). The plates were incubated for 1 hour at 37ºC and DAPI fluorescence of the invaded cells read from the bottom at excitation and emission wavelengths of 485 and 530 nm, respectively.

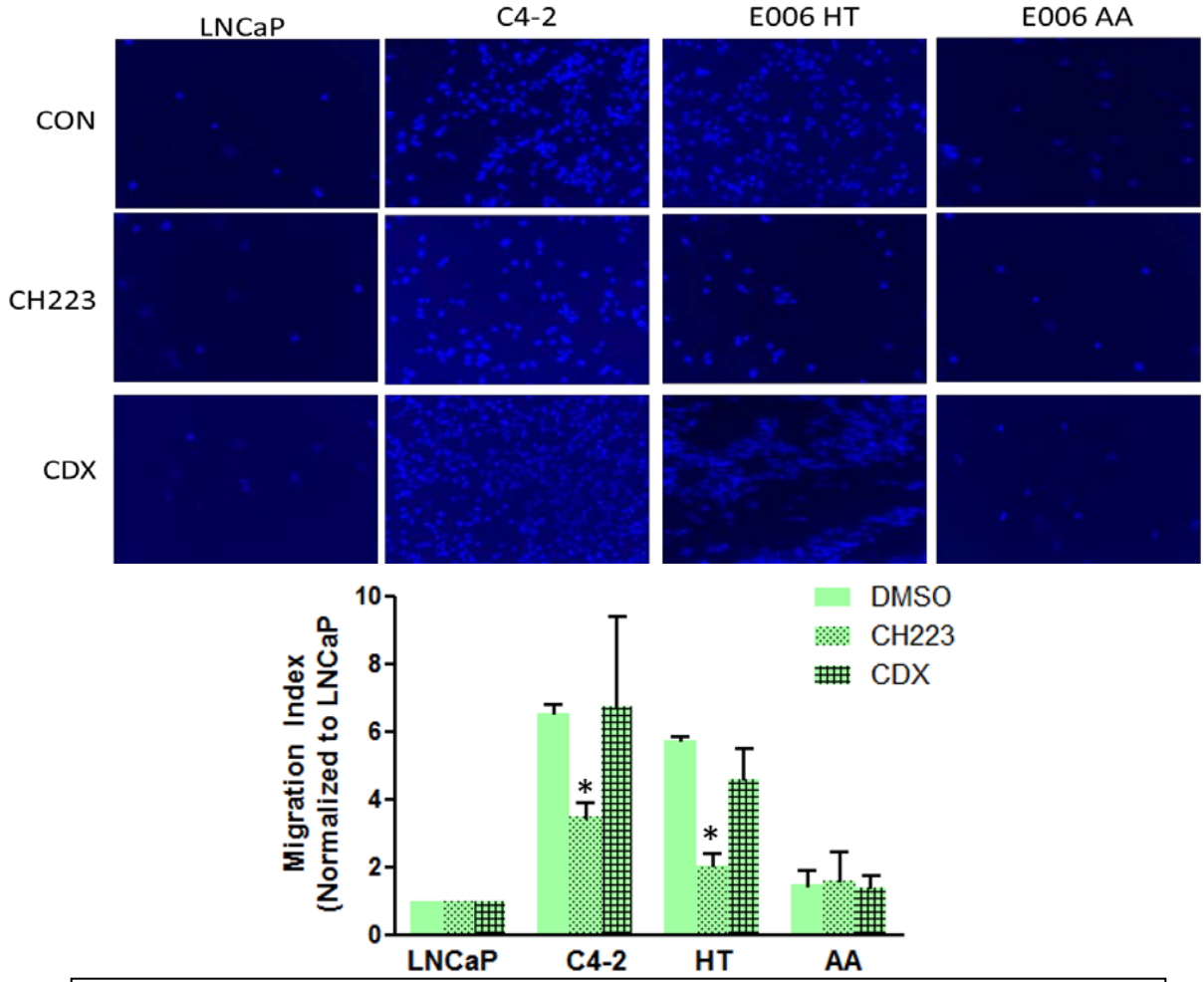


Figure 9: (A) Migration of prostate cancer cell lines in the presence and absence of AhR inhibitor, CH223191 (CH223) and androgen receptor inhibitor, casodex (CDX). Immunofluorescence expressions exhibited a blue-stained nucleus after 24 hours incubation. Bar graphs represent mean ± SD of three separate experiments.

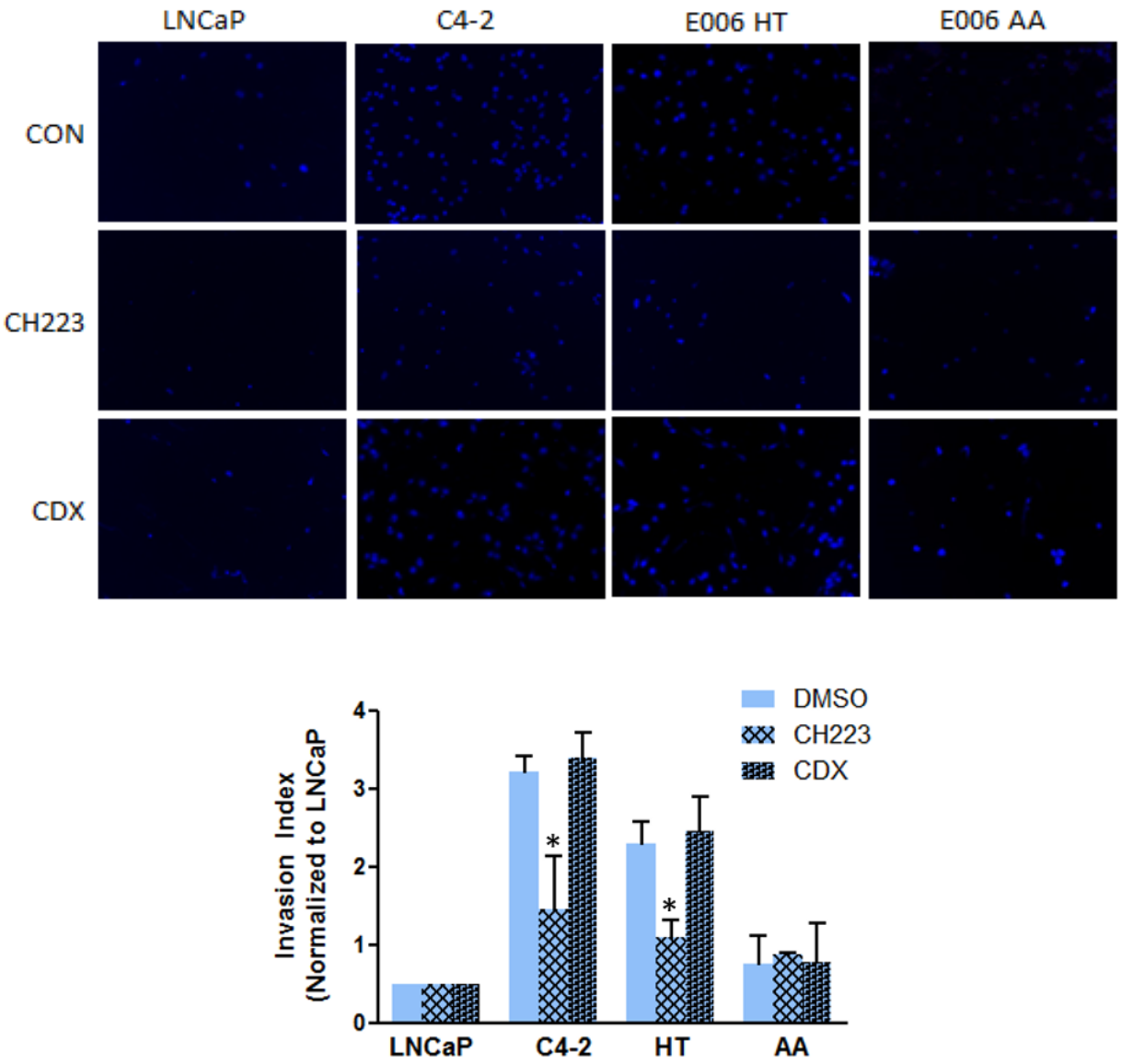


Figure 10: Invasion of prostate cancer cell lines in the presence and absence of AhR inhibitor, CH223191 (CH223) and androgen receptor inhibitor, casodex (CDX). Immunofluorescence expressions exhibited a blue-stained nucleus after 48 hours incubation. Bar graphs represent mean ± SD of three separate experiments.

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.

Principal Investigator attend the tenth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved in Atlanta, Georgia on September 25-28, 2017.

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.

Nothing to Report.

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.

During the next reporting period, we will re-establish E006HT (-AhR) clones which were lost due to mycoplasma contamination. Upon completion of this set of studies, we will submit manuscripts for results from Specific Aim 1. We will also 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 varying clinical PSA levels using AhR antibodies.

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

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "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.

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), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

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:

Nothing to Report.

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.

The original clones of E006HT cells derived from shRNA mediated knock-down of AhR were contaminated with mycoplasma. In an effort to limit the spread of contamination, the cell cultures were destroyed. Therefore, we were unable to complete Specific Objective 2 within the proposed timeline.
We are re-establishing the knock-out clones using the same methods. We will test for contamination weekly using the LookOut Mycoplasma PCR Detection Kit (Sigma-Aldrich).

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 of biohazards and/or select agents

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).*

Nothing to Report

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.*

Nothing to Report.

- **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: Joann Powell
Project Role: PI
Contribution to Project: No change

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:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (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.