

Award Number: W81XWH-15-1-0639

TITLE: The Role of BMI1 in CRPC

PRINCIPAL INVESTIGATOR: Qi Cao

CONTRACTING ORGANIZATION: METHODIST HOSPITAL RESEARCH INSTITUTE  
HOUSTON, TX 77030

REPORT DATE: October 2017

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

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

*Form Approved*  
*OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE</b> October 2017			<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED</b> 21 Sep 2016 - 20 Sep 2017	
<b>4. TITLE AND SUBTITLE</b>  The Role of BMI1 in CRPC					<b>5a. CONTRACT NUMBER</b>	
					<b>5b. GRANT NUMBER</b> W81XWH-15-1-0639	
					<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Qi Cao  E-Mail: qcao@houstonmethodist.org					<b>5d. PROJECT NUMBER</b>	
					<b>5e. TASK NUMBER</b>	
					<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  METHODIST HOSPITAL RESEARCH INSTITUTE 6565 Fannin St. Houston, TX 77030					<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012					<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
					<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b>  Approved for Public Release; Distribution Unlimited						
<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> Our preliminary data strongly suggest that BMI1 is a master regulator of castration-resistant prostate cancer (CRPC) progression. Our objective is to determine how BMI1 interacts with epigenetic complexes and with AR to regulate tumor suppressor gene expression. We aim to identify novel binding partners and regulators of oncogene expression, which will lead to a better understanding of AR signaling and dysfunction. Specifically, we will identify how BMI1 and PRC1 proteins mediate their oncogenic functions by recruiting AR and distinct binding partners to promote castration-resistance of PCa. Furthermore, we will evaluate the therapeutic efficacy of targeting BMI1 and of combinational targeting of BMI1 and AR in castration-resistant prostate cancer. During the first year of this project, we discovered that BMI1 directly binds to Androgen Receptor and prevents it from MDM2-mediated protein degradation. We further demonstrated that inhibiting BMI1 decreased prostate cancer tumor growth in VCaP murine xenograft.						
<b>15. SUBJECT TERMS: Polycomb, BMI1, Androgen Receptor, ubiquitination, PRC1, castration-resistant prostate cancer</b>						
<b>16. SECURITY CLASSIFICATION OF:</b>				<b>17. LIMITATION OF ABSTRACT</b>  Unclassified	<b>18. NUMBER OF PAGES</b>  16	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b>  Unclassified	<b>b. ABSTRACT</b>  Unclassified	<b>c. THIS PAGE</b>  Unclassified	<b>19b. TELEPHONE NUMBER</b> (include area code)			

## Table of Contents

	<u>Page</u>
<b>1. Introduction.....</b>	<b>3</b>
<b>2. Keywords.....</b>	<b>3</b>
<b>3. Accomplishments.....</b>	<b>4</b>
<b>4. Impact.....</b>	<b>12</b>
<b>5. Changes/Problems.....</b>	<b>12</b>
<b>6. Products.....</b>	<b>13</b>
<b>7. Participants &amp; Other Collaborating Organizations.....</b>	<b>14</b>
<b>8. Special Reporting Requirements.....</b>	<b>N/A</b>
<b>9. Appendices.....</b>	<b>N/A</b>

## **INTRODUCTION**

Each year, over 240,000 American men are diagnosed with prostate cancer (PCa). B lymphoma Mo-MLV insertion region 1 homolog (BMI1) have been shown associating with metastatic prostate cancer by cDNA microarray analyses and tissue microarray analysis. BMI1 is an epigenetic component of a Polycomb Repressive Complex 1 (PRC1), maintaining gene repression. We have demonstrated that BMI1 promotes prostate cancer progression by repressing multiple tumor suppressors. However, its precise role in castration-resistant prostate cancer (CRPC) remains unclear. Our preliminary data strongly suggest that BMI1 is a master regulator of castration-resistant prostate cancer (CRPC) progression. Our objective is to determine how BMI interacts with epigenetic complexes and with AR to regulate tumor suppressor gene expression. We aim to identify novel binding partners and regulators of oncogene expression, which will lead to a better understanding of AR signaling and dysfunction. Specifically, we will identify how BMI1 and PRC1 proteins mediate their oncogenic functions by recruiting AR and distinct binding partners to promote castration-resistance of PCa. Furthermore, we will evaluate the therapeutic efficacy of targeting BMI1 and of combinational targeting of BMI1 and AR in castration-resistant prostate cancer.

## **KEYWORDS**

BMI1, Prostate Cancer, Polycomb Repressive Complex, Androgen Receptor, Castration-Resistant Prostate Cancer, small molecule inhibitor

## ACCOMPLISHMENTS

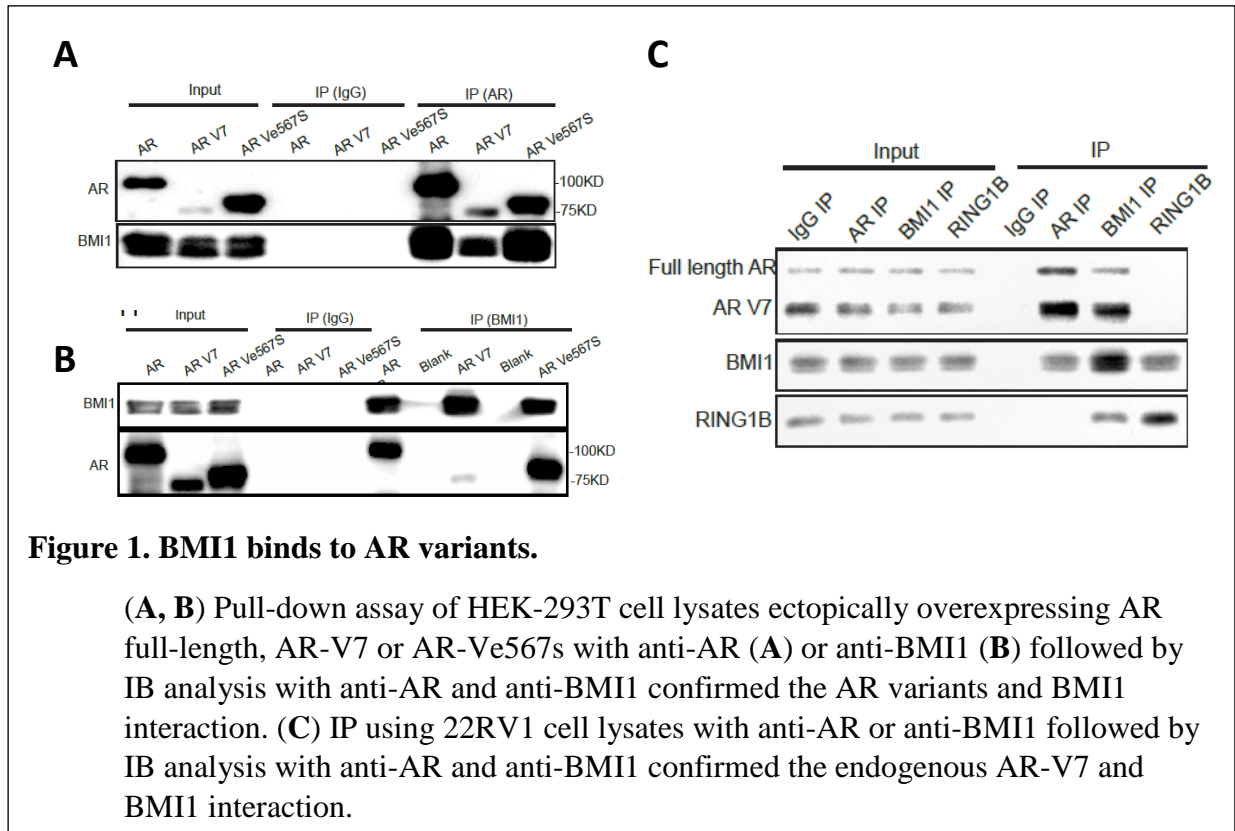
### A. What were the major goals of the project?

	Months	Percentage of completion
<b>Major Task 1: to elucidate the mechanism by which BMI1 interacts with AR and recruits AR</b>	1-24	100%
<b>Major Task 2: to dissect how BMI1 plays its role in androgen signaling</b>	1-36	70%
<b>Major Task 3: to evaluate BMI1 as a therapeutic target for advanced prostate cancer patient treatment.</b>	1-36	70%
<b>Milestone(s) Achieved: discovery of critical domains for AR and PRC1 protein interactions; determination of binding affinity of AR and PRC1, and set-up of a high-throughput platform for small molecule inhibition screening</b>	24	90%
<b>Milestone(s) Achieved: identification and characterization of novel binding partners and downstream targets of BMI1 and AR in androgen-dependent and -independent PCa cells, in the presence and absence of androgen.</b>	36	80%
<b>Milestone(s) Achieved: evaluation of BMI1 as a therapeutic target for CRPC patients and rationale for combinatorial targeting of AR and BMI1 in clinic trials; publication of 1-2 peer reviewed papers</b>	36	70%

### B. What was accomplished under these goals?

#### 1. BMI1 binds to AR variants

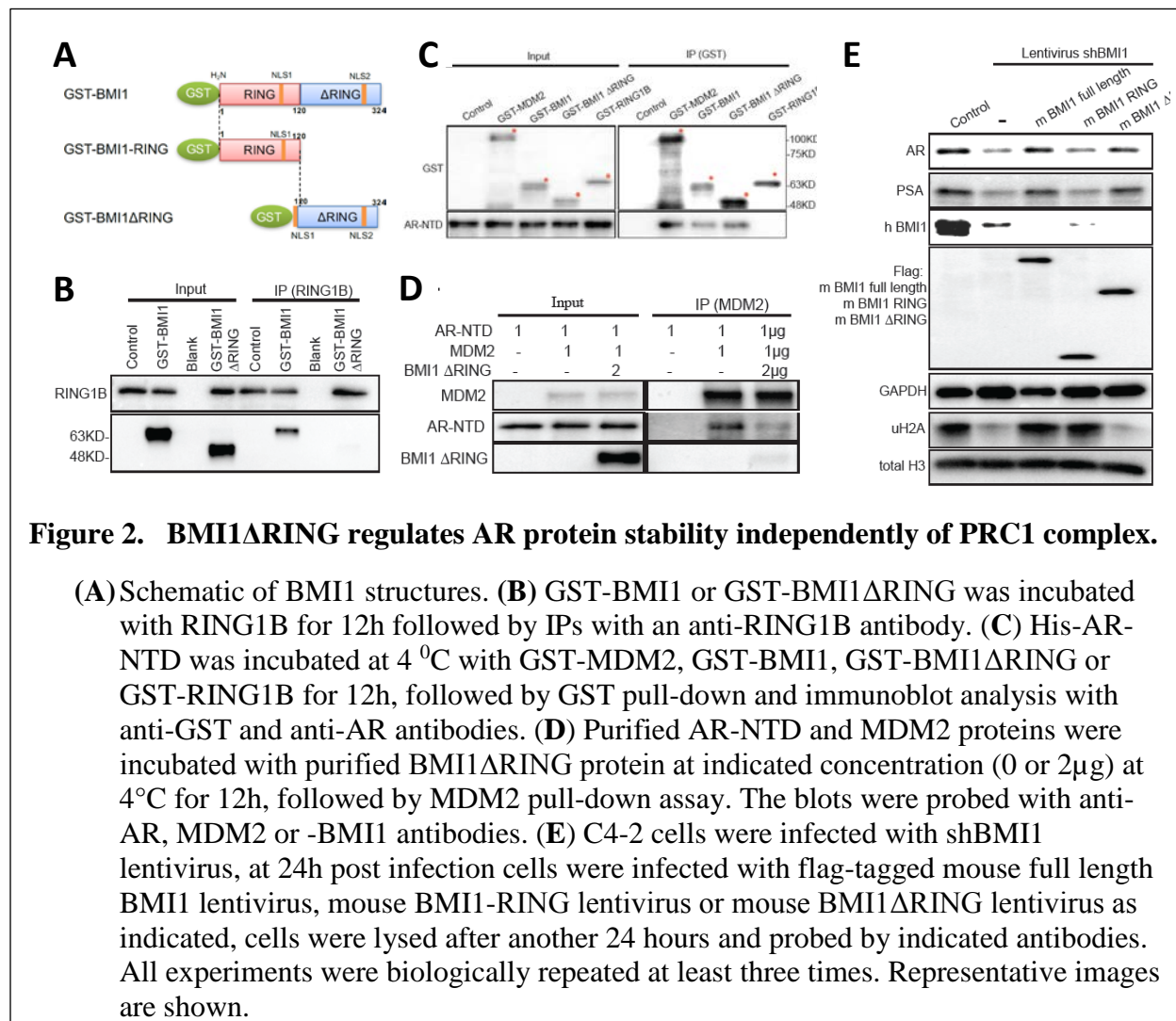
As reported last year, BMI1 binds to AR N-terminal domain (NTD). Since all AR variants still have NTD domain, we tested if BMI1 binds to these AR variants. Here we used AR-V7 and AR-Ve567s as examples. We overexpressed AR full-length (FL), AR-V7 or AR-Ve567s into 293T cells and then performed immunoprecipitation (IP) with anti-AR (Fig. 1A) or anti-BMI1 (Fig. 1B) and demonstrated that BMI1 interacts with full-length AR, as well as the AR variants. Further, we demonstrated the interaction between endogenous BMI1 and AR-V7 as well as AR full-length in 22RV1 cells (Fig. 1C) which harbors AR-V7 and AR-FL.



We generated BMI1 truncated mutants BMI1-RING and BMI1 $\Delta$ RING (Fig. 2A). As previously reported, we showed that BMI1 $\Delta$ RING did not bind to RING1B (Fig. 2B). However, BMI1 $\Delta$ RING, as well as BMI1 full-length and MDM2 (a previously reported AR binding protein) interacted with AR, but RING1B did not interact with AR (Fig. 2C).

## 2. BMI1 $\Delta$ RING inhibits MDM2-mediated AR degradation.

We next performed in vitro interaction competition assays using the purified AR-NTD, MDM2 and BMI1 $\Delta$ RING proteins. As shown in Fig. 2D, the presence of BMI1 $\Delta$ RING disrupted the interaction between MDM2 and AR. More importantly, ectopic overexpressing RNAi-resistant BMI1 $\Delta$ RING or BMI1 could completely restore the decrease of AR induced by BMI1 knockdown (Fig. 2E), demonstrating a novel PRC1-independent function of BMI1 through its  $\Delta$ RING domain.



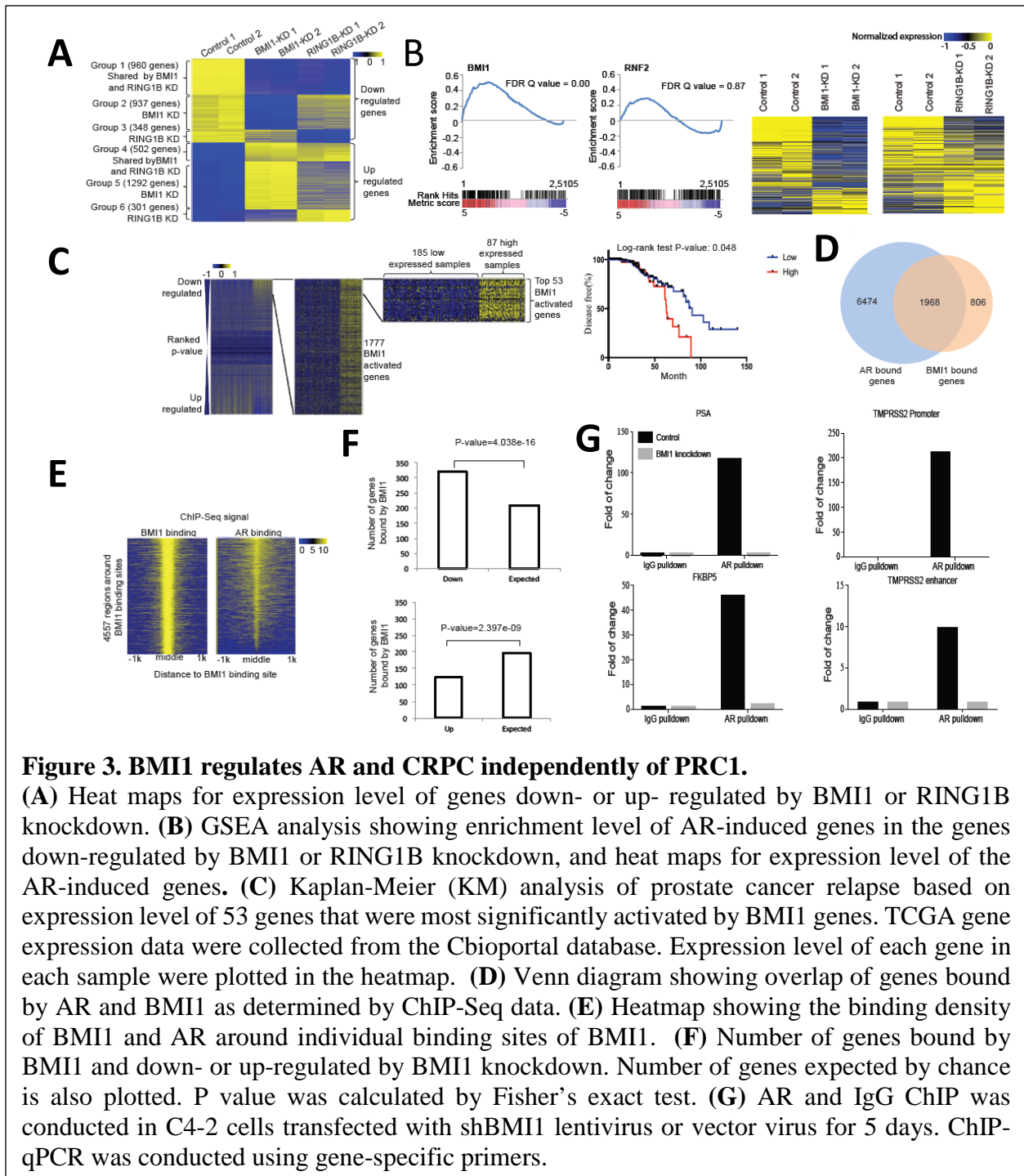
### 3. BMI1 regulates AR and prostate cancer progression independent of PRC1

To better understand the functions of BMI1 in CRPC, we performed RNA-Seq analysis using BMI1 or RING1B knockdown C4-2 cells. We observed that 1,794 genes were upregulated, while 1,897 genes were downregulated by knockdown of BMI1, suggesting that BMI1 functions as a transcriptional activator along with its well-known transcriptional repression role in cancer. Intriguingly, while a majority (69.2%) of RING1B-regulated genes were also regulated by BMI1, only 39.6% of BMI1 regulated genes were also regulated by RING1B, indicating that BMI1 plays an additional role separate from its canonical functions as a component of PRC1. Gene expression profiling analysis further revealed that genes regulated by BMI1 and/or RING1B could be clustered into six groups: downregulated (group 1) or upregulated (group 4) by both BMI1 and RING1B knockdown; downregulated (group 2) or upregulated (group 5) by BMI1, but not RING1B knockdown; and downregulated (group 3) or upregulated (group 6) by RING1B, but not BMI1 knockdown (Fig 3A). We analyzed 113 AR-regulated genes derived

from cell lines, human prostate cancer, and castration-resistant prostate cancer tissues, and found their expression levels significantly dysregulated by BMI1 knockdown, but not RING1B. Gene Set Enrichment Analysis (GSEA) using androgen-induced genes further confirmed that AR-activated genes were significantly enriched in genes down regulated in BMI1 knockdown, but not RING1B knockdown (Fig. 3B). Gene expression heat maps also confirmed that AR-induced genes were significantly down regulated by BMI1 knockdown but not RING1B (Fig. 3B). Intriguingly, KEGG pathway enrichment analysis revealed that BMI1 activated genes, but not BMI1 repressed genes, were associated with protein lysine degradation, prostate cancer, and several other cancers. Importantly, survival analysis of two prostate cancer gene expression data sets both revealed that higher expression levels of BMI1 activated genes (those downregulated by BMI1 knockdown) were significantly associated with poorer disease-free survival (Figure 3C). In addition, patients with mutations or copy-number alterations of these BMI1-activated genes also had shorter disease-free survival time, compared to patients without these genomic alterations.

To further investigate how BMI1 plays its role in AR signaling, we performed ChIP-Seq analysis using anti-AR and anti-BMI1 antibodies. Our analysis revealed that AR and BMI1 were recruited to 8,442 and 2,774 genes, respectively. Intriguingly, AR was recruited to 70.9% (2.11 fold larger than random expectation) of BMI1 occupied genes (Fig. 3D) and they shared the binding sites on most of these genes (Fig. 3E), suggesting that BMI1 may directly regulate AR targets. In addition, the genes bound by BMI1 significantly overlapped with BMI1-activated genes, but not with BMI1-repressed genes, suggesting that the binding of BMI1 on chromatin is more likely to activate transcription (Fig. 3F). ChIP-qPCR analysis further confirmed that BMI1 knockdown decreased the enrichments of AR and BMI1 in upstream regions of well-known AR (Fig. 3G) and BMI1 target genes.





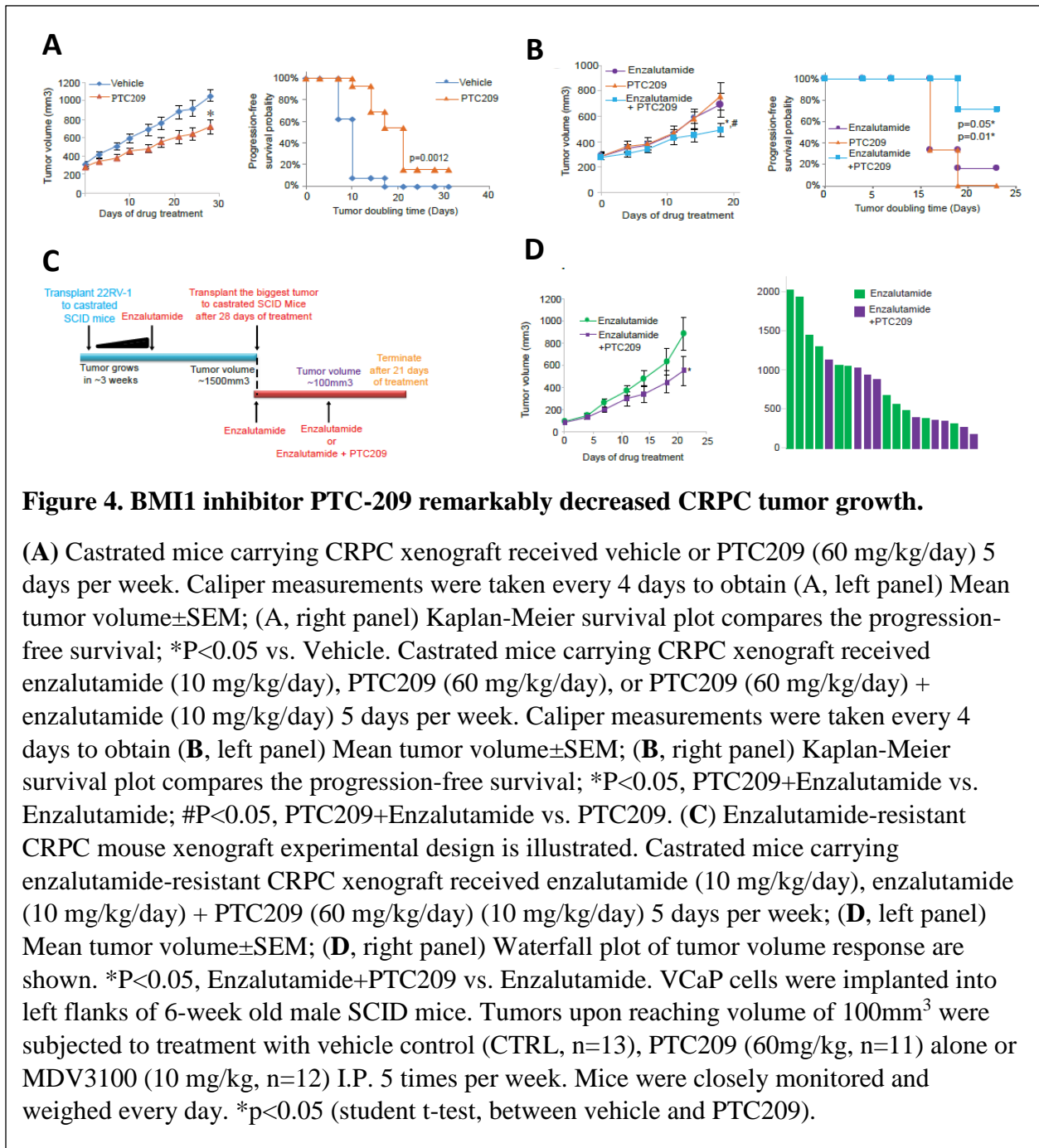
**Figure 3. BMI1 regulates AR and CRPC independently of PRC1.**

(A) Heat maps for expression level of genes down- or up- regulated by BMI1 or RING1B knockdown. (B) GSEA analysis showing enrichment level of AR-induced genes in the genes down-regulated by BMI1 or RING1B knockdown, and heat maps for expression level of the AR-induced genes. (C) Kaplan-Meier (KM) analysis of prostate cancer relapse based on expression level of 53 genes that were most significantly activated by BMI1 genes. TCGA gene expression data were collected from the Cbioportal database. Expression level of each gene in each sample were plotted in the heatmap. (D) Venn diagram showing overlap of genes bound by AR and BMI1 as determined by ChIP-Seq data. (E) Heatmap showing the binding density of BMI1 and AR around individual binding sites of BMI1. (F) Number of genes bound by BMI1 and down- or up-regulated by BMI1 knockdown. Number of genes expected by chance is also plotted. P value was calculated by Fisher's exact test. (G) AR and IgG ChIP was conducted in C4-2 cells transfected with shBMI1 lentivirus or vector virus for 5 days. ChIP-qPCR was conducted using gene-specific primers.

#### **4. BMI1 inhibitor PTC209 decreased tumor progression of CRPC xenograft.**

To assess the therapeutic effect of BMI1 in AR-positive PCa and CRPC, we utilized several mouse xenograft models. First, in the VCaP model we observed that, compared to vehicle control treatment, PTC209 treatment significantly inhibited tumor growth with no effect on body weight. Since our data strongly support that BMI1 regulates AR signaling in CRPC cells, and treatment of CRPC is still limited, we used a castration-resistant VCaP xenograft mouse model to evaluate therapeutic potential of BMI1 inhibition in CRPC, and PTC209 treatment significantly reduced tumor growth compared to vehicle control treatment (Fig. 4A left panel). An aggregate analysis of the initial tumor doubling time showed a significant decrease in the PTC209 treatment group compared with the vehicle group (Fig. 4A right panel). Mice bearing castration-resistant tumors demonstrated markedly faster progression of the disease compared with the treatment group. Next, we evaluated a potential synergistic effect of combination treatment with PTC209 and enzalutamide on CRPC tumor growth. While PTC209 showed similar inhibition potential as enzalutamide (Fig. 4B), combinatorial treatment of PTC209 and enzalutamide showed significantly better outcomes compared to each treatment alone and had no effect on body weight.

The rapid development of therapy resistance in prostate cancer patients subjected to enzalutamide treatment is becoming a major clinical challenge. One of the important mechanisms of enzalutamide-resistance is the increased expression of AR variants lacking the ligand binding-domain, the best characterized of which is AR-V7. To mimic enzalutamide-resistance in vivo, 20 castrated male SCID mice carrying xenografts of 22Rv1 tumors were treated with enzalutamide for 4 weeks, then sections of the biggest tumor (~1500 mm<sup>3</sup>) among these mice were transplanted to next passages of castrated male SCID mice treated with enzalutamide to maintain the enzalutamide-resistance. These mice were randomly divided into two groups when the tumors reached 100 mm<sup>3</sup>. One group was treated with PTC209 plus enzalutamide for 21 days, whereas the other group was treated with enzalutamide alone to serve as a control (Fig. 4C). Compared to the control, PTC209 treatment significantly decreased enzalutamide-resistant CRPC tumor growth (Fig. 4D), which suggests that BMI1 is a novel therapeutic target for clinically enzalutamide-resistant CRPC. There was no difference in body weight between these two groups



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

**Award/grant**

2017-2020, DoD PCRP Idea-Development Award (Established Investigator Option)  
2017-2022, R01 (NCI)

**National/International conferences attended**

Oct. 26-29, 2016, 23rd Annual Prostate Cancer Foundation Scientific Retreat, Carlsbad, CA

April 1- 5, 2017, AACR Annual Meeting, Washington DC

Jun 29-Jul 2, the 16th SCBA International Symposium, Hangzhou, China

**D. How were the results disseminated to communities of interest?**

Nothing to Report.

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

We are continuously working on this project and pursue the aims.

Besides the xenograft assays reported here (Fig. 4), we are evaluated if BMI1 inhibitor PTC-209 could inhibit the tumor growth using the patient-derived xenografts (PDXs). In addition, we are evaluating if the newly developed BMI1 inhibitor PTC596, which is in the phase I clinic trial, could inhibit tumor growth of CRPC and drug-resistant CRPC using the PDX models.

## **IMPACT**

- A. What was the impact on the development of the principal discipline(s) of the project?**

*Nothing to Report*

- B. What was the impact on other disciplines?**

*Nothing to Report*

- C. What was the impact on technology transfer?**

*Nothing to Report*

- D. What was the impact on society beyond science and technology?**

*Nothing to Report*

## **CHANGES/PROBLEMS**

- A. Changes in approach and reasons for change**

*Nothing to Report*

- B. Actual or anticipated problems or delays and actions or plans to resolve them**

*Nothing to Report*

- C. Changes that had a significant impact on expenditures**

*Nothing to Report*

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

*Nothing to Report*

- E. Significant changes in use or care of human subjects**

*Nothing to Report*

- F. Significant changes in use or care of vertebrate animals.**

*Nothing to Report*

- G. Significant changes in use of biohazards and/or select agents**

*Nothing to Report*

## PRODUCTS:

### A. Publications, conference papers, and presentations

#### a. Journal publications.

*Two manuscripts are under review/revision*

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

*Nothing to Report*

#### c. Other publications, conference papers, and presentations.

Poster presentation

1) Sen Zhu, Jungsun Kim, Bingnan Gu, Weihua Jiang, Lin Yan, Ladan Fazli, Jonathan Zhao, Xuesen Dong, Jindan Yu, Qi Cao. A Novel Role of BMI1 in Androgen Receptor Pathway. 23<sup>rd</sup> Prostate Cancer Foundation Annual Scientific Retreat, Oct 26-29, 2016, Carlsbad, CA

Oral presentations

Jun 1, 2016 The Coordinated Regulation of Polycomb Group Proteins in Cancer, Department of Pharmacology, University of Maryland School of Medicine, Baltimore, MD

Jun 13, 2017 A Novel Role for BMI1 in Prostate Cancer, Department of Urology, Peking University First Hospital, Beijing, China

Jun 23, 2017 Epigenetic Regulation of Prostate Cancer, Center for Regeneration Medicine and Stem Cell Engineering, Sun Yat-sen University Zhangshan School of Medicine, Guangzhou, China

Jul 1, 2017 A Novel Role for BMI1 in Prostate Cancer, the 16th SCBA International Symposium, Hangzhou, China

Sep 28, 2017 A Novel Role for BMI1 in Prostate Cancer, Annual Summit on Cell Signaling and Cancer Therapy, Chicago, IL

### B. Website(s) or other Internet site(s)

*Nothing to Report*

### C. Technologies or techniques

*Nothing to Report*

### D. Inventions, patent applications, and/or licenses

*Nothing to Report*

### E. Other Products

*Nothing to Report*

## PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

### A. What individuals have worked on the project?

Name:	<i>Qi Cao</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	<i>Conceive the idea, lead the project, design experiments and analyze the data</i>
Funding Support:	<i>DoD PCRP IDA, Prostate Cancer Foundation, American Cancer Society, Start-up</i>

Name:	<i>Sen Zhu</i>
Project Role:	<i>Post-Doctoral</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	<i>Perform major experiments and analyze the data</i>
Funding Support:	<i>DoD PCRP IDA, Prostate Cancer Foundation, American Cancer Society, Start-up</i>

Name:	<i>Weihua Jiang</i>
Project Role:	<i>Post-Doctoral</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	<i>Perform xenograft experiments and analyze the data</i>
Funding Support:	<i>Prostate Cancer Foundation, American Cancer Society, Start-up</i>

Name:	<i>Lin Yan</i>
-------	----------------

Project Role:	<i>Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	5
Contribution to Project:	<i>Help Drs. Zhu and Jiang perform xenograft experiments and molecular and cellular assays, and analyze the data</i>
Funding Support:	<i>Prostate Cancer Foundation, American Cancer Society, Start-up</i>

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

**C. What other organizations were involved as partners?**

1. Collaborator Names: Jindan Yu and Jonathan Zhao  
**Organization Name:** Northwestern University Feinberg School of Medicine  
**Location of Organization:** Chicago, IL, USA  
**Partner's contribution to the project:** **Collaboration**
  
2. Collaborator Names: Xuesen Dong and Ladan Fazli  
**Organization Name:** Vancouver Prostate Centre and Department of Urologic Sciences, University of British Columbia  
**Location of Organization:** Vancouver, BC, Canada V6H 3Z6  
**Partner's contribution to the project:** **Collaboration, Facilities**
  
3. Collaborator Names: Ming Hung  
**Organization Name:** Department of Urology, University of Washington  
**Location of Organization:** Seattle, WA, USA  
**Partner's contribution to the project:** **Collaboration**
  
4. Collaborator Names: Xiaobing Shi  
**Organization Name:** Department of Epigenetics, MD Cancer Center  
**Location of Organization:** Houston, TX, USA  
**Partner's contribution to the project:** **Collaboration**
  
5. Collaborator Names: Kaifu Chen, Dongyu Zhao, Jie Lu  
**Organization Name:** Center for Cardiovascular Sciences, Houston Methodist Research Institute  
**Location of Organization:** Houston, TX, USA  
**Partner's contribution to the project:** **Collaboration**