AWARD NUMBER: W81XWH-16-1-0532

TITLE: Epigenetic Machinery Regulates Alternative Splicing of Androgen Receptor (AR) Gene in Castration-Resistant Prostate Cancer (CRPC)

PRINCIPAL INVESTIGATOR: Hsieh, Jer-Tsong

CONTRACTING ORGANIZATION: University of Texas Southwestern Medical Center
Dallas, TX 75390

REPORT DATE: September 2017

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2017	Annual	1 Sep 2016 -31 Aug 2017

4. TITLE AND SUBTITLE

Epigenetic Machinery Regulates Alternative Splicing of Androgen Receptor (AR) Gene in Castration-Resistant Prostate Cancer (CRPC)

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0532

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jer-Tsong Hsieh

E-Mail: jt.hsieh@utsouthwestern.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

UT Southwestern Medical Center
5323 Harry Hines Blvd.,
Dallas, TX 75390

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

Conventional chemotherapy with cell killing en mass often targets mitotic cells with less specificity, which likely leads to undesirable side effect. Knowing specific molecular defects in cancer cells has led to discover new chemotherapeutic agents. Thus, combined agents targeting different defected pathways in cancer cells have a better chance to eradicate tumor completely. Thus, to achieve a cure, a comprehensive targeting strategy needs to be implemented. In addition, improved methods for monitoring drug delivery and tumor response in a nearly real-time manner should offer a safe and effective treatment. This project carried out by a team of chemist, radiologist, and molecular tumor biologist is to develop a novel drug delivery system with new small molecular therapeutic agents assisted with new imaging probe is expect to bring a new frontier for prostate cancer (PCa) management. Our objective is to develop dendrimer-based theranostic agent with prostate cancer specificity and positron emission tomography imaging capability that can prevent the early onset of PCa metastasis or delay the progression of metastasis. The mission of my project is to design small peptide derived from tumor suppressor DAB2 family as therapeutic agent and examine its biology activities.

15. SUBJECT TERMS

None listed.

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
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

1. INTRODUCTION

The subject of our research is to identify the molecular mechanism of the drug resistance in castration-resistant prostate cancer (CRPC). Our preliminary data suggest that one of the mechanisms of the resistance is the emergence of constitutively active androgen-receptor (AR) variants such as AR-V7. Previous finding indicated that histone lysine demethylase KDM4B is associated with androgen receptor signaling (1) and the elevated KDM4B is found in metastatic PCa (2). KDM4s are known to be co-activators of AR (3-5). It appears that epigenetic regulation can couple with alternative gene splicing (6). Our goals are to demonstrate that KDM4B regulates AR-V7 via alternative splicing and to test the efficacy of our newly identified KDM4B inhibitor(s) as a monotherapy or combined with approved anti-androgen agents in AR-V7-expressing CRPC in pre-clinical animal models of CRPC.

REFERENCES

1. Gao L & Alumkal J (2010) Epigenetic regulation of androgen receptor signaling in prostate cancer. Epigenetics. 5, 100-104.
2. Duan LL, Rai G , Roggero C, Zhang Q-J, Wei Q, Ma SH, Zhou YY, Santoyo J, Martinez ED, Xiao G, Raj GV, Jadhav A, Simeonov A, Maloney DJ, Rizo J, Hsieh JT, and Liu ZP (2015) KDM4/JMJD2 histone demethylase inhibitors block prostate tumor growth by suppressing the expression of AR and BMYBregulated -genes, Chem & Bio, 22,1185-96.
3. Coffey K, Rogerson L, Ryan-Munden C, Alkharaif D, Stockley J, Heer R, Sahadevan K, O'Neill D, Jones D, Darby S, Staller P, Mantilla A, Gaughan L, Robson CN (2013) The lysine demethylase, KDM4B, is a key molecule in androgen receptor signaling and turnover. Nucleic Acids Res., 41, 4433- 4446.
4. Berry WL, Janknecht R (2013). KDM4/JMJD2 histone demethylases: epigenetic regulators in cancer cells. Cancer Res. 73, 2936-42.
5. Shin S and Janknecht R (2007) Activation of androgen receptor by histone demethylases JMJD2A and JMJD2D. Biochem. Biophys. Res. Commun., 359, 742-746.
6. Fiszbein A, Kornblihtt AR. (2016) Histone methylation, alternative splicing and neuronal differentiation. Neurogenesis (Austin) 3, e1204844.

2. KEYWORDS

Histone lysine demethylase, castration-resistant prostate cancer, alternative splicing, AR-V7, KDM4B, small molecule inhibitors.

3. ACCOMPLISHMENTS

Major goals and accomplishments

Aim 1. To establish that KDM4B promotes AR-V7 expression and identify the regulatory mechanisms.

Major Task 1: Determine the role of KDM4B in promoting AR-V7 expression in various PCa cell lines, including those resistant to enzalutamide.

This task has been completed (see Dr. Liu's report).

Major Task 2: Determine how KDM4B binds to the spliceosome associated with pre-mRNA.

This task has been completed (see Dr. Liu's report).

In summary, overexpression of KDM4B can promote PCa cell growth under androgen –deprived condition (Fig. 1). In contrast, knockdown (KD) of *KDM4B* can inhibitetumor growth in castrated mice and abolish AR-V7 expression (Fig. 2).

Milestone #1: Co-author manuscript on KDM4B-RNA interaction.

A manuscript has been submitted to Cancer Cell.

Aim 2. To evaluate the clinical application of KDM4B inhibitors on CRPC tumors expressing AR-Vs.

Major Task 5: Identify two lead compounds using CPRC cell lines and optimizing their dosage and schedule in xenograft models.

In summary, based on the in vitro $IC5_0$ of KDM4B inhibitor (i.e., B3), we first used Osmotic pump to deliver B3 (50 mg/kg daily dosage for 7 days) in 22RV1 subcutaneous model. Because the presence of AR-V7 in 22RV1 cells, this tumor is expected to resist anti-androgen (Fig. 3A). Nevertheless, there is a significant inhibition of tumor growth compared with control group (Fig. 3A). Apparently, AR-V7 expression is diminished in tumor cells treated with B3 (Fig. 3B), which validates the drug target from in vivo.

What opportunities for training and professional development have the project provided?
This project provides excellent training opportunities for tumor biology, experimental therapy and pathohistologic techniques.

How were the results disseminated to communities of interest?
By coordinating with leading PI, we are preparing abstract for presenting in PCa national conference and manuscript for peer-reviewed scientific journals.

What do you plan to do during the next reporting period to accomplish the goals?
Currently, our progress is right on the target based on original SOW. For the next reporting period, I expect to examine (1) Knockdown Brm and/or KDM4B in VCaP cells and assay its effect on AR-V7 expression and (2) Determine synergistic effect of KDM4 inhibitors combined with anti-androgen agents (enzalutamide).

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project? What was the impact on other disciplines?
Alternative splicing is emerging as an important determinant of tumorigenesis, response to treatment, and drug resistance, thus representing an important vulnerability

with potential to be exploited for therapeutic purposes. Epigenetic changes including histone modifications have long been recognized to play a role in tumor initiation and progression. In my project, I have established several in vivo xenograft models to demonstrate the role of KDM4B-ovexpressing PCa cells in supporting androgen independent growth, which underlies clinical manifestation of CRPC. In addition, using a novel small molecular inhibitor, I have explored an experimental therapy to demonstrate the potential therapeutic benefit of this molecule, which highlights KDM4B as a druggable target and potential new therapeutic strategy of CRPC patients.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to report.

5. CHANGES/PROBLEMS
Nothing to report.

6. PRODUCTS
Nothing to report.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS
Name: Jer-Tsong Hsieh
Project Role: PI
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 1.2
Project: Design experiments, supervise technician, communicate with leading PI, analyze data, and write progress report.
Funding Support: Cancer prevention and research institute of Texas (CPRIT), DOD, NIH

Name: Andrew Dang
Project Role: Research Technician
Researcher Identifier (e.g. ORCID ID): none
Nearest person month worked: 6
Project: Perform tissue culture, tumor model, animal surgery, drug treatment and immunostaining.
Funding Support: NIH

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to Report.

What other organizations were involved as partners?
Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

Please see Leading PI Zhi-Ping Liu's progress report.

9. Appendices

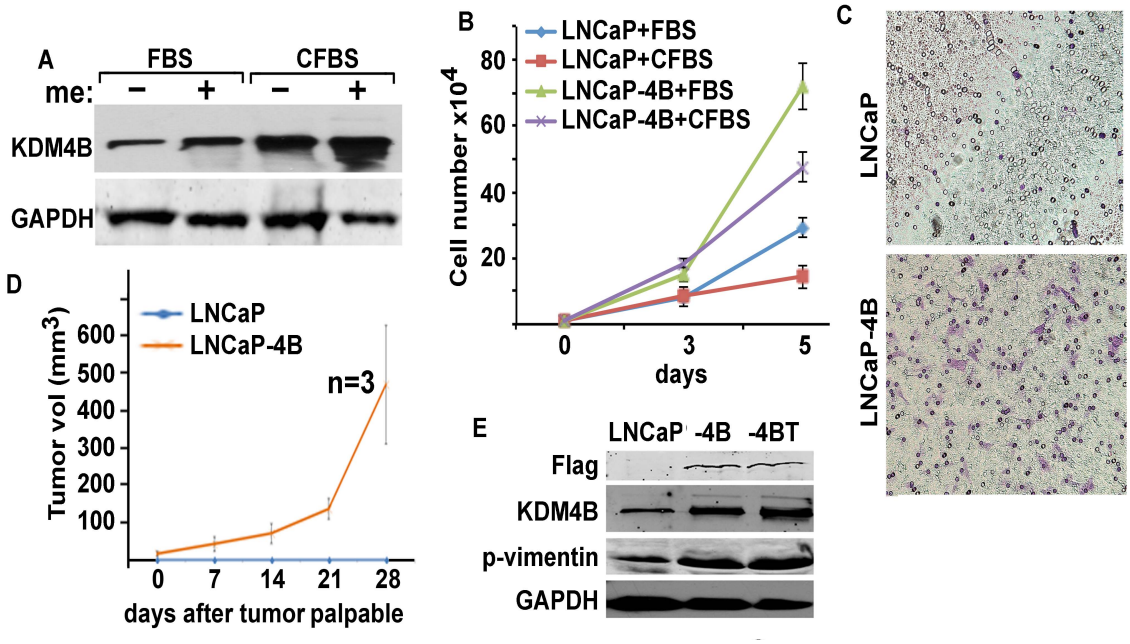

Figure 1. KDM4B promotes prostate tumorigenicity. (A) KDM4B expression in LNCaP cell culture without (-) or with methylcellulose (me) in the presence of FBS or CFBS. (B) Growth curves of LNCaP and LNCaP-4B cells in FBS or CFBS. (C) Transwell assay showing that LNCaP-4B cells (stained with hematoxylin, in blue) are significantly more migratory. (D) Growth curves of xenografts derived from LNCaP-4B and LNCaP cells (n=10/group, mean± SD). 30% of tumor take-up rate was observed for LNCaP-4B xenograft. (E) Western blot of lysates from LNCaP, LNCaP-4B (4B), and cells re-plated from LNCaP-4B xenograft tumor (-4BT).

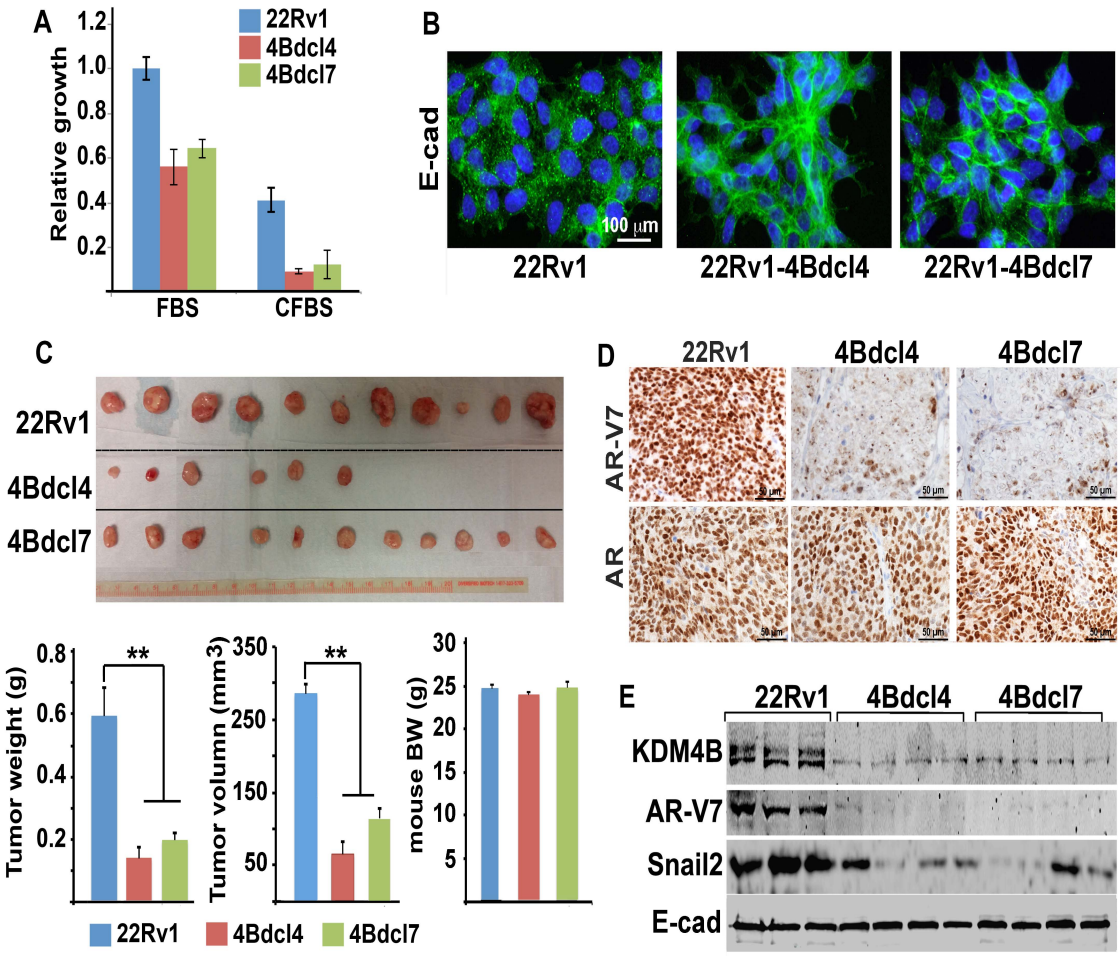

Figure 2. KDM4B knockdown inhibits CRPC growth in vivo and AR-V7 expression. (A) Relative growth of 22Rv1, and clonal 22Rv1 cells with KDM4B-heterozygously deleted (4Bdcl4 and 4Bdcl7). Equal amounts of cells were seeded in medium supplemented with FBS or CFBS. MTT assays were performed 3 days later. (B) Immunofluorescence micrographs of cells in (A) stained with E-cadherin (E-cad, green) and DAPI (blue). (C) Tumor xenografts derived from 22Rv1, 4Bdcl4, and 4Bdcl7 cells (upper panel). The lower panel shows tumor weight and volume, and castrated host mice weights at time of sacrifice. **, $p<0.01$. (D) Representative IHC staining of AR-V7 and AR in tumors of (C). (E) Western blot of indicated proteins from tumors of (C).

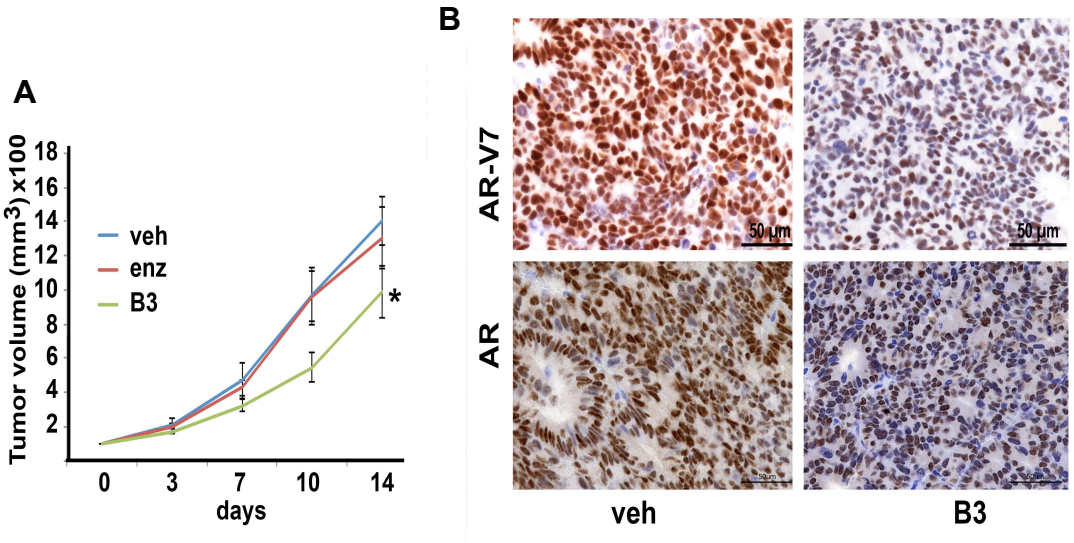

Figure 3. KDM4B inhibitor B3 sensitizes the resposnse of CRPC cell lines to enzalutamide and inhibits castration-resistant tumor growth. (A) Tumor growth curves of 22Rv1-xenografts treated with veh (n=6), enzalutamide (n=8), or B3 (n=11). Mean ± SEM, $p<0.05$. (B) IHC of AR and AR-V7 in tumors from (C).