AWARD NUMBER:
W81XWH-18-1-0163

TITLE:
The Role of DHEAS in Abiraterone Resistant Prostate Cancer

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REPORT DATE:
July 2019

TYPE OF REPORT:
Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2019	Annual report	1 Jul 2018 - 30 Jun 2019

4. TITLE AND SUBTITLE
The Role of DHEAS in Abiraterone Resistant Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0163

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Prostate cancer (PCa) is a leading cause of cancer deaths in American men. Androgen deprivation therapy (ADT), lowers to castrate levels the androgens that drive PCa growth through androgen receptor (AR) signaling. Despite initial success, castration-resistant PCa (CRPC) develops within 24 months of ADT and remains primarily driven by androgens. Abiraterone acetate, a CYP17A1 inhibitor, reduces circulating androgen levels by preventing the synthesis of dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS), an androgen precursor. Although most patients respond well to abiraterone acetate, survival is prolonged by only about 4-5 months, and secondary resistance develops. Hypoxia is a hallmark of many types of human tumors and implicated in clinical behavior and treatment response. Preclinical study indicate that hypoxia promotes both local aggressiveness and metastasis via mediating metabolism of cancer cells to facilitate the rapid growth of tumor. Previous study suggest that hypoxia plays a critical role in prostate cancer development. Here I further determined how hypoxia affected DHEA metabolism via regulating the expression of HSD3B1 as well as co-factors required for HSD3B1 activity. I established hypoxia-resistant cell lines which can survive under hypoxia forever to simulate the hypoxic condition that exists in a tumor. My results indicate that while acute hypoxia leads to deaccelerated conversion of DHEA to downstream metabolites due to reduced co-factors, long-term hypoxia significantly increases the expression of HSD3B1 at both mRNA and protein level. HSD3B1 exhibits higher stability under hypoxia. Moreover, my study suggest that hypoxia-resistant cells exhibit faster conversion of DHEA to downstream metabolites than hypoxia-sensitive cells after reoxygenation.

15. SUBJECT TERMS
CRPC, abiraterone resistance, DHEA, hypoxia, HSD3B1

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U Unclassified	U Unclassified	U Unclassified	U Unclassified	12	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

Prostate cancer (PCa) is a leading cause of cancer deaths in American men and is responsible for 27,000 deaths annually. Androgen deprivation therapy (ADT), the first-line treatment for advanced PCa, lowers to castrate levels the androgens that drive PCa growth through androgen receptor (AR) signaling. Despite initial success, castration-resistant PCa (CRPC) develops within 24 months of ADT and remains primarily driven by androgens. Abiraterone acetate, a CYP17A1 inhibitor, is a second-line therapy used to treat metastatic CRPC and reduces circulating androgen levels by preventing the synthesis of dehydroepiandrosterone (DHEA) and its sulfated form (DHEAS), an androgen precursor. Although most patients respond well to abiraterone acetate, survival is prolonged by only about 4-5 months, and secondary resistance develops. Despite blockade of CYP17A, significant serum concentrations of DHEAS persist after abiraterone treatment. Our preliminary data indicate that abiraterone-resistant cells have the machinery to transport and utilize DHEAS, and loss of *HSD3B1*, which encodes 3BHSD1 affects DHEAS uptake, complementing our previous studies that highlight the importance of this enzyme in androgen metabolism. Therefore, DHEA as well as its precursor DHEAS may serve as the major remaining androgen pool for the synthesis of potent androgen such as testosterone and DHT in abiraterone-resistant PCa.

Hypoxia is not only a feature of many types of human tumors but also implicated as an important modulator of clinical behavior and treatment response. Preclinical study indicate that hypoxia promotes both local aggressiveness and metastasis via mediating metabolism of cancer cells to facilitate the rapid growth of tumor. Previous study suggest that hypoxia plays a critical role in prostate cancer development. My preliminary data also indicated that DHEA metabolism was affected by hypoxia. Here I further determined how hypoxia affected DHEA metabolism via regulating the expression of HSD3B1 as well as co-factors required for HSD3B1 activity. Although the prostate cancer cell lines I used showed adaptation to hypoxia—only modest loss of cell viability after 24h, prolonged hypoxia (48h) finally killed the majority. Since cells in a tumor experience long-term hypoxia due to inconsistent levels of oxygen and nutrient supply resulting from disorganized vasculature, I established hypoxia-resistant cell lines which can survive under hypoxia forever to simulate the hypoxic condition that exists in a tumor. Different prostate cancer cell lines were grown under cyclic 12h hypoxia and 12h reoxygenation for several weeks. After several passages, the cells became resistant to hypoxia. My results indicate that while acute hypoxia leads to deaccelerated conversion of DHEA to downstream metabolites due to reduced co-factors, long-term hypoxia significantly increases the expression of HSD3B1 at both mRNA and protein level. HSD3B1 exhibits higher stability under hypoxia. Moreover, my study suggest that hypoxia-resistant cells exhibit faster conversion of DHEA to downstream metabolites than hypoxia-sensitive cells after reoxygenation.

KEYWORDS:

CRPC, abiraterone resistance, DHEA, hypoxia, HSD3B1

ACCOMPLISHMENTS:

What were the major goals of the project?

Training-Specific Tasks:

<u>Milestone(s) Achieved</u>:

1: Responsible Conduct of Research Training (12 months)

2: Monthly Prostate Cancer Working Group and Seminar Series (12 months)

3: Weekly lab meetings and journal clubs (12 months)

4: Cleveland Clinic Department of Cancer Biology weekly seminars and journal clubs (12 months)

Research-Specific Tasks:

Specific Aim 1: Determine the role of DHEAS uptake and HSD3B1 expression in ABI resistance.

Major Task 1: Establish long-term DHEAS- and ABI-treated cells.

Milestone(s) Achieved: Establishment of long-term treated cell lines that survive ABI treatments. (4 months)

Major Task 2: Evaluate changes in DHEAS uptake.

Milestone(s) Achieved: Link increased DHEAS uptake with ABI-resistance status. (12 months)

Specific Aim 2: Define the metabolic pathway and regulatory enzymes of DHEAS metabolism to DHT.

Major Task 3: Measure metabolism of long-term ABI-treated LNCaP and LAPC4 cells.

Milestone(s) Achieved: Determination of the effect of hypoxia on DHEA metabolism. (12 months)

What was accomplished under these goals?

Accomplished tasks:

Specific Aim 1: Determine the role of DHEAS uptake and HSD3B1 expression in ABI resistance.

Major Task 1: Establish long-term DHEAS- and ABI-treated cells.

Milestone(s) Achieved: Establishment of long-term treated cell lines that survive ABI treatments. (4 months)

Results:

I have treated LNCaP cells with 200nM DHEAS with or without 5μM ABI for ≥ 4 months, and cells treated with both agents have greater uptake. In addition to LNCaP cells that harbor mutated AR, LAPC4 cells (WT AR, gift from Charles Sawyers, Howard Hughes Medical Institute) was also treated with DHEAS with or without 5μM ABI for ≥ 4 months. The establishment of these ABI-resistant cells allows me to determine whether ABI-resistant CRPC utilizes DHEAS.

Major Task 2: Evaluate changes in DHEAS uptake.

Milestone(s) Achieved: Link increased DHEAS uptake with ABI-resistance status. (12 months)

Results:

I compared the uptake of [^{3}H]-DHEAS in both normal prostate cancer cells and ABI-resistant cells established by culturing the cells for ≥ 4 months in 10% fetal bovine serum (FBS) phenol red-free RPMI media containing 200nM DHEAS with 5μM ABI. 200nM DHEAS falls within the range of serum concentrations of DHEAS observed in patients after ADT and ABI combination treatment. The cells were serum starved for 48 h with 10% charcoal stripped fetal bovine serum (CSS) phenol red-free RPMI to get rid of the residual steroid in the cells, and then incubated with 200μM DHEAS spiked with [^{3}H]-DHEAS. My result suggested that ABI treatment conferred the cells increased DHEAS uptake (Figure 1).

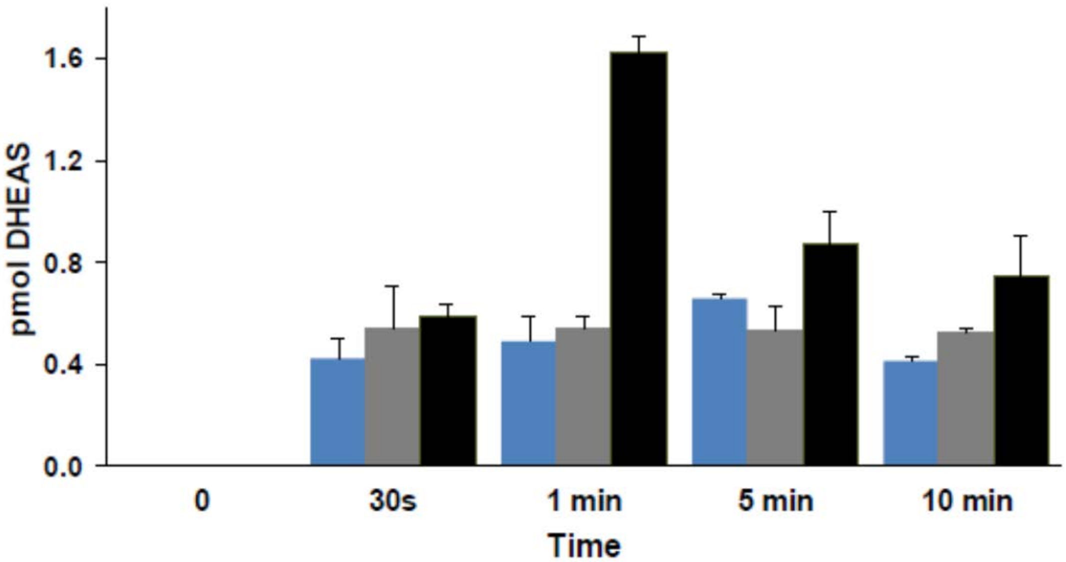


Figure 1. Uptake of [3H]-DHEAS in untreated LNCaP cells (blue), long-term DHEAS-treated LNCaP cells (gray), and long-term DHEAS-abiraterone-treated LNCaP cells (black). Cells were incubated with 200nM DHEAS spiked with [^{3}H]-DHEAS for the indicated time points upon washing with ice cold buffer. The cell samples were lysed and measured on a liquid scintillation counter. Bars represent pmol DHEAS in the cell lysate. Error bars = SD.

Specific Aim 2: Define the metabolic pathway and regulatory enzymes of DHEAS metabolism to DHT.

Major Task 3: Measure metabolism of long-term ABI-treated LNCaP and LAPC4 cells.

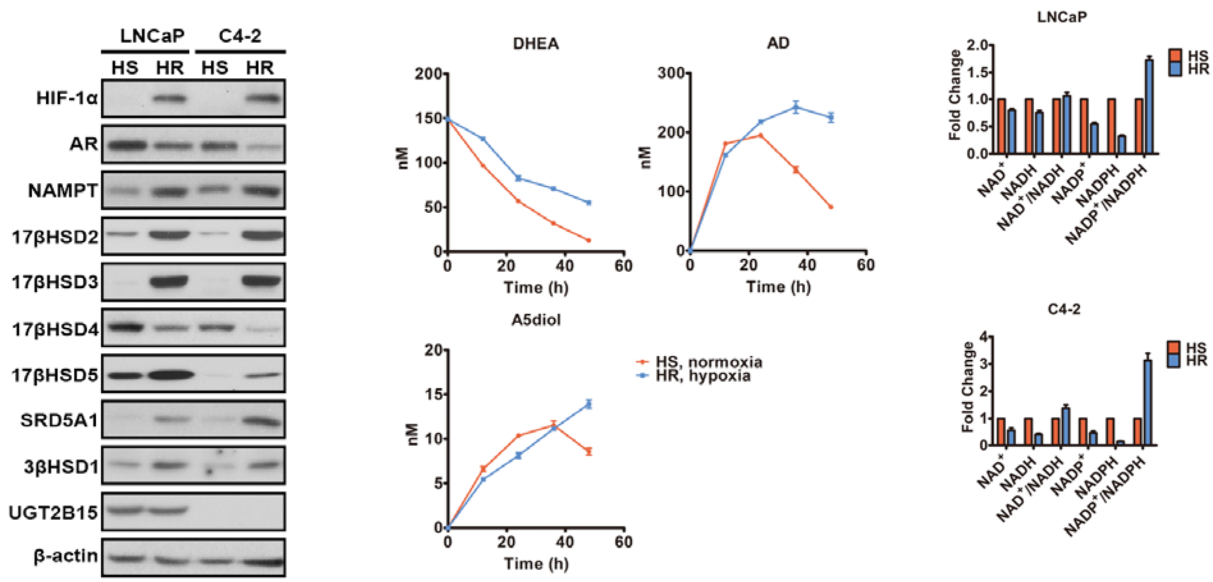
Milestone(s) Achieved: Determination of the effect of hypoxia on DHEA metabolism. (12 months)

Results:

Since cells in a tumor experience long-term hypoxia due to inconsistent levels of oxygen and nutrient supply resulting from disorganized vasculature, I established hypoxia-resistant cell lines which can survive under hypoxia forever to simulate the hypoxic condition that exists in a tumor. Previous studies suggest that long-term hypoxia leads to the proliferation of more aggressive, treatment-resistant cancer cell in patients. Different prostate cancer cell lines were grown under cyclic 12h hypoxia and 12h reoxygenation for several weeks. After several passages, the cells became resistant to hypoxia. I first compared the protein level of the enzymes involved in androgen synthesis from DHEA in both hypoxia-resistant cells and their counterparts which are sensitive to normoxia. Interestingly, most enzymes I detected were dramatically upregulated in hypoxia-resistant cells, except 17βHSD4 and UGT2B15 (Figure 2A). The significant alteration of the protein level of these enzymes in hypoxia-resistant cells may suggest their critical roles for the survival of these androgen-dependent cells under chronic hypoxia.

To understand the significance of the altered expression profile of the enzymes involved in androgen metabolism in hypoxia-resistant cells, I compared the metabolism of DHEA in normal cells under normoxia and hypoxia-resistant cells under hypoxia. The most amazing observation in hypoxia-resistant cells under chronic hypoxia is the much longer preservation of DHEA compared with normal cells under normoxia, although same amount of (or more) downstream metabolites were finally produced (Figure 2B). Such slower conversion of DHEA to their downstream metabolites was inconsistent with the much higher level of the enzymes involved in androgen synthesis. Therefore, we compared the level of the cofactors in normal and hypoxia-resistant cells. Not surprisingly, our results revealed that the level of all 4 cofactors was drastically lower in hypoxia-resistant cells compared with normal cells (Figure 2C), which indicates the decisive role of cofactor rather than the protein level of the enzymes in androgen synthesis from DHEA.

I examined whether reoxygenation can accelerate the metabolism of DHEA in HR cells. I compared the metabolism of DHEA in both normal and hypoxia-resistant cells under normoxia. Interestingly, hypoxia-resistant cells exhibited accelerated conversion of DHEA to the major downstream metabolites than normal cells under normoxia (Figure 3A). To explore the mechanism of the altered metabolism, I measured the level of cofactors involved. 12h reoxygenation led to significant upregulation of all 4 cofactors, especially the two reduced forms: NADH and NADPH (Figure 3B). Cyclic hypoxia-reoxygenation, a common feature of tumor, confers on HR cells efficient utilization of DHEA. During hypoxia under which the cells do not have enough nutrient to proliferate, DHEA persists much longer, and the enzyme levels are higher than those in the cells under normoxia, then consequent reoxygenation facilitates androgen production by rapidly increasing cofactor level without reducing protein level of the enzymes in short term.



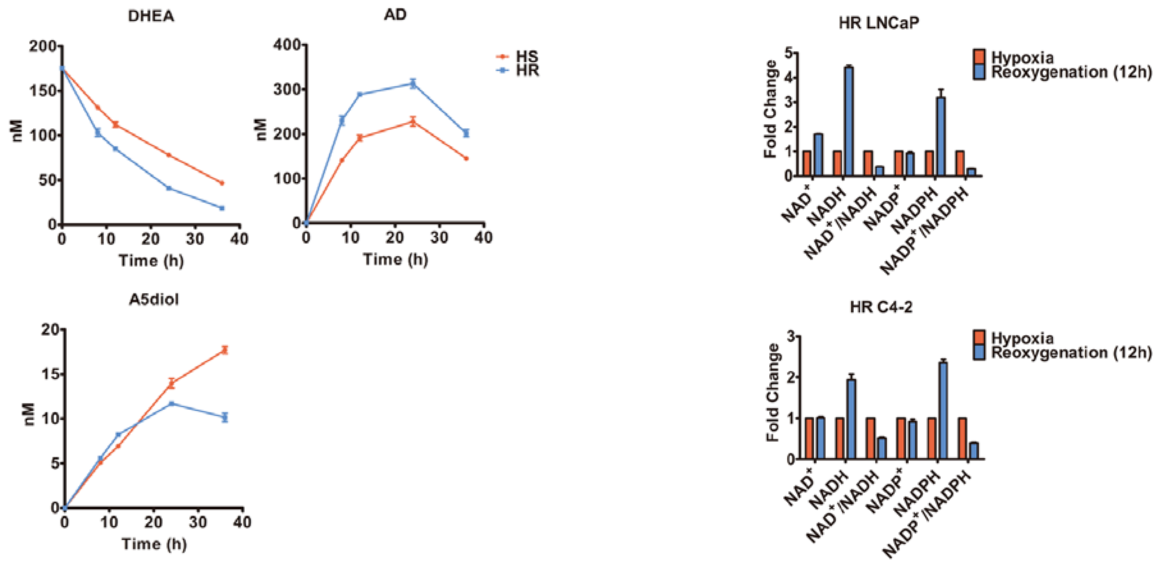
A B C

Figure 2. Conversion of DHEA to downstream metabolites was deaccelerated in hypoxia-resistant cells due to decreased cofactor level.

A. Comparison of the protein level of the enzymes involved in androgen metabolism in hypoxia-sensitive (HS) and hypoxia-resistant (HR) cells by immunoblot.

B. Conversion of DHEA to AD and A5diol in HS C4-2 cells under normoxia, and in HR C4-2 cells under hypoxia in time course.

C. Comparison of NAD^+, NADH, $NADP^+$, and NADPH levels in HS cells under normoxia and HR cells under hypoxia.

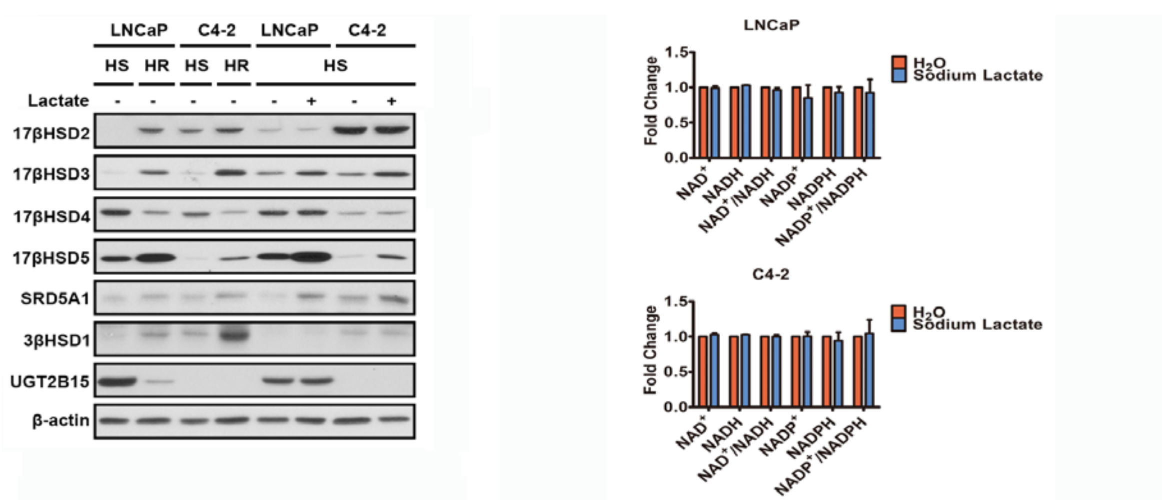


A B

Figure 3. Hypoxia-resistant cells exhibited accelerated conversion of DHEA to downstream metabolites.
A. Conversion of DHEA to AD and A5diol in both HS and HR C4-2 cells under normoxia in time course.
B. Comparison of NAD^+, NADH, $NADP^+$, and NADPH levels in HR cells under hypoxia and reoxygenation.

Other achievements:

I observed that the medium with phenol red as a pH indicator turned to yellow from pink very rapidly when it was used for cell culture under chronic hypoxia. Such rapid color change indicated robust increase of hydrogen ions as a product of glycolysis induced by hypoxia. The majority of pyruvate is converted to lactate as the last step of glycolysis. Increasing studies indicate lactate functions not only as a waste of glycolysis, but also a signaling molecule involved in many cellular events. Therefore, we determined whether lactate plays a role in altered androgen metabolism under chronic hypoxia. Surprisingly, the treatment of normal cell with sodium lactate significantly increased the protein level of many enzymes involved in androgen metabolism, simulating that occurring in hypoxia-resistant cells (Figure 4A). The level of the cofactors was not affected by lactate (Figure 4B).



A B

Figure 4. Lactate upregulates the key enzymes responsible for DHEA metabolism.
A. Comparison of the protein level of the enzymes involved in DHEA metabolism in HS and HR cells treated with or without sodium lactate by immunoblot.
B. Comparison of NAD^+, NADH, $NADP^+$, and NADPH levels in LNCaP and C4-2 cells treated with solvent or sodium lactate.

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

1. Attended Conduct of Research and Human Subjects training.
2. Attended monthly Prostate Cancer Working Group and Seminar Series
3. Attended and presented research at the weekly lab meetings and journal clubs
4. Attended and presented work at the weekly CCF Department of Cancer Biology seminars

How were the results disseminated to communities of interest?

1. Presented my work at weekly lab meetings

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

1. Determine the activation of AR activity by DHEA in hypoxia-resistant cells after reoxygenation, compared with normal prostate cancer cells. Both normal and hypoxia-resistant cells should be fasted first to get rid of intracellular androgen, and then treated with DHEA to measure the mRNA level of androgen-responsive genes.
2. Determine the mechanism of the upregulation of 3βHSD1, AKR1C3, as well as SRD5A1. Determine both the protein stability and transcription of 3βHSD1 in normal and hypoxia-resistant cells.
3. Determine the tumorigenic ability of hypoxia-resistant cells after reoxygenation *in vivo*, such as xenograft, compared with normal prostate cancer cells.

IMPACT:

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."

Although intensive study has been done, prostate cancer remains a leading cause of cancer deaths in American men. Androgen deprivation therapy as the first-line treatment for advanced PCa leads to castration-resistant prostate cancer after 24 months, despite initial success. Abiraterone acetate, a CYP17A1 inhibitor, only prolongs patient survival by about 4-5 months. Despite blockade of CYP17A, significant serum concentrations of DHEAS persist after abiraterone treatment. My study indicates that abiraterone-resistant cells uptake and utilize DHEAS to synthesize potent androgen such as testosterone and DHT in abiraterone-resistant PCa.

Hypoxia as a hallmark of many types of human tumors plays an important role in tumorigenesis. However, how hypoxia is involved in androgen synthesis in prostate cancer is largely unknown so far. My study here suggests that hypoxia alters DHEA metabolism via regulating the expression of HSD3B1 as well as co-factors required for HSD3B1 activity.

Therefore, my study laid the foundation for future studies of the clinical significance of DHEA metabolism in prostate cancer cells, with the eventual goal of developing new clinical treatment strategies, as well as a potential biomarker for personalized treatment.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

My project revealed that 3βHSD1 as well as the co-factors play critical roles in DHEA metabolism under the regulation of hypoxia. Hypoxia level could be a potential biomarker for ABI-resistant prostate cancer diagnosis.

What was the impact on society beyond science and technology?

Nothing to Report

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report

Changes that had a significant impact on expenditures

Nothing to Report

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

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers, and presentations.

Nothing to Report

Website(s) or other Internet site(s)

Nothing to Report

Technologies or techniques

Nothing to Report

Inventions, patent applications, and/or licenses

Nothing to Report

Other Products

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Liang Qin*
Project Role:	*Principal investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-3928-8956*
Nearest person month worked:	8
Contribution to Project:	*Liang Qin is responsible for designing, performing and interpreting experiments and manuscript preparation.*
Funding Support:	**National Cancer Institute R01CA168899** Investigator: Nima Sharifi **American Cancer Society 12–038-01-CCE** Investigator: Nima Sharifi **National Cancer Institute R01CA172382** Investigator: Nima Sharifi **National Cancer Institute R01CA190289** Investigator: Nima Sharifi **Howard Hughes Medical Institute Physician-Scientist Early Career Award** Investigator: Nima Sharifi

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

No Change.

What other organizations were involved as partners?

Nothing to Report.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Nothing to Report.

APPENDICES: