

AWARD NUMBER: W81XWH-13-1-0202

TITLE: Functional Genomics to Identify Therapeutic Targets in Cancer Stem Cells
Using a Novel Murine CRPC Model

PRINCIPAL INVESTIGATOR: Dr. Guocan Wang

CONTRACTING ORGANIZATION: The University of Texas MD Anderson Cancer Center
Houston, TX 77030

REPORT DATE: SEPTEMBER 2014

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: **A:** Approved for public release; distribution is
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.					
1. REPORT DATE SEPTEMBER 2014		2. REPORT TYPE Annual		3. DATES COVERED 6 Aug 2013 - 5 Aug 2014	
4. TITLE AND SUBTITLE Functional Genomics to Identify Therapeutic Targets in Cancer Stem Cells Using a Novel Murine CRPC Model				5a. CONTRACT NUMBER W81XWH-13-1-0202	
				5b. GRANT NUMBER W81XWH-13-1-0202	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Guocan Wang E-Mail: GWang6@mdanderson.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas MD Anderson Cancer Center 1515 Holcombe Boulevard Houston, TX 77030-4009				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 is the most common noncutaneous malignancy in men in the United States ¹ . Prostate cancer after Androgen deprivation therapy (ADT) will invariably become refractory to castration, resulting in the development of lethal castration resistant prostate cancer (CRPC), which remains incurable. It is generally believed that prostate stem cells can be the cell of origin for prostate cancers accounting for the development of CRPC, but the identity of prostate cancer stem cells in CRPC remains elusive. Here I used a metastatic Pten ^{pc/-} Smad4 ^{pc/-} mouse prostate cancer model to study the mechanisms for CRPC and the biology of cancer stem cells. As compared to Pten ^{pc/-} tumors, I discovered that Pten ^{pc/-} Smad4 ^{pc/-} tumors are resistant to ADT, showed both basal and luminal phenotypes with increased proliferation. Furthermore, ADT in Pten ^{pc/-} Smad4 ^{pc/-} mice may promote massive lung metastasis. Interestingly, by integrative analysis of microarray datasets I identified pathways that may play a role in the resistance to ADT and cancer stem cells, including the Rb/E2Fs and Yap1, which will be subjected to functional validation in vitro and in vivo. Thus our metastatic mouse prostate cancer model will facilitate the process of identifying novel therapeutic targets for CRPC using a functional genomics approach.					
15. SUBJECT TERMS Prostate cancer, Genetically engineered mouse model, Castration resistance prostate cancer, cancer stem cell.					
16. SECURITY CLASSIFICATION OF: U			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Overall Project Summary	4
4. Key Research Accomplishments	11
5. Conclusion	11
6. Publications, Abstracts, and Presentations	12
7. Inventions, Patents and Licenses	12
8. Reportable Outcomes	12
9. Other Achievements	12
10. References	12
11. Appendices	13

1. **INTRODUCTION:** Prostate cancer is the most common noncutaneous malignancy in men in the United States[1]. Even though androgen deprivation therapy (ADT), the mainstay of systemic therapy for prostate cancer, has an initial success rate of almost 90%[2], prostate cancer invariably becomes refractory to castration, resulting in the development of lethal castration resistant prostate cancer (CRPC). Although several novel therapies, such as CYP17 inhibitor Abiraterone[3] and the next generation AR inhibitor MDV3100[4], significantly prolong the survival of CRPC patients, there remains a critical need for a better understanding the molecular mechanisms for the development of CRPC. Despite the debatable nature of the prostate stem cells, it is generally believed that prostate stem cells can be the cell of origin for prostate cancers accounting for the development of CRPC. However, the identity of prostate cancer stem cells in CRPC remains elusive, in part due to the lack of amenable animal models with defining properties of human CRPC, including castration-resistance and lethal metastasis. Our pilot study in our metastatic *Pten^{pc/-}Smad4^{pc/-}* mouse model demonstrated a significant tumor growth delay and better survival in the castrated cohort as compared with the control, which recapitulates the temporary benefits of ADT for human prostate cancer patients. However, the castrated mice eventually developed CRPC with metastatic spread to lymph nodes and distant organs (data not shown). Furthermore, BrdU labeling shows an increased in proliferating cells in castrated *Pten^{pc/-}Smad4^{pc/-}* prostate tumors as compared to *Pten^{pc/-}*. Interestingly, it has been demonstrated that Smad4 interacts with AR and regulates AR signaling[5], suggesting a potential role for Smad4 in the development of CRPC. Thus our metastatic prostate cancer model will allow us to generate a humanized CRPC mouse model which will facilitate the process of identifying novel therapeutic targets for CRPC using a functional genomics approach.
2. **KEYWORDS:** Prostate cancer, Genetically engineered mouse model, Castration resistance prostate cancer, cancer stem cell.
3. **OVERALL PROJECT SUMMARY:** Summarize the progress during appropriate reporting period (single annual or comprehensive final). This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW in a summary of Current Objectives, and a summary of Results, Progress and Accomplishments with Discussion. Key methodology used during the reporting period, including a description of any changes to originally proposed methods, shall be summarized. Data supporting research conclusions, in the form of figures and/or tables, shall be embedded in the text, appended, or referenced to appended manuscripts. Actual or anticipated problems or delays and actions or plans to resolve them shall be included. Additionally, any changes in approach and reasons for these changes shall be reported. **Any change that is substantially different from the original approved SOW (e.g., new or modified tasks, objectives, experiments, etc.) requires review by the Grants Officer's Representative and final approval by USAMRAA Grants Officer through an award modification prior to initiating any changes.**
Aim 1. Biological characterization of CRPC and tumor-repopulating cells.
Current objects: (a) To characterize the response of *Pten^{pc/-}Smad4^{pc/-}* mutant mice to ADT. We have performed castration in a larger cohort of mice and generated cumulative

survival curve for this cohort (Fig.1). We have also collected prostate tissues and non-

prostate tissue from major organs such as bone, lung, and lymph nodes from these mice. In addition, lung and bone tissues from *Pten^{pc/-}Smad4^{pc/-}* mutant mice with lethal CRPC will be examined for increased lung metastasis and bone metastasis. (b) To characterize cancer stem cells in the treatment naïve prostate tumors and CRPC tumors.

Methodology: Wild type, *Pten^{pc/-}*, and *Pten^{pc/-}Smad4^{pc/-}* mice are subjected to surgical castration or in combination with MDV3100 treatment (10 mg/kg daily oral gavage). 5 mice will be sacrificed at 2, 4, 8, and 12 weeks post ADT and tumors and other tissues, such as lung, bones, and lymph

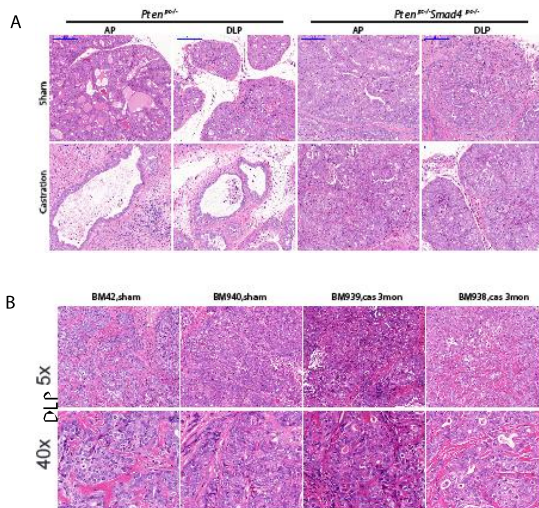


Fig 1. H & E staining of tumors from sham control and castrated mice (*Pten^{pc/-}Smad4^{pc/-}* and *Pten^{pc/-}* mice) at 1 month (A) and 3 months (B) post-ADT

nodes, are harvested for histopathological analysis, RNA isolation, Chromatin immunoprecipitation, Western blot analysis, and cancer stem cell sphere assay.

Results:

1. *Pten^{pc/-}Smad4^{pc/-}* tumors are resistant to surgical castration as well as surgical castration plus MDV3100 (also called Enzalutamide). I performed surgical castration alone or in combination with MDV3100 treatment in *Pten^{pc/-}Smad4^{pc/-}* and *Pten^{pc/-}* mice. Tumors

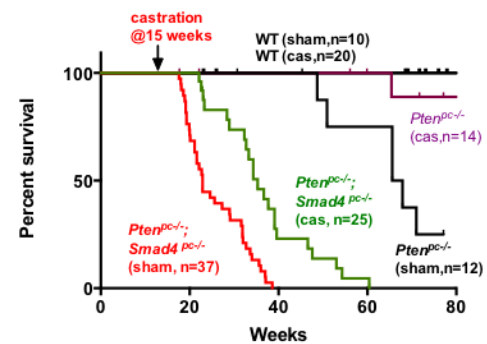


Fig 3. Kaplan Meier survival analysis.

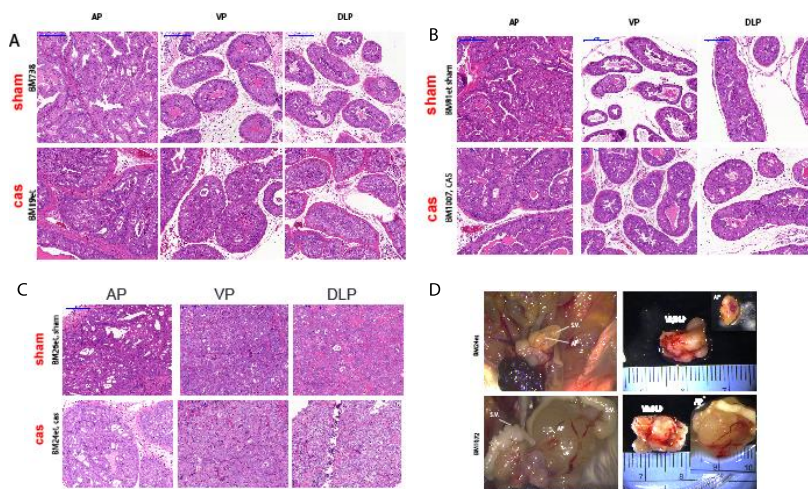


Fig 2. in *Pten^{pc/-}Smad4^{pc/-}* tumors are resistance to MDV3100. (A) 2 weeks, (B) 4 weeks, and (C-D) 12 weeks post-castration+MDV3100 treatment.

were harvested at 2, 4, 8, and 12 weeks post-ADT and subjected to histopathological analysis. In consistent with previous report [6, 7], *Pten^{pc/-}* tumors are sensitive to ADT (Fig 1A). A significant amount of normal epithelium was identified in castrated *Pten^{pc/-}* mice at 1 and 2 months post-castration, suggesting a clear response to castration. Interestingly, the stroma in

the castrated *Pten*^{pc/-} mice is significantly expanded, which may account for the emergence of CRPC in *Pten*^{pc/-} mice 3 months post-castration[6, 7]. On the contrary, *Pten*^{pc/-}*Smad4*^{pc/-} mice are resistant to castration as compared to *Pten*^{pc/-} as shown by

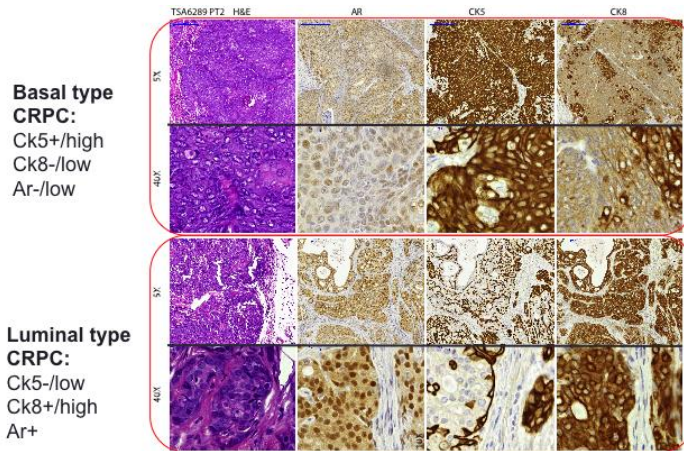


Fig 4. *Pten*^{pc/-}*Smad4*^{pc/-} CRPC tumors display basal and luminal phenotype.

histopathological analysis by H & E staining (Fig 1A) and MRI analysis (data not shown). There is no noticeable normal epithelium in the castrated *Pten*^{pc/-}*Smad4*^{pc/-} mice 1, 2, and 3 months post-castration (Fig 1), suggesting loss of *Smad4* in a *Pten*-null genetic context confers a de novo resistance to ADT. Furthermore, *Pten*^{pc/-}*Smad4*^{pc/-} prostate tumors are also resistant to a comprehensive AR blockade using surgical castration plus treatment with Enzalutamide, a potent AR inhibitor used in clinic

for metastatic CRPC, as shown by H & E staining (Fig 2) and MRI analysis (data not shown). Although ADT did slightly prolong the survival of *Pten*^{pc/-}*Smad4*^{pc/-} mice, they eventually succumbed to lethal CRPC (Fig 3), suggesting that this unique model may allow the identification of novel pathways that may play a role in the development of CRPC as well as resistance to MDV3100.

2. *Pten*^{pc/-}*Smad4*^{pc/-} CRPCs display both luminal and basal phenotypes. To fully

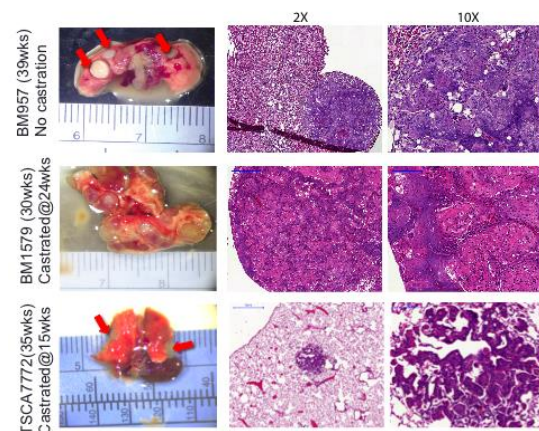


Fig 5. ADT promote massive lung metastasis.

characterize the lethal CRPC developed at ~3 months post castration, I performed H & E staining and immunohistochemical staining using various verified antibodies for prostatic epithelial origin, such as luminal cell markers (CK8, CK18, Nkx3.1, and AR), and basal cell markers (CK14, CK5, p63). In addition, I also performed cyclin D1 and Ki67 staining to assess the proliferation of these CRPCs. Interestingly, IHC staining shows these CRPCs from *Pten*^{pc/-}*Smad4*^{pc/-} mice display both luminal and basal phenotypes, which are CK18⁺/AR⁺CK5^{low} and CK18^{low}/AR^{low} CK5^{hi} respectively (Fig 4). Consistent with human CRPCs, Ar is reactivated in *Pten*^{pc/-}*Smad4*^{pc/-} tumors as shown by the nuclear staining of Ar (Fig 4). Interestingly, in the treatment

naïve prostate *Pten*^{pc/-}*Smad4*^{pc/-} tumors, Ar is expressed in the majority of the cells (data not shown). However, some AR⁺ tumor cells also expressed strong basal cell markers Ck5 (data not shown), suggesting that such basal type AR⁺ cells downregulate Ar expression in the CRPC tumors.

3. ADT may promote tumor progression a subset of aggressive *Pten*^{pc/-}*Smad4*^{pc/-} tumors. Given the *Pten*^{pc/-}*Smad4*^{pc/-} mice developed lung metastasis, I examined all control and

castrated *Pten*^{pc/-}*Smad4*^{pc/-} mice for lung metastasis. Interestingly, a subset of *Pten*^{pc/-}*Smad4*^{pc/-} mice (n=2) developed massive metastasis in the lung (Fig 5. Middle panel), which is not observed in control mice (Fig 5, top panel), suggesting that although ADT provide some survival benefits for the *Pten*^{pc/-}*Smad4*^{pc/-} mice, it can possibly promote dissemination or the growth of the metastatic tumor cells.

4. Castrated *Pten*^{pc/-}*Smad4*^{pc/-} tumors display increased proliferation as compared to *Pten*^{pc/-} tumors. To assess the proliferative potential of the prostate tumor

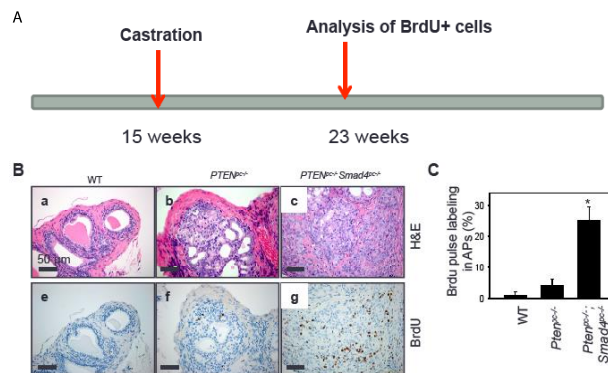


Fig 6. BrdU incorporation experiment in castrated mice. (A) IHC staining for BrdU and (B) quantification of BrdU positive cells.

cells undergone ADT, I performed BrdU incorporation assay. Briefly, BrdU was injected into mice at the age of 15 weeks (wild type, *Pten*^{pc/-}, and *Pten*^{pc/-}*Smad4*^{pc/-} mice) and BrdU incorporation was determined by IHC using a BrdU specific antibody. As shown in Fig 6, castrated *Pten*^{pc/-}*Smad4*^{pc/-} tumors contained significantly more BrdU positive cells than the castrated *Pten*^{pc/-} tumors, which is in line with the de novo resistance to ADT observed in the *Pten*^{pc/-}

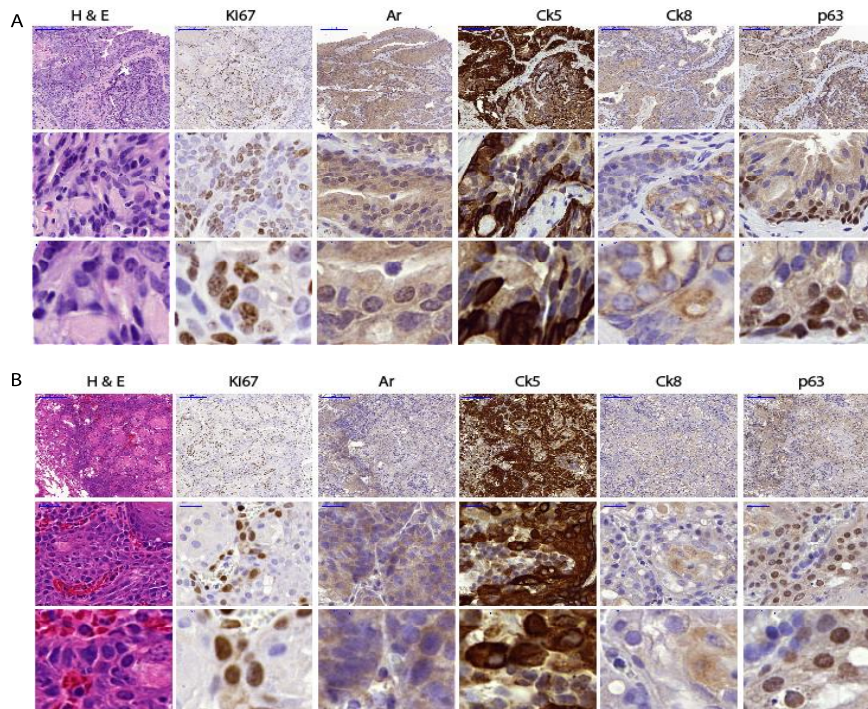


Fig 7. H & E staining and Ki67, Ar, Ck5, Ck9, p63 IHC CRPC primary tumors (A) and lung metastasis (B)

Smad4^{pc/-} tumors. Furthermore, Ki67 staining of the primary tumor and the lung metastasis from the castrated *Pten*^{pc/-}*Smad4*^{pc/-} mice shows that *Pten*^{pc/-}*Smad4*^{pc/-} CRPCs are highly proliferative (Fig 7). In addition, p63+ cells are significantly increased in the castrated *Pten*^{pc/-}*Smad4*^{pc/-} mice (Fig7), suggesting the basal cells contribute significantly to the aggressiveness of

the *Pten*^{pc/-}*Smad4*^{pc/-} CRPCs.

5. Cyclin D1 positive cells are mainly CK5+ cells. Since Cyclin D1 has been shown to be overexpressed in the treatment naïve *Pten*^{pc/-}*Smad4*^{pc/-} tumors (ref, Ding 2011), I

performed IHC for cyclin D1, Ar, and CK5, in the *Pten^{pc/-}Smad4^{pc/-}* CRPCs. Interestingly, Cyclin D1 is highly expressed in the *Pten^{pc/-}Smad4^{pc/-}* CRPCs, which also display high CK5 expression and low Ar expression, suggesting that the majority of the cyclin D1+ cells in the *Pten^{pc/-}Smad4^{pc/-}* CRPCs are of basal cell properties, which also renders them less dependent on Ar signaling and thus are resistant to ADT.

Progress, accomplishment and discussion:

I have generated a larger experimental cohort and have completed the Kaplan Meier

Survival analysis. The mouse prostate tumors have been subjected to extensive histopathological analysis. I will further characterize the cancer stem cells in the treatment naïve and castration resistant prostate tumors from *Pten^{pc/-}Smad4^{pc/-}* mice.

Aim 2. Genomic and transcriptomic analysis of CRPC and cancer stem cells.

Current objects: The goal of this aim is to utilize powerful genomic profiling technologies to identify novel genes and

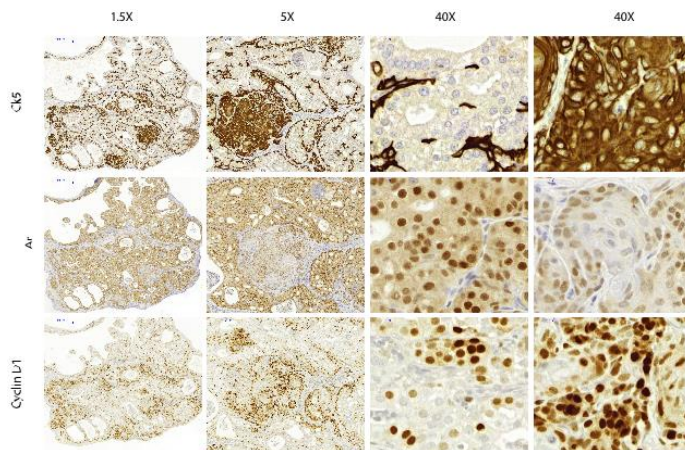


Fig. 8. Cyclin D1 positive cells are mainly CK5+ cells.

pathways that drive the development of CRPC. **Methodology:** To enable unambiguous distinction between tumor and stroma, we incorporated a dual fluorescent reporter allele, Rosa26-Lox-tdTomato-Lox-EGFP (a.k.a. mTmG)26, into the PB-Cre+ *Pten^{L/L} Smad4^{L/L}* model. This allele allows Cre-dependent GFP expression in prostate epithelial cells as well as ubiquitous tdTomato expression in all other cells (Figure 9). We now can (1) visualize tumor and stroma distinctly by fluorescence imaging, (2) visually quantify metastases in lymph node and lung without laborious histological inspection, and (3) easily isolate tumor and stromal cells using flow cytometry or Laser Capture Microdissection. Furthermore, to enable noninvasive bioluminescence imaging (BLI) of tumor and metastasis development,

we have further incorporated Rosa26-Lox-STOP-Lox-Luciferase allele and have confirmed the prostate-specific luciferase signal (Figure 9). Thus, we now have established a robust metastatic PCa model: PB-Cre+ *Pten^{L/L} Smad4^{L/L} mTmG^{L/+} Luc^{L/+}*, which will facilitate monitoring of tumor development and tumor-stroma interactions through dual fluorescence and bioluminescence imaging. In parallel, we have also established a comparable model with only *Pten* loss: PB-Cre+ *Pten^{L/L} mTmG^{L/+} Luc^{L/+}*. It is important to note

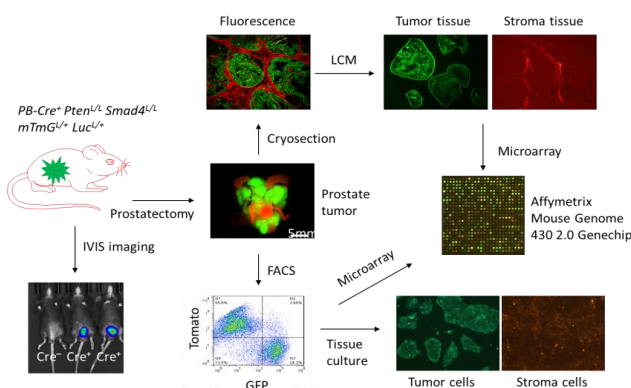


Figure 9. A novel metastatic prostate GEMM with fluorescence and luciferase reporters for noninvasive tracking of tumor growth as well as phenotypic and molecular dissection of tumor and stroma.

that we have now backcrossed these strains to C57BL/6 congenic background, which enables syngeneic transplantation.

Results:

1. Transcriptomic profiling of GFP+ tumor cells and Tomato+ stromal cells from *Pten^{pc/-}* *Smad4^{pc/-}* shows a distinct expression pattern for these two populations. We isolated GFP+

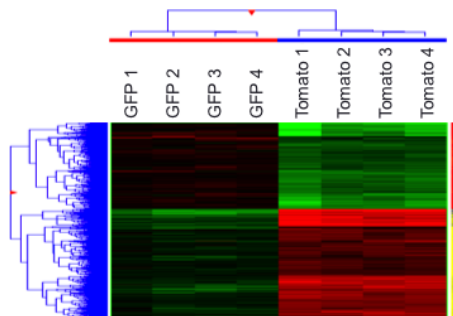


Fig 10. Hierarchical Clustering Analysis of GFP+ tumor cells and Tomato+ stromal cells.

tumor cells and Tomato+ stromal cells, from which RNAs were isolated and subjected to microarray analysis. As expected, tumor and stroma showed distinct expression pattern by Hierarchical Clustering Analysis (Figure 10), and IPA indicated that while tumor cells are enriched for pathways involving cell adhesion molecules and tight junction (consistent with their epithelial nature), stromal cells display activation of more diverse signaling pathways involved in chronic inflammation, such as cytokine/cytokine receptor interaction, chemokine signaling pathway, Jak-STAT pathway, TCR pathway, and BCR pathway

($p < 0.01$, data not shown).

2. Integrative transcriptomic analysis identified tumor and stroma specific genes which may play a role in CRPC and cancer stem cells. I performed an integrative analysis of our published dataset GSE25140 (*Pten^{pc/-}* *Smad4^{pc/-}* vs. *Pten^{pc/-}*) and my new dataset (tumors vs. stroma (Tomato+)).

- Ar activity is suppressed in *Pten*/*Smad4* tumors as compared to *Pten* (Fig 11) as shown by Gene Enrichment Analysis (GSEA). This pathway is identified as the

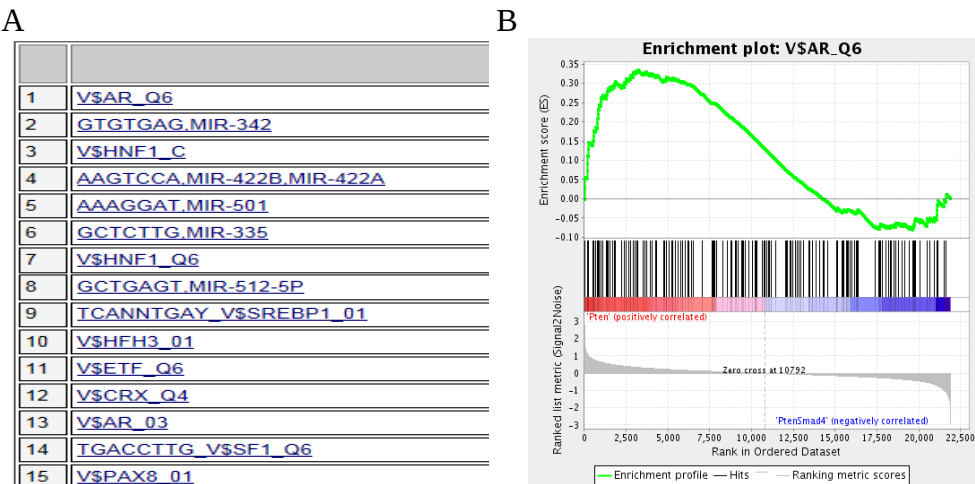


Fig 11.

top pathways when compared *Pten^{pc/-}* *Smad4^{pc/-}* to *Pten^{pc/-}*, which is in line with the histopathological findings described previously. Given the potential cross-talk between Tgfbeta/Smads pathways with AR signaling as Smad4 and Smad3 can

interact with AR, it's possible that loss of Smad4 leads to a reprogramming of AR signaling pathways which renders the *Pten^{pc/-}Smad4^{pc/-}* tumors less dependent on AR signaling in ADT. Interestingly, total Smad3/Smad2 and phosphor-Smad2/3 expression are increased in *Pten*/Smad4 as compared to *Pten* tumors.

- Rb/E2Fs pathways are deregulated in the *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors (Fig 12 B-C). Rb/E2Fs pathway deregulation has been shown

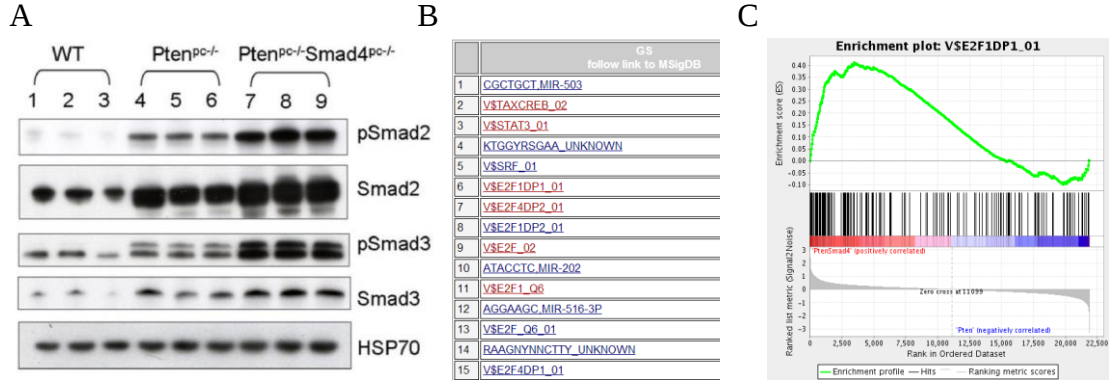


Fig 12. (A). Increased expression of Smad2/Smad3 and phosphor-Smad2/3 in *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors. **(B)** Top 15 pathways activated in *Pten^{pc/-}* tumors as compared to the *Pten^{pc/-}Smad4^{pc/-}* tumors. **(C)** AR activation is suppressed in *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors.

to play an important role in human CRPC[8]. Interestingly, GSEA analysis identified multiple E2Fs signatures as the top 15 activated pathways in the *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors, which is in line with the de novo resistance to ADT observed in the *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors.

- YAP1 is activated in the *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors. In addition to the Rb/E2Fs pathways, a novel pathway, Hippo/YAP1, is

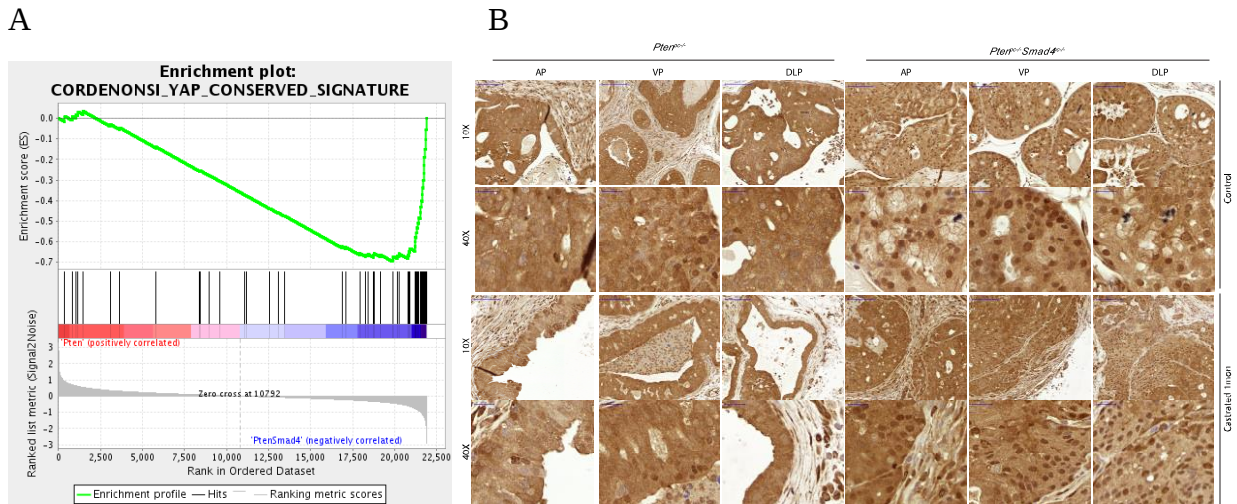


Fig 13. YAP1 pathways may play a role in the de novo resistance to ADT in the *Pten^{pc/-}Smad4^{pc/-}* tumors. **(A)** YAP1 signature identified in the *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors. **(B)** IHC staining of YAP1 in treatment naïve and castrated mice shows an increase in nuclear of YAP1 in *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to *Pten^{pc/-}* tumors.

identified as one of the top 10 activated oncogenic pathway signatures (GSEA C6 category, Fig 13 A). Furthermore, IHC staining for YAP1 shows a significant increase in the nuclear staining of YAP1 in the treatment naïve and CRPCs from *Pten^{pc/-}Smad4^{pc/-}* tumors as compared to the *Pten^{pc/-}* tumors (Fig 13B), suggesting that Hippo/YAP1 pathways may play a direct role in the de novo resistance to ADT for the *Pten^{pc/-}Smad4^{pc/-}* tumors. Given the role of YAP1 in stem cells[9, 10], it's possible that deregulation of Hippo/YAP1 pathways may play a role in the prostate cancer stem cells, which contribute to the observed CRPC phenotypes in *Pten^{pc/-}Smad4^{pc/-}* tumors.

Progress, accomplishment and discussion:

I have performed integrative analysis of our previous published microarray dataset GSE25140 & this tumor/stroma microarray dataset identified novel pathways that may play an import role in tumor progression, resistance to castration and cancer stem cells. Tumors from castrated mice and sham control mice will be subjected to RNA-seq analysis and additional candidate genes for CRPC and cancer stem cells will be subjected to functional validation in vitro and in vivo. In addition, I will continue to investigate the role of the novel Hippo/YAP1 pathway in the development of CRPC and cancer stem cells.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Completed the Kaplan-meier survival analysis of Pten/Smad4 mice with a large cohort.
- Comprehensive histopathological analysis of CRPC in the Pten/Smad4 mice suggests an expansion of the prostate tumor cells that express basal cell markers, such as Ck5 and p63. These basal type CRPC cells are highly proliferative, suggesting it may contain the cancer stem cell population.
- Transcriptome profiling of GFP+ tumor cells and Tomato+ cells from my refilled Pten/Smad4/mTmG model identified tumor- and stroma-specific genes and pathways
- Integrative analysis of our published microarray dataset GSE25140 and the new Pten/Smad4 tumor/stroma dataset identified novel pathways that may play an important role in prostate cancer progression, metastasis, and resistance to ADT and resistance to MDV3100, such as Rb/E2Fs, AR, and Hippo/YAP1 pathways.

5. **CONCLUSION:** The understanding the role of cancer stem cells in the development of castration resistant prostate cancer and identified genes that play an important role in prostate cancer stem cells will not only offer potential therapeutic targets but also biomarkers for CRPC. I have collected CRPC tumor samples for proofing. In addition, I have identified novel pathways that may plan an important role in prostate cancer progression, metastasis, and CRPC. I will continue to characterize the cancer stem cell in the Pten/Smad4 mouse model. In addition, genomic/transcriptomic profiling of the CRPC and cancer stem cells will result in candidate genes for functional validation. Furthermore, the novel genes and pathways which were identified by integrative analysis of our published microarray dataset GSE25140 and our tumor/stroma microarray dataset will be subjected to functional validation for their involvement in cancer stem cells and CRPC.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

- a. List all manuscripts submitted for publication during the period covered by this report resulting from this project.
Nothing to report
- b. List presentations made during the last year (international, national, local societies, military meetings, etc.).
Nothing to report

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report

8. REPORTABLE OUTCOMES: Nothing to report

9. OTHER ACHIEVEMENTS: Nothing to report

10. REFERENCES: List all references pertinent to the report using a standard journal format (i.e., format used in *Science*, *Military Medicine*, etc.).

- 1. Jemal, A., et al., Cancer Statistics, 2010. CA: A Cancer Journal for Clinicians, 2010. 60(5): p. 277-300.
- 2. Bubley, G.J. and S.P. Balk, Treatment of Androgen-Independent Prostate Cancer. Oncologist, 1996. 1(1 & 2): p. 30-35.
- 3. Attard, G., et al., Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. J Clin Oncol, 2009. 27(23): p. 3742-8.
- 4. Tran, C., et al., Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science, 2009. 324(5928): p. 787-90.
- 5. Kang, H.Y., et al., Differential modulation of androgen receptor-mediated transactivation by Smad3 and tumor suppressor Smad4. J Biol Chem, 2002. 277(46): p. 43749-56.
- 6. Lunardi, A., et al., A co-clinical approach identifies mechanisms and potential therapies for androgen deprivation resistance in prostate cancer. Nat Genet, 2013. 45(7): p. 747-55.
- 7. Wang, G., et al., Zbtb7a suppresses prostate cancer through repression of a Sox9-dependent pathway for cellular senescence bypass and tumor invasion. Nat Genet, 2013. 45(7): p. 739-46.
- 8. Sharma, A., et al., The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. J Clin Invest, 2010. 120(12): p. 4478-92.
- 9. Camargo, F.D., et al., YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol, 2007. 17(23): p. 2054-60.
- 10. Zhao, B., K. Tumaneng, and K.L. Guan, The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nat Cell Biol, 2011. 13(8): p. 877-8

11. APPENDICES: Nothing to report

NOTE:

TRAINING OR FELLOWSHIP AWARDS:

1) Training from intra- and inter-laboratory interactions: I cannot ask for a better training environment than DePinho lab. In both DFCI and MDACC, we have weekly lab meeting and journal club with post-docs and graduate students from Ron DePinho, Lynda Chin and Giulio Draetta labs, which provide great opportunities to present my work in a critical academic setting and to learn about emerging discoveries from other talented post-docs and graduate students. Since Dr. DePinho took the position of President of MDACC, I was exposed to a whole array of facilities and cores, and I've got the chance to talk to some of the directors who are willing to assist me in different aspects of my research. In addition, I have maintained a good relationship with bioinformaticians and biostatisticians led by Dr. Chin and the scientists from the Institute of Applied Cancer Science led by Dr. Draetta. By collaborating and exchanging ideas with these scientists and physicians, I not only benefit from their great science, but will also build up a strong network for my future independent career.

2) Grant writing: With the help of Drs. DePinho and Chin, I successfully got the Department of Defense Prostate Cancer Research Program Postdoctoral Training Award. In addition, I'm helping Dr. DePinho to put together a CIPRIT grant on the novel prostate cancer mouse model generated in our lab. These experiences are invaluable, as Dr. DePinho will work closely with me to make sure I learn and understand the craft of grant writing. I also attended "Grant Writing 101 Workshop" provided by DFCI and will attend two workshops provided by MDACC named "Write Winning Grants" and "So You Want a Career Development Award". These training activities will help me obtain funding for my independent research laboratory.

3) Scientific communication in writing: I'm working closely with Dr. DePinho in writing my first-author research article on the novel molecular mechanisms for CRPC in the Pten/Smad4 mouse model. I expect to write another two co-first-author papers on several novel mouse PCa models in collaboration projects with Dr. Xin Lu in our lab before I finish my postdoctoral training here. Furthermore, I attended the "How to Write a Scientific Paper" workshop in DFCI and will attend "Writing and Publishing Scientific Articles Workshop" in MDACC. These training experiences will help me to write and publish high-profile papers of my own laboratory.

4) Scientific communication in speaking: I routinely present my work in the DePinho lab meeting every other month in DFCI and MDACC. I also regularly present my work in the joined weekly lab meeting among Draetta lab/Chin lab/DePinho lab/IACS and in the weekly Cancer Biology Department Seminar. In addition, I present regularly in the weekly Department of Cancer Biology Journal club. Moreover, I took the "Communication and Presentation Skills Workshop" sponsored by Harvard Medical School (HMS) and will take the workshops provided by MDACC such as "Giving Presentations: Learn Presentation Skills and Tips That Will Help You Keep Your Audience Enthralled" and on similar topics. I believe these training activities will make presentation skill one of my strengths and help me interview for and attain a faculty position.

5) Lab management/Mentoring training: The DePinho lab is big, so all of the post-docs participate heavily in ensuring that various aspects of the lab are running smoothly. I have trained 3 undergraduate intern students, 2 technicians and one medical student. These experiences have provided me the great opportunity to practice my mentoring and management

skills. In terms of education for myself, I attended the “Lab Management Workshop” in DFCI and will attend “Faculty Mentoring Academy Series” and “Faculty Leadership Academy Series” in MDACC. Finally, I will continue to learn from Dr. DePinho, who is a master of mentoring himself, to improve a wide range of skills on mentoring and management.

6) Seminar series/Conferences/Workshops: Both DFCI/Harvard communities and MDACC offer a wide range of top-notch seminars/series/conferences, where I keep abreast of current research findings around the world. I will present my findings on mechanisms of CRPC identified in our mouse model in the IMPACT meeting of the Prostate Cancer Program of Department of Defense in 2015. I also plan to attend various international conferences including but not limited to AACR Mouse Modeling Symposium, CSHL Mechanisms & Models of Cancer Meeting and Keystone Symposium-Tumor Immunology. In addition to the workshops mentioned in other sections, I will attend “Faculty Development Workshop and Seminar Series” of MDACC regularly to help me prepare the transition to independent PI.

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as “Proprietary Data” and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the GOR to obtain approval. REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE. It is the responsibility of the Principal Investigator to advise the GOR when restricted limitation assigned to a document can be downgraded to “Approved for Public Release.” DO NOT USE THE WORD “CONFIDENTIAL” WHEN MARKING DOCUMENTS. See term entitled “Intangible Property – Data and Software Requirements” and https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting for additional information.