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**TITLE:** CHD1-Deficiency Engenders a Distinct Epigenetic Profile in Castration-Resistant Prostate Cancer

**PRINCIPAL INVESTIGATOR:** Joseph Gawdzik

**CONTRACTING ORGANIZATION:** University of Wisconsin System, Madison, WI

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**6. AUTHOR(S)**
**Joseph Gawdzik**

David Jarrard
E-Mail: jarrard@urology.wisc.edu

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University of Wisconsin System
21 N. Park St., STE. 6401
Madison, WI 53715-1218

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**14. ABSTRACT**

This proposal addresses specific gaps in prostate cancer research and treatment by addressing two overarching challenges: 1) Developing treatments that improve outcomes for men with lethal prostate cancer and 2) Defining the biology of lethal prostate cancer to reduce death. Recent data highlights an important role for epigenetic dysregulation in CRPC cells. This proposal can potentially define an aspect of the biology underlying lethal prostate cancer which can lead to fewer annual deaths from this disease. Furthermore, epigenetic enzyme dysregulation represents a therapeutic target. However, knowing how these changes are driven represents a significant barrier to improved therapies. The research proposed here addresses this knowledge gap so that next-generation CRPC treatments can be developed to improve outcomes for men with lethal prostate cancer.

**15. SUBJECT TERMS**
None listed.

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Title: **CHD1-Deficiency Engenders a Distinct Epigenetic Profile in Castration-Resistant Prostate Cancer**

## 1. Introduction

Prostate cancer (PC) is one of the most frequently diagnosed cancer types in American men, accounting for an estimated 26,000 deaths in 2017. The majority of these deaths occur after PC has transitioned into the hormone insensitive form of the disease, castration-resistant prostate cancer (CRPC). Epigenetic misregulation is a common occurrence in CRPC, and can lead to altered expression of genes known to control tumor growth and metastasis. Physicians are able to detailed information relating to a tumor's genotype as genomic profiling becomes more frequently used in clinical settings. Hypothesis: This proposal postulates that a tumors genomic profile can be used to predict the types of epigenetic alterations seen in CRPC. This proposal tests if the genetic status of a gene encoding an epigenetic modifying protein commonly deleted in PC, Chromodomain helicase-DNA-binding protein 1 (CHD1), predicts a unique epigenetic profile. Preliminary data suggests there is an epigenetic profile specific to CHD1-deficient CRPC. An analysis of LuCaP Patient Derived Xenografts (PDX) showed decreased global H3.3K27K36 methylation in CHD1-deficient CRPC PDX samples. A reduction in NSD2 expression, a histone methyltransferase, is associated with the decrease in H3.3 methylation. A mechanistic relationship between CHD1-status, NSD2 expression, and levels of H3.3 methylation will be examined for using an in vitro model of CHD1-deficient CRPC. Levels of CHD1 and NSD2 protein will also be examined in prostate cancer patient samples to determine if the relationship between CHD1, NSD2 and H3.3K27K36 methylation exists beyond LuCaP PDXs.

Aim1: Determine if deletion of CHD1 causes reduced NSD2 expression, and alters H3.3K27K36 methylation in CRPC. An in vitro model of the CHD1-deficient CRPC epigenetic profile will be generated to test the hypothesis that downregulation of NSD2 expression specifically accounts for the presence of this profile in CRPC cell lines. This aim will validate the existence of this CHD1-deficient epigenetic profile and directly implicate NSD2 downregulation as a critical process in its formation. Aim 2: Determine if CHD1 regulates NSD2 expression via NF-κβ signaling. This aim investigates if NF-κβ activity regulates NSD2 expression in a CHD1-status dependent manner in CRPC. This aim can potentially identify CHD1 as a molecular determinant allowing for NSD2 to be regulated by the NF-κβ pathway in CRPC. Aim 3: Examine if levels of CHD1, NSD2, and H3.3K27K36 methylation, correlate in tissue samples from CRPC patients. This aim tests if the association between CHD1 and NSD2 expression levels observed in the analyzed PDX samples also exists in the greater PC patient population. These aim will validate the relationship found within the small cohort of PDX samples and expand it to a larger number of CRPC patients. Impact This proposal addresses specific gaps in prostate cancer research and treatment by addressing two overarching challenges: 1) Developing treatments that improve outcomes for men with lethal prostate cancer and 2) Defining the biology of lethal prostate cancer to reduce death. Recent data highlights an important role for epigenetic dysregulation in CRPC cells. This proposal can potentially define an aspect of the biology underlying lethal prostate cancer which can lead to fewer annual deaths from this disease. Furthermore, epigenetic enzyme dysregulation represents a therapeutic target. However, knowing how these changes are driven represents a significant barrier to improved therapies. The research proposed here addresses this knowledge gap so that next-generation CRPC treatments can be developed to improve outcomes for men with lethal prostate cancer.

## 2. Keywords

Prostate cancer, CHD1, castration-resistant prostate cancer, epigenetic enzyme dysregulation, NSD2.

**3. Accomplishments**

**SPECIFIC Aim 1: Determine if CHD1 inactivation downregulates NSD2 expression and H3.3K27K36 methylation in CRPC.**

**Aim 1A: Delete *CHD1* in CRPC cell lines, examine *NSD2* expression and histone methylation.**

***Subtask 1: Create CHD1-null 22Rv1, DU145, and PC-3 CR cell lines by transfecting vectors expressing Cas9 and CHD1 targeted gRNAs.***

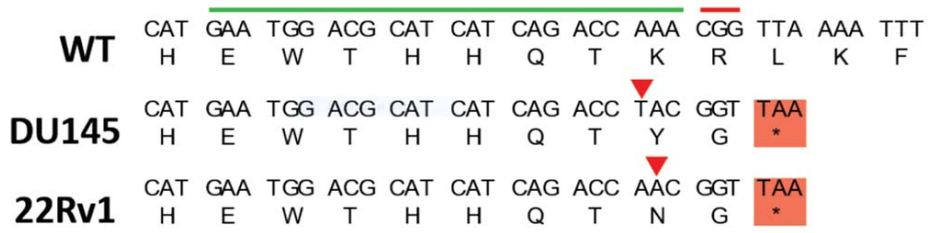
- CHD1-KO CRPC DU145 and 22Rv1 cell lines were generated using CRISPR-Cas9 gene editing. Confirm CHD1 deletion by immunoblotting for protein, RT-qPCR for mRNA. Cells transfected with a Cas9 expression vector alone will serve as a control.

**-COMPLETED**

We have generated three CHD1-KO cell lines, two CRPC (DU145 and 22Rv1), and one AR-sensitive (LNCaP) cell lines using CRISPR technique to induce sites of mutation and new stop codons in CHD1 exon 12. PC-3 cell line was replaced by LNCaP, as we think it is necessary to compare the alteration between CRPC and AR-sensitive cells after CHD1 inactivation.

We have demonstrated that CHD1-KO shows decreased NSD2 and EZH2 expression in both CRPC and AR-sensitive cell lines. (**Figure 1**).

**A.**




**B.**

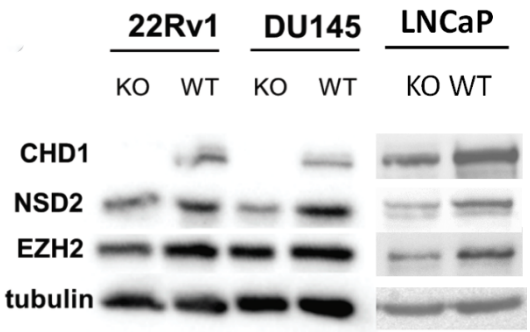



**Figure 1. Levels of NSD2 and EZH2 protein were measured in CHD1-KO CRPC DU145, 22Rv1 and AR-sensitive LNCaP cell lines.** A) Codons 4-16 of CHD1 exon 12 are shown for WT, and the DU145 and 22Rv1 CHD1-KO cell lines. Red and green bars indicate PAM and sgRNA target sites respectively. Red arrows and red boxes indicate sites of indels and new stop codons. B) Immunoblots of CHD1, NSD2 and EZH2 in 22Rv1, DU145 and LNCaP CHD1 WT or KO lines.

***Subtask 2: Analyze growth rates, apoptosis, colony forming abilities, HMT activity in CHD1- null 22Rv1, DU145, and PC-3 cells following NSD2 overexpression. Identical cells transfected with the functionally inactive NSD2 mutant or empty vector will serve as controls.***

**-ONGOING**

***Subtask 3: Analyze global H3.3K27K36 methylation and at specific loci in CHD1-null 22Rv1, DU145, and PC-3 cells.***

- CHD1-null cells will be generated in the 22Rv1, DU145, and PC-3 CRPC cell lines. CHD1-intact cells will serve as controls. Levels of NSD2 expression, colony formation, growth rates, HMT activity, histone methylation levels, and H3.3K27K36 methylation levels at specific gene promoters will be made between CHD1-intact and CHD1-null CRPC cells.

**-COMPLETED**

We have generated three CHD1-KO cell lines, two CRPC (DU145 and 22Rv1), and one AR-sensitive (LNCaP) cell lines, histone modification was then performed in these cell lines using LC-MS/MS. Triplicate values were obtained. We have determined that CHD1-KO CRPC cell lines show decreased H3.3K27K36 methylation **(Figure 2) consistent with a decrease in histone methylase activity overall.**

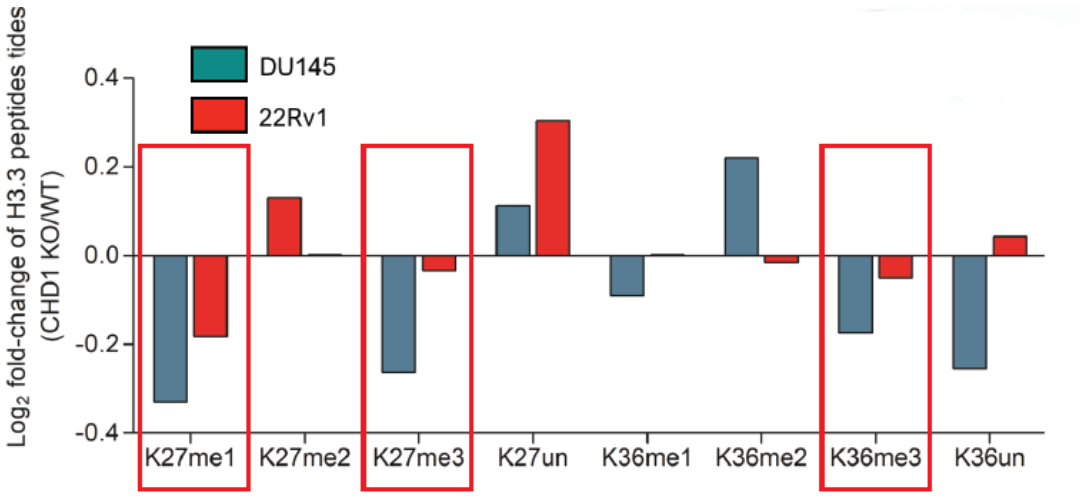



**Figure 2. CHD1-KO CRPC cell lines show altered H3.3K27K36 methylation.** An analysis of H3.3K27 and K36 modifications was performed using LC-MS/MS on CHD1-WT and KO CRPC cell lines. Graph depicts averaged KO/WT fold-change of H3.3K27 and H3.3K36 modification for two replicates of each condition.

**Aim 1B: Test effect of NSD2 knockdown on H3.3K27K36 methylation levels and HMTase activity in CHD1-intact CRPC cells.**

***Subtask 1: Test shRNAs to confirm efficient (>60%) knockdown of NSD2 expression in CHD1-intact 22Rv1, DU145, and PC-3 cells.***

- NSD2 knockdown was performed using NSD2 shRNA and confirmed by examining NSD2 protein levels by immunoblotting, mRNA levels by qRT-PCR. Identical cells transduced with a scrambled shRNA sequence or empty vector will serve as controls**.**

**-COMPLETED**

We have knocked down NSD2 in Du145 and LNCaP cells using two NSD2 shRNAs, scrambled shRNA was served as a control. NSD2 silencing was confirmed at protein level by western blot and mRNA level by qRT-PCR **(Figure 3)**.

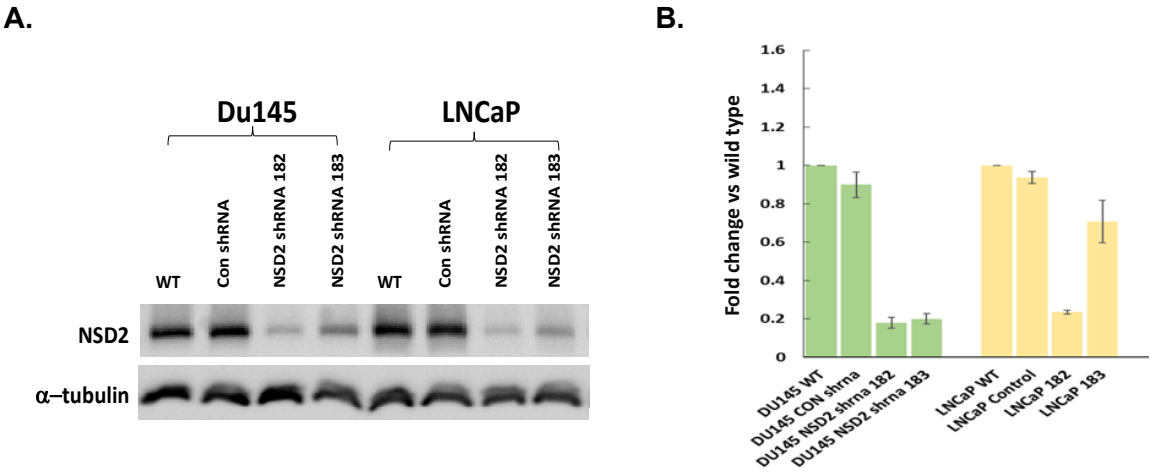



**Figure 3. Confirm silencing of NSD2 in PCa cell lines.** Du145 or LNCaP cells were transduced scramble shRNA, NSD2 shRNA # 182 or 183 shRNA, all these are lenti-viral constructs. After 48 hours, stable cells were selected with Puromycin. The cells were then collected for protein lysates or RNA extraction for confirmation of NSD2 silencing after 10 days selection.

***Subtask 2: Analyze growth rates, apoptosis, colony forming abilities, HMT activity in CHD1- intact 22Rv1, DU145, and PC-3 cells following NSD2 knockdown.***

- Cell growth rates are quantitated with a fluorescent DNA assay.
- Cell growth is also detected using Colony-Forming Assay.

**-COMPLETED**

We have evaluated cell growth in CHD1-intact Du145 and LNCaP with or without NSD2 knockdown. Cell growth was quantified with a fluorescent DNA assay. Briefly, 2 x $10^3$ LNCaP or 1x $10^3$ Du145 cells transduced with either scramble shRNA or NSD2 shRNA were plated in 96-well culture plates. After different period of NSD2 knockdown, plates were washed with 25% PBS once, and then added 100 µl of sterilized nuclease-free water and frozen at -80$^o$ C. Subsequently plates were thawed, incubated with Hoechst 33258 dye at final concentration of 6.7 µg/mL in high salt TNE buffer, and scanned on a plate reader using 360 nm excitation / 460 nm emission. NSD2 knockdown slowed down the growth of Du145 and LNCaP cells. Colony-Forming Assay was also used to determine the cell growth in these cells with Crystal violet stain. Four hundred LNCaP or 200 Du145 cells
were split into 6-well plate, after get enough colony formed, the cells were stained with 0.5% crystal violet in 25% methanol.

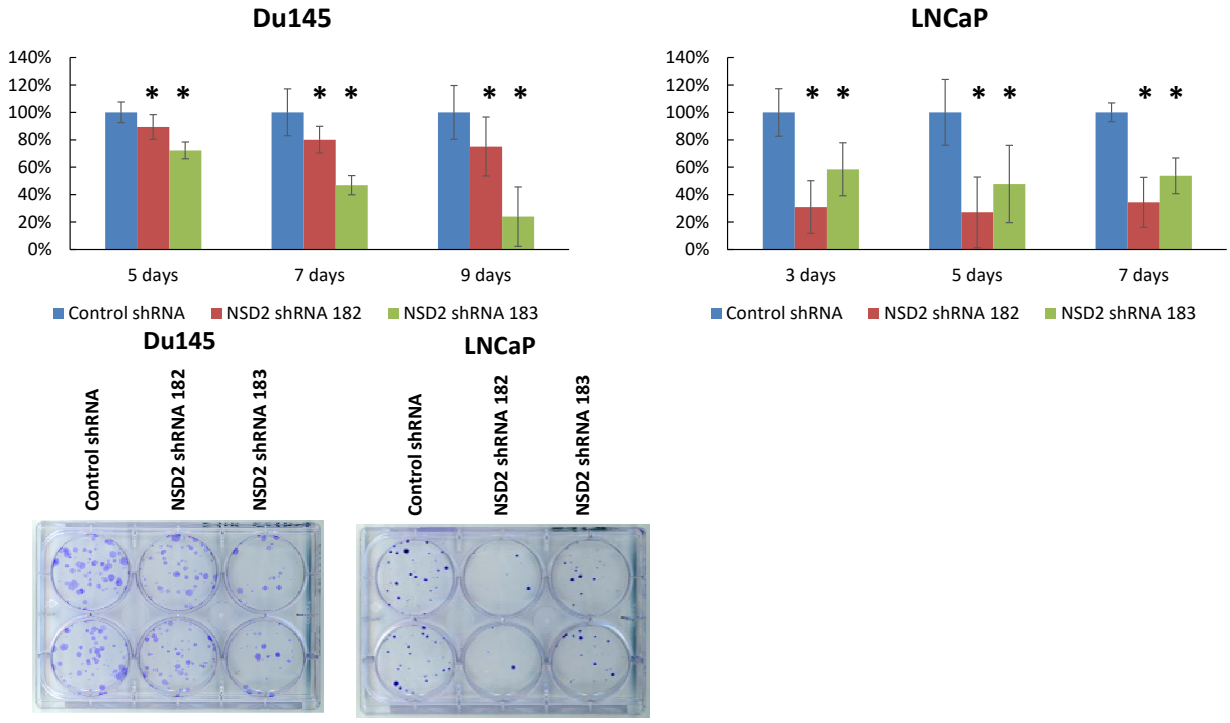



**Figure 4: Cell growth in CHD1-intact PCa cells following NSD2 knockdown**. A) Cell growth quantified with DNA assay, scramble shRNA served as control. Data shown as percentage to the control group, Mean ± SD. Eight replicates in each group, three independent experiments. B) Colony formation tested in the same group of cells with crystal violet stain. LNCaP cells were stained at 21 days, and Du145 were stained at 17 days.

***Subtask 3: Analyze global H3.3K27K36 methylation and at specific loci in CHD1-intact 22Rv1, DU145, and PC-3 cells using LC-MS/MS following NSD2 knockdown.***

**-NOT STARTED**

**Aim 1C: Determine if NSD2 overexpression reverses loss of H3.3K2736 methylation in CHD1- null CRPC cells.**

***Subtask 1: Generate expression vectors for NSD2 coding sequence, and functionally inactive NSD2 mutant control. Transfect into CHD1-null 22Rv1, DU145, and PC-3 cells, confirm overexpression by immunoblotting and RT-qPCR. Identical cells transfected with an empty vector will serve as controls. 4 replicates/condition/cell line, experiments done in duplicate.***

**-NOT STARTED**

***Subtask 2: Analyze growth rates, apoptosis, colony forming abilities, HMT activity in CHD1- null 22Rv1, DU145, and PC-3 cells following NSD2 overexpression. Identical cells***

***transfected with the functionally inactive NSD2 mutant or empty vector will serve as controls. 4 replicates/condition/cell line, experiments done in duplicate.***

**-NOT STARTED**

***Subtask 3: Analyze global H3.3K27K36 and loci specific methylation in CHD1-null 22Rv1, DU145, and PC-3 cells using LC-MS/MS following NSD2 overexpression. Identical cells transfected with the functionally inactive NSD2 mutant or empty vector will serve as controls. 4 replicates/condition/cell line, experiments done in duplicate.***

**-NOT STARTED**

**Specific Aim 2: Determine if CHD1 is necessary for NF-κβ regulation of NSD2 expression in CRPC.**

**Aim 2A: Characterize NF-κβ activity and binding at NSD2 promoter in CHD1-intact and CHD1-null cells.**

***Subtask 1: Analyze baseline NF- κβ activity in CHD1-null and CHD1-intact 22Rv1, DU145, and PC-3 cell lines by transfecting an NF- κβ reporter plasmid (Promega, Madison WI) into cell lines and performing luciferase expression assay. Identical cells transfected with a plasmid without NF- κβ response elements will serve as a control, non-specific IgG and primers amplifying loci known to be devoid of NF- κβ will serve as controls for ChIP-qPCR and RT- qPCR.***

**-NOT STARTED**

***Subtask 2: Analyze baseline NF- κβ binding at NSD2 locus in CHD1-null and CHD1-intact 22Rv1, DU145, and PC-3 cell lines Non-specific IgG and primers amplifying loci known to be devoid of NF- κβ will serve as controls for ChIP-qPCR and RT- qPCR. Milestone Achieved: Baseline NF- κβ activity and binding at the NSD2 locus will be determined in CHD1-null and intact 22Rv1, DU145, and PC-3 cell lines.***

**-NOT STARTED**

**Aim 2B: Determine if NF-κβ directly regulates NSD2 expression in CRPC cell lines.**

***Subtask 1:*** **Repress NF- κβ activity by transfecting vector overexpressing IκBα into CHD1- intact 22Rv1, DU145, and PC-3 cell lines. Confirm IκBα expression by immuoblotting and RT- qPCR. Confirm repressed NF- κβ activity by transfecting an NF-κβ reporter plasmid (Promega, Madison WI) into cell lines and performing luciferase expression assay. Specific methods in ref 45,47,67. An empty vector will be used to control for IκBα overexpression, a plasmid without NF- κβ response elements will serve as a control for NF- κβ activity.**

**-NOT STARTED**

***Subtask 2*****: Analyze NSD2 expression, NF- κβ binding, HMT activity, in CHD1-intact 22Rv1, DU145, and PC-3 cells using immunoblotting and RT-qPCR following repression of NF- κβ activity. An empty vector will be used to control for IκBα overexpression.**

**-NOT STARTED**

***Subtask 3:*** **Analyze NSD2 expression, NF- κβ binding, HMT activity in CHD1-null 22Rv1, DU145, and PC-3 cells using immunoblotting and RT-qPCR following TNFα treatment to stimulate NF- κβ activity. Vehicle alone will serve as a control for TNFα treatment. 4 replicates/condition/cell line, experiments done in duplicate. Milestone Achieved: This aim will determine if repressed NF- κβ signaling alters NSD2 expression, NF- κβ binding at NSD2 locus, and alters HMT activity in CHD1-intact 22Rv1, DU145, and PC-3 cells. This aim will also determine if NF- κβ activation rescues NSD2 expression, NF- κβ binding at NSD2 locus, and alters HMT activity in CHD1-intact 22Rv1, DU145, and PC-3 cells.**

**-NOT STARTED**

**Specific Aim 3: Determine if NSD2 and CHD1 protein and mRNA expression levels correlate in CRPC patients.**

***Subtask 1: Obtain Institutional Review Board (IRB) and Human Resource Protection Office (HRPO) approval from the Department of Defense for research using human specimens.***

**-COMPLETED**

***Subtask 2: Analyze levels of CHD1, NSD2, and H3K36me2 using immunohistochemistry on tissue microarrays from local hormone refractory tumors. These tissue microarrays will be analyzed using the VECTRA system. At least 100 tumors will be analyzed. Correlations between CHD1, NSD2 amd H3K36me2 will be examined for.***

- CHD1, NSD2, EZH2 and H3K36me2 levels are measured in tissue microarrays created from CRPC patient tumor tissues (n=51) using immunohistochemistry in conjunction with automated VECTRA image analysis for quantification. We found a clear correlation between NSD2 and CHD1 levels. E cadherin is utilized as a control gene to identify cancer cells.

**-COMPLETED**

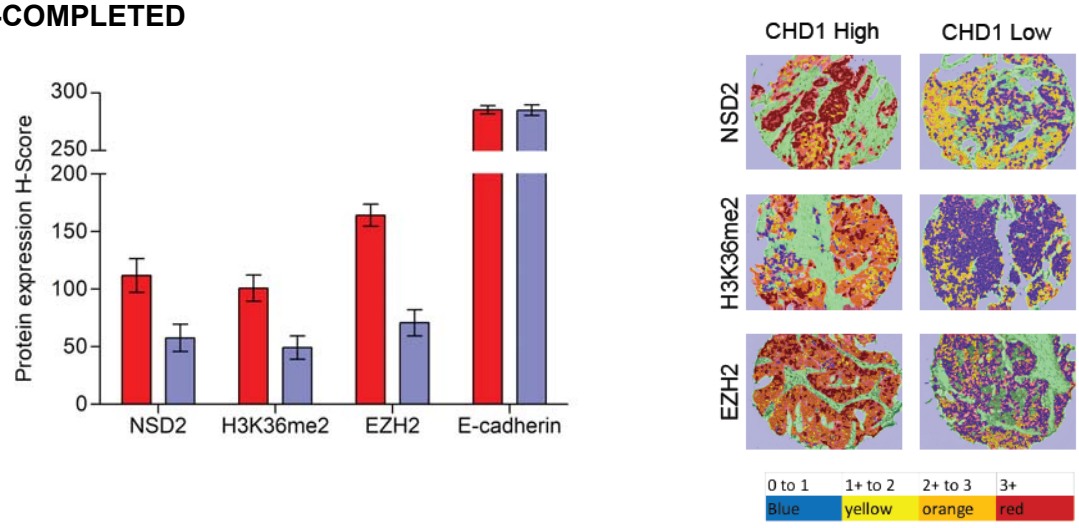



**Figure 5: Protein levels of NSD2, H3K36me2 and EZH2 associate with levels of CHD1 protein expression in CRPC patient tumor tissue array.** The levels of NSD2, EZH2, and H3K36me2 were measured in epithelial cell nuclei in CRPC biopsy cores that had been designated as being either CHD1 high or low expression based on being in the top 40% or bottom 40% of CHD1 H-scores, respectively. A) H-scores for NSD2, EZH2 and H3K36me2 are significantly reduced in CHD1 low expressors (n=20) compared to CHD1 high expressers (n=20) Student's t-test p*<0.05. B) Representative images of CRPC biopsie cores from CHD1 high and low expressors with VECTRA quantification of indicated proteins. Color scheme shown below, unanalyzed stromal tissue shown in green.

***Subtask 3: Analyze levels of CHD1 and NSD2 mRNA expression in CRPC patient databases***

***(MSKCC, SU2C, Fred Hutchinson). Determine if these mRNA levels correlate. Also, examine if expression associates with overall cancer specific outcomes.***

**-NOT STARTED**

***Subtask 4: Treat CHD1-intact and null 22Rv1, DU145, and PC-3 cells with NSD2 inhibitors to determine if inhibitor treatment affects growth rates, apoptosis, and colony forming abilities.***

**-NOT STARTED**

**Opportunities for training and professional development?**

Joe Gawdzik had presented research at the monthly Prostate Cancer Research Group and Gene Expression, Chromatin and Transcription Mechanism Research Club. Participated in Gee-Wisc genome engineering club. Dr Gawdzik had plans to present at the AACR meeting but this was cancelled due to the pandemic.

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

Abstract presentation at the Society for Basic Urologic Research Meeting (11/2019 in New Orleans, LA).

**• What do you plan to do during the next reporting period to accomplish the goals and objectives?**

Dr Gawdzik and the laboratory had to shut down in late February/early March because of the pandemic. Given these events Dr Gawdzik decided to take a job in the biotech working for a cancer research company. It is our hope to hire a new Postdoctoral student to continue this work and publish the results.

**4. Impact**

This proposal addresses specific gaps in prostate cancer research and treatment by addressing two overarching challenges: 1) Developing treatments that improve outcomes for men with lethal prostate cancer and 2) Defining the biology of lethal prostate cancer to reduce death. Recent data highlights an important role for epigenetic dysregulation in CRPC cells. This proposal can potentially define an aspect of the biology underlying lethal prostate cancer which can lead to fewer annual deaths from this disease. Furthermore, epigenetic enzyme dysregulation represents a therapeutic target. However, knowing how these changes are driven represents a significant barrier to improved therapies. The research proposed here addresses this knowledge gap so that next-generation CRPC treatments can be developed to improve outcomes for men with lethal prostate cancer.

## 5. Changes Problems

- PC-3 cell line was replaced by LNCaP, as we think it is necessary to compare the epigenetic alteration between CRPC and AR-sensitive cells.
- Joseph Gawdzik resigned 6 months after the funding started and the pandemic began to take a job in the biotech industry. The project has been paused. We have another interested individual who potentially could undertake finishing this work.

## 6. Products

Abstract presentation at the Society for Basic Urologic Research Meeting (11/2019 in New Orleans, LA).

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**Senior key personnel have been working on the project since the initiation of the project with no changes. As noted Dr Gawdzik has resigned at the beginning of the pandemic.**

**The following individuals have worked on the project:**

Name: David F. Jarrard, MD
Project Role: Principal Investigator
Researcher Identifier (e.g., ORCID ID): 0000-0001-8444-7165
Nearest person month worked: 1.2
Contribution to Project: David Jarrard has conceived and designed the study, reviewed all of the data and the analysis of all of the results on the project, wrote and revised the manuscript.

## 8. Special Reporting Requirements

NA

## 9. APPENDICES

### References


1. Zhang Z, Zhou C, Li XL, Spencer SD, Deng S, et al (2020). Loss of CHD1 Promotes Heterogeneous Mechanisms of Resistance to AR-Targeted Therapy via Chromatin Dysregulation. Cancer Cell 13;37(4):584-598.
2. Aytes A, Giacobbe, A, Mitrofanova A, Ruggero, K, Cyrta, J., et al (2018). NSD2 is a conserved driver of metastatic prostate cancer progression. Nat Commun 5;9(1):5201.