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

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14. ABSTRACT

Scientific Objective and Rationale: Prostate cancer is one the most common malignancy of adult males in the United States; there are currently 3.3 million diagnosed men, and roughly 160,000 new cases will be made in 2017. The advancement of next-generation sequencing technology has made looking at DNA changes in tumors more common in clinical settings. Information relating to tumor DNA changes is highly valuable as it can be used to predict patient response to prostate cancer therapies. This proposal will examine the role one of the most frequent gene alterations, in CHD1 (Chromodomain-helicase DNA binding protein 1), plays as a driver of cancer. We will test whether it alters a series of enzymes that may be targets for cancer therapy.

15. SUBJECT TERMS

NONE LISTED

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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.

2. KEYWORDS

castration-resistant prostate cancer (CRPC), epigenetics, CHD1, NSD2, histone modifications, NF-κβ signaling

3. ACCOMPLISHMENTS

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

Major Task 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.

-Completed

CHD1-KO CR DU145 and 22Rv1 PC cells have been generated. Cells transfected with a Cas9 expression vector alone served as a control. CRISPR-mediated CHD1 gene editing and single cell fluorescence activated cell sorting (FACS) were performed as planned. Together these strategies resulted in the identification of at least two clones from each cell line harboring an inactivating mutation within the CHD1 allele as revealed by Sanger sequencing. Control cells were derived from cells that had undergone the same transfection and sorting procedures yet retained a WT CHD1 allele, **(Fig. 1A).** CHD1-KO was then confirmed by immunoblotting (**Fig. 1B).**

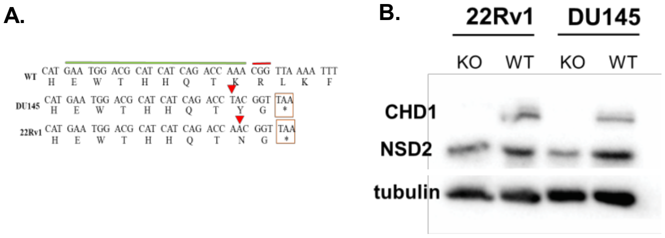

Figure 1: Great human PC cell lines with CHD1 knockout. A) Codons 4-16 of CHD1 exon 12 are shown for WT, and the DU145 and 22Rv1 CHD1 KO cells. Red and green bars indicate PAM and sgRNA target sites, respectively. Red arrows and red boxes indicate sites of indels and new stop codons. **B)** Immunoblots showing CHD1 and NSD levels in DU145 and 22Rv1 CHD1 WT and CHD1 KO cells.

Subtask 2: Analyze NSD2 expression, growth rates, colony formation, apoptosis, and HMT activity CHD1-null 22Rv1, DU145, and PC-3 cells.

-Completed

In order to test if a loss of CHD1 gene function caused a reduction of NSD2 levels, expression of NSD2 protein was examined in these CHD1-KO cells and control cells. Immunoblotting showed a noticeable decrease in NSD2 protein levels in CHD1-KO cells relative to controls in both cell lines **(Fig. 1B).**

Additionally, we analyzed the growth rate of CHD1 WT with CHD1 KO cells by colony formation assay and DNA proliferation assay. We noticed that cells with CHD1 KO condition grew slower than CHD1 WT conditions in both cell lines **(Fig. 2A and 2B).**

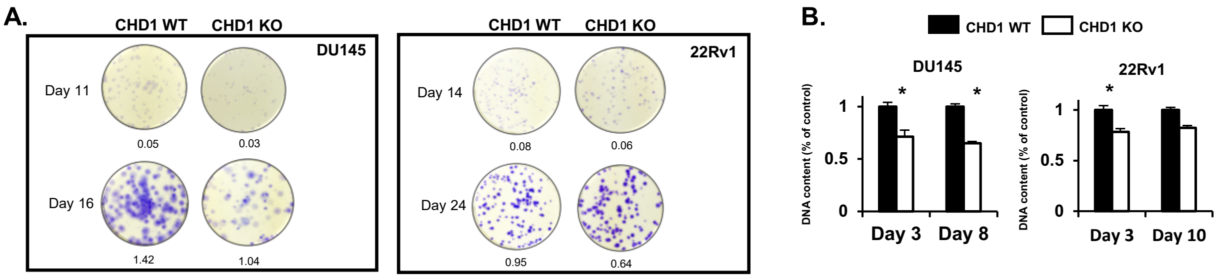

Figure 2. CHD1 KO decreased PCa cells growth. A) Representative images of colony formation assay with quantification and **B)** Cell proliferation assay in DU145 and 22Rv1 CHD1 WT and CHD1 KO cells, respectively. Student's t-test, p*<0.05.

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

Milestones: CHD1-null cells will be generated in the 22Rv1, DU145, and PC-3 CRPC cell lines. 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.

-Ongoing

To test if a loss of CHD1 gene function caused a reduction in histone H3.3 methylation levels, LC-MS/MS was performed to analyze H3.3K27K36 methylation in CHD1-KO cells and controls. Initial experiments showed that in both cells lines levels of H3.3K27me1, me3 and K36me3 were reduced by approximately 10% compared to controls. **(Fig. 3)**

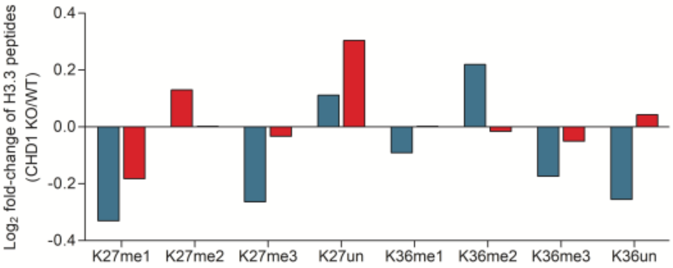

Figure 3. An analysis of H3.3K27 and K36 modifications was performed using LC-MS/MS on CHD1-WT and KO Du145 cell lines. Graph depicts averaged KO/WT fold-change of H3.3K27 and H3.3K36 modification for two replicates of each condition.

Future work will focus on confirming these LC-MS/MS results with additional samples and analyses. Additionally, the levels of H3.3K27K36 methylation at gene promoters known to play a role in PC progression, and the levels of histone methyltransferase activity in CHD1-KO cells will also be investigated.

Major Task 1B: Test effect of NSD2 knockdown on H3.3K2736 methylation 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 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.

-Pending

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

-Pending

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.

Milestones: Knockdown of NSD2 expression will be confirmed in in the 22Rv1, DU145, and PC-3 CRPC cell lines. Colony formation, growth rates, HMT activity, histone methylation levels, and H3.3K27K36 methylation levels at specific gene promoters will be made between NSD2 knockdown or controls**.**

-Pending

Major Task 1C: Determine if NSD2 overexpression rescues H3.3K2736 methylation in CHD1-deficient 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.

-Pending

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.

-Pending

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.

Milestones: Overexpression of NSD2 will be confirmed in in the CHD1-null 22Rv1, DU145, and PC-3 CRPC cell lines. Colony formation, growth rates, HMT activity, histone methylation levels, and H3.3K27K36 methylation levels at specific gene promoters will be made between NSD2 knockdown or controls.

-Pending

SPECIFIC AIM 2: Determine if CHD1 is necessary for NF-κβ regulation of NSD2 expression in CRPC

Major Task 2A: Characterize NF-κβ activity and binding at NSD2 promoter in CHD1-intact and deficient CRPC 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.

-Pending

Subtask 2: Analyze baseline NF- κβ binding at NSD2 locus in CHD1-null and CHD1-intact 22Rv1, DU145, and PC-3 cell lines.

Milestones: 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.

-Pending

Major Task 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. 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.

-Pending

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.

-Pending

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.

Milestones**:** 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.

-Pending

SPECIFIC AIM 3: Determine if NSD2 and CHD1 protein and mRNA expression levels correlate and predict outcomes 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.

We have received IRB approved protocol from University of Wisconsin.

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.

-Completed

Tissue microarrays containing CRPC and HSPC biopsy tissue have been stained using IHC for CHD1, NSD2, EZH2 and H3K36me2. These tissue microarrays have been subsequently imaged, and then the chromagen intensity analyzed using VECTRA automated analysis as described in the proposal. CHD1, NSD2, EZH2 and H3K36me2 levels have been examined using tissue microarrays containing CRPC tumor biopsies. In an initial assessment, these CRPC biopsies were stratified based on CHD1 expression levels. Expression of NSD2, EZH2 and H3K36me2 levels were compared in biopsies with high CHD1 expression to biopsies with low CHD1 expression. This analysis showed that NSD2, EZH2 and H3K36me2 levels were all significantly reduced in low CHD1 expressing biopsies relative to high CHD1 expressing biopsies ($p<0.01$), **Figure. 4.**

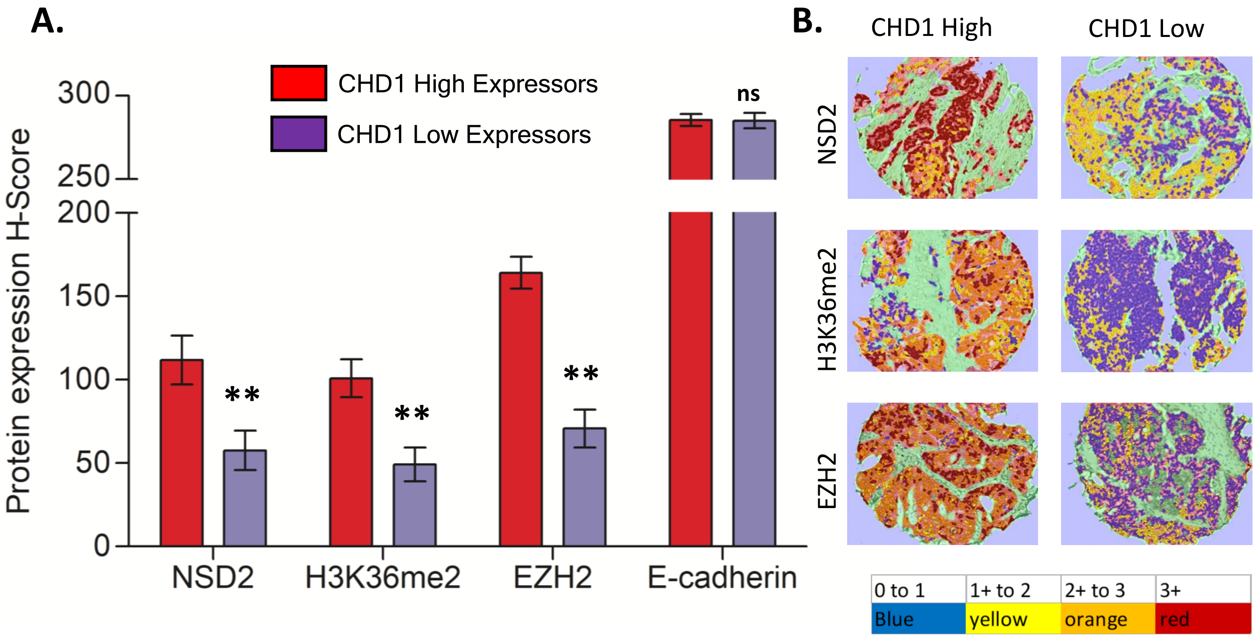

Figure 4: The levels of NSD2, EZH2, and H3K36me2 in human CRPC biopsy cores. These protein expression were measured in epithelial cell nuclei in that had been designated as being either CHD1 high or low expression based on being in the top 40% or bottom 40% of CHD1 H-scores, respectively. **A)** H-scores 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.

During this analysis we also found a coregulation of NSD2 and EZH2 expression identified an aggressive subvariant of prostate cancer. This data was published (Filon, Gawdzik et al. BJC 2021).

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.

-Ongoing
An assessment of CHD1 and NSD2 expression using online patient databases (cBioportal.org) has also begun. To date, this analysis has shown an increased expression of NSD2 in CRPC patients relative to HSPC patients ($p<0.05$). Future work will focus on examining the relationship between CHD1 and NSD2 expression levels and patient outcomes in CRPC.

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.

Milestones: Will have characterized the correlation of CHD1, H3K36me2, and NSD2 proteins in the greater CRPC patient population along with examining expression of CHD1 and NSD2 mRNA in CRPC patient databases. Finally, will have tested NSD2 currently being developed for their ability to impede CRPC cell growth and proliferation.

-Pending

Summary:

During the limited time that Dr Gawdzik was working on this grant he was able to make several important advances. He discovered that CHD1 loss did not alter growth in PTEN deficient cell lines. In addition, CHD1 loss decreased H3.K36me2 methylation suggesting an important link between CHD1 and the regulation of this oncogenic permissive epigenetic change. The laboratory is continuing to work on this discovery and a graduate student is currently involved in this. These studies will be published in the next year.

Dr Gawdzik was able to work with the group to generate an important discovery regarding NSD2, a histone methylase involved in H3.K35me2 methylation being investigated as part of this project. They found that increased EZH2/NSD2 co-expression is overrepresented in CRPC, metastases and associates with shorter disease-free survival in PC patients. Coregulation of these two histone methyltransferases is a biomarker for aggressive PC and licenses them as therapeutic targets. (BJCan 2021)

Opportunities for training and professional development?

Dr. Joseph Gawdzik, audited a statistics, research ethics, and oncology course at UW-Madison to fulfill this aspect of the training grant. He attended and presented at local prostate cancer cancer center meetings and also at a national meeting. However, because of the pandemic the laboratory was shut down and Dr Gawdzik opted to pursue other options.

How were the results disseminated to communities of interest?

Abstract presentation at the Society for Basic Urological Research meeting 11/2019 New Orleans, LA.

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

We plan to adhere to the proposed SOW with the exceptions noted under accomplishments. Dr Gawdzik opted to pursue a job in biotech and left the laboratory after 7 months of effort on this grant was performed.

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

We didn't use PC3 cell line in Aim1 since some researcher has shown the amplification of CHD1 in PC3 cells (Cancer Cell Line Encyclopedia, Novartis/Broad, Nature 2012).

6. Products

Abstracts:

Screening of histone post-translational modifications in castration resistant prostate cancer reveals CHD1 gene deficiency engenders a distinct epigenetic profile. Joseph Gawdzik, Eric Armstrong, Bing Yang, Rehann Machhi, John Denu, David Jarrard (Society for Basic Urological Research meeting 11/2019 New Orleans, LA.)

Papers: Filon M, **Gawdzik J**, Truong A, Allen G, Huang W, Khemees T, Machhi R, Lewis P, Yang B, Denu J, Jarrard D. Tandem histone methyltransferase upregulation defines a unique aggressive prostate cancer phenotype. Br J Cancer. 2021 Jul;125(2):247-254. doi: 10.1038/s41416-021-01398-7. Epub 2021 May 11. PMID: 33976366; PMCID: PMC8292389.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

N/A