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TITLE: Frequent loss of CHD1 in the prostate cancer of African Americans and its potential role in increased sensitivity to platinum or PARP inhibitor-based therapy

PRINCIPAL INVESTIGATOR:

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CONTRACTING ORGANIZATION:
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14. ABSTRACT African American individuals with prostate cancer have significantly worse clinical outcome than European Americans with the same disease. In our preliminary analysis, we identified a specific molecular aberration that is present in African American prostate cancer cases two to three times more frequently than in European Americans with the same disease. This aberration, the loss of the CHD1 gene, is thought to lead to impaired homologous recombination and increased genomic instability. Homologous recombination (HR) deficiency can be therapeutically exploited by either platinum or PARP inhibitor-based therapy. Therefore,we are performing a comprehensive analysis of the clinical consequences of the increased prevalence of CHD1 loss in the prostate cancer of African American individuals. We are investigating the relationship between CHD1 loss and the HRD mutational signatures. A robust association between CHD1 loss and HR deficiency may be the mechanistic basis for effective, platinum or PARP inhibitor-based personalized therapy for African American prostate cancer cases.

15. SUBJECT TERMS
African American men, prostate cancer, CHD1 gene, PARP inhibitor, platinum-based therapy

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Introduction:

African American individuals with prostate cancer have significantly worse clinical outcome than European Americans with the same disease. In our preliminary analysis, we identified a specific molecular aberration that is present in up to 40% of African American prostate cancer cases, two to three times more frequently than in European Americans with the same disease. This aberration, the loss of the CHD1 gene, is thought to lead to impaired homologous recombination and increased genomic instability. Homologous recombination (HR) deficiency can be therapeutically exploited by either platinum or PARP inhibitor-based therapy. The main aim of this proposal is to establish a firm link between CHD1 loss, increased homologous recombination deficiency and increased sensitivity to targeted therapy. This could lead to improved therapy for African American individuals with advanced prostate cancer.

Keywords:

African American men, prostate cancer, CHD1 gene, PARP inhibitor, platinum-based therapy

Accomplishments:

Major developments mainly associated with Children's Hospital, Boston as outlined in Specific Aim 2: Investigating the relationship between CHD1 loss and the HRD mutational signatures

Subtask 3: Download and process whole genome sequencing data from all available prostate cancer cases. Extract the various homologous recombination deficiency (HRD) measures from the sequencing data and correlate those with CHD1 loss.

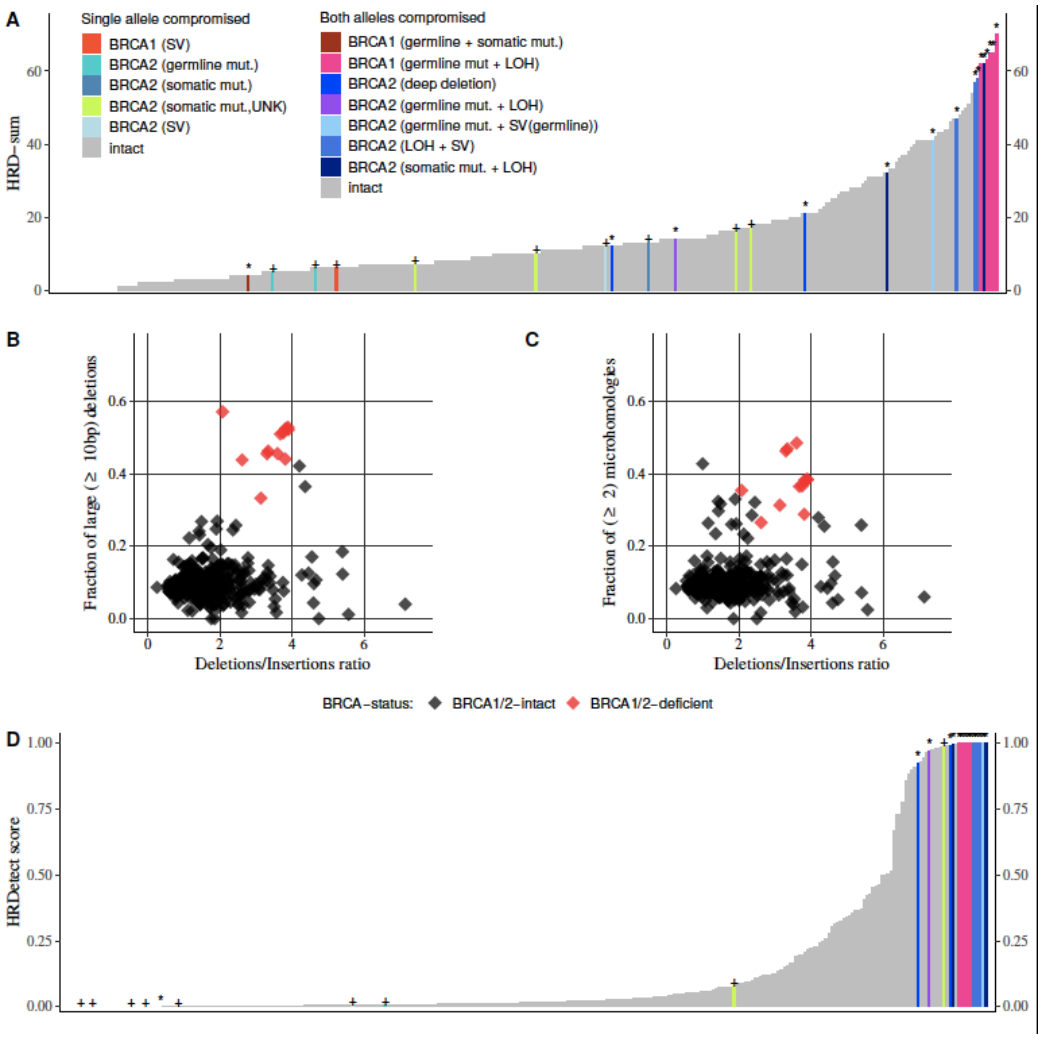
Subtask 4: Catalogue all mutational signatures in the PRAD WGS data sets and identify those cases whose HRD signatures cannot be associated with a loss of function mutation, deletion in an HRD associated gene. Identify other possible causes of HR deficiency by RNASeq analysis and other strategies.

1) Major activity: We have downloaded and processed raw whole genome sequencing data (BAM files) from 311 prostate cancer samples. We have called germline mutations with HaplotypeCaller, we determined somatic point-mutations and short indels using Mutect2 (GATK 3.8). The high fidelity of the reported variants was ensured by the application of additional hard filters on top of the tools' default ones. Allele-specific copy number profiles had been estimated by using Sequenza. Structural Variants were called using BRASS (v6.0.0 - https://github.com/cancerit/BRASS).
The mutations were annotated using InterVar. Variants predicted as pathogenic or likely pathogenic were considered deleterious, while variants with unknown significance were marked differently. Copy number status of BRCA1/2 were based on Sequenza results.

Somatic point-mutational signatures were determined with the deconstructSigs R package, by using the cosmic signatures as a mutational-process matrix. The extraction of rearrangement signatures was executed as described in (Nik-Zainal S et al. Nature 2016;534:47–54.)
The calculation of the genomics scar scores (loss-of-heterozygosity: LOH, large scale transitions: LST and number of telomeric allelic imbalances: ntAI.) were determined using the scarHRD R package.
Due to the lack of sufficient numbers of bona fide HR-deficient cases within the prostate cancer cases, a prostate-specific HRDetect model could not be created at this time. Instead, the weights of the original, breast cancer-specific, whole genome-based HRDetect model (Davies H, Nat Med 2017;23:517–25) were used to calculate the HRDetect scores of the WGS prostate samples.
It should be noted that the processing of a single WGS case is about 2-3 weeks (depending on coverage). Of course, we are parallel processing the cohorts. Nevertheless, the computational investment should be acknowledged.

2) Specific objectives: The main objective was to set up robust methods to detect and quantify homologous recombination deficiency in prostate cancer. HR deficiency was mainly studied so far through mutational signatures in breast and ovarian cancer and we had to validate and benchmark all the previously described methods for the analysis of prostate cancer derived next generation sequencing data (both in EA and AA patients.) Such analysis would then allow us to correlate CHD1 loss with the downstream mutational signatures of HR deficiency.

3) Significant results: First, we determined that we had nine bona fide HR deficient cases (both active copies of BRCA1 or BRCA2 are lost). These cases (marked with red in the figure below) show all signs of HR deficiency induced mutational signatures. They had high deletions/insertion ratio, had high fraction of microhomology mediated deletions and had a high ratio of >10 bp long deletions. They also had high values of the composite mutational signature scores, HRD score and HRDetect. Notably, there were thirteen cases in our analysis with likely intact BRCA1 and BRCA2 function that also had a higher Deletions/Insertions ratio than the BRCA1/2 deficient cases. Three of the cases had a similar fraction of microhomology associated cases to those with BRCA2 deficiency and two of those three cases had a similar ratio of >10bp deletions to those with BRCA2 deficiency as well. This suggests that there are BRCA1/2 intact cases that display the mutational signatures usually associated with BRCA deficiency in prostate cancer. Several of those cases (panel D) had almost maximal HRDetect values as well. This has led to the question whether the CHD1 loss cases are those with these high HRDetect values. This analysis is currently in progress. We have also started the RNAseq analysis on the same cohort.
In summary, in year 1 we laid all the groundwork for all the whole genome sequencing based mutational signature analysis for the entire project.



Tasks specific for the Subaward at Henry M. Jackson Foundation for the Advancement of Military Medicine:

Task #1: Detection of prevalence of CHD1 deletion and its correlation to more aggressive prostate cancer subtype in cohort of Center for Prostate Disease Research.

- Obtain IRB approval (mths 1-4)
- Construct tumor tissue microarrays (TMA), TMA-1: 42AA and 59CA and TMA-2 100AA and 100CA prostate cancers (mths 5-10)
- Assess CHD1 deletion frequency by Fluorescence In Situ Hybridization (FISH), compare to other cancer gene defects (ERG, PTEN, LSAMP) (mths 11-16)
- Perform association analyses with clinical-pathological features (mths 17-21)

Task #2: Detection of prevalence of CHD1 deletion in carboplatin treated cohort of castration resistant PCa cases from Dana Farber Cancer Institute.

- Prepare TMA from prostate specimens of patients treated with carboplatin at DFCI and assess by FISH and IHC (mths 6-7)

Task #3 (Subaward PI): Whole Genome Sequencing, data analysis of 35 CPDR AA PCa patients.

- Obtain IRB approval (mths 1-4)
- Complete Whole Genome Sequencing of prostate tumors of 35 AA patients (mths 5-16)

Task #4 (PI and Subaward PI): Assess patient derived AA patient derived cell lines with CHD1 loss for PARP inhibitor and platinum sensitivity.

- Characterize nine AA patient derived CPDR cell lines for CHD1 and for other cancer genes by FISH and IHC (mths 19-30)

1) Major activity: to obtain IRB approvals to perform Tasks (mths 1-9) followed by the initial and secondary reviews and approval by the USAMRMC, Office of Research Protections, Human Research Protection Office (mths 9-12)

Specific objectives: to obtain IRB approval from the Walter Reed National Military Medical Center IRB for: 1) generating TMA from prostate cancer tissue specimens archived in the CPDR Biobank; 2) IRB approval for using patient derived immortalized prostate cancer cell lines from the CPDR Biobank; 3) approval for utilizing whole genome sequences that have been generated under a CPDR data banking protocol. For the use of follow up data up to 20 years, we also obtained approval from the Uniform Services University of Health Sciences.

2) The significant result of these IRB reviews was the outcome letter that determined the protocol to be "research not involving human subjects as defined by 32 CFR 219.102(e) because the research involves the use of de-identified specimens and data not collected specifically for this study" and the approval from the USAMRMC Human Research Protection Office.

3) Other achievements: Toward tasks that require reliable reagents and tools we have completed the assessments, and quality control of fluorescence in situ hybridization probes and selected a probe that is a critical tool for the detection of CHD1 deletion. FISH analysis of the CHD1 locus was performed on TMAs. CHD1 FISH probe was obtained from CytoTest Inc (Rockville, MD, USA). The CHD1 locus-specific probe covers a chromosomal region which includes the entire CHD1 gene located on chromosome band 5q15-21. A chromosome specific probe D5S23, D5S721, covers the chromosomal region between the STS marker D5S23 and D5S721 and the region upstream and downstream of the two markers. Before use on tissue samples, locus-specific and control probes were mapped to normal human peripheral blood lymphocyte metaphases to confirm location and performance in interphase nuclei.

For generating TMAs 50 AA and 50 CA patients were selected with at least 10 years of follow up for non-biochemical recurrence and/or metastasis with at least 1 cc index tumor volume with a morphology of well differentiated (1/3), moderately differentiated (1/3) and poorly differentiated (1/3) cells. Random positions on TMAs were assigned to the patient derived archived FFPE prostate cancer tissues and the code was secured by the database team (the research/pathologist team blinded for this study).

Completion of CHD1 FISH assays for 50AA and 50CA derived tumor TMAs: Whole-mounted prostate sections were pre-warmed in oven at 180°C for one hour. Then, the sections were de-paraffinized with xylene. The depaffinized sections were dehydrated with ethanol. The air-dried sections were immersed in Trizma Base + EDTA (pH 7.0). The sections were rinsed with Phosphate-Buffered Saline (PBS) solution. The air-dried sections were digested with Digest All III (Invitrogen, Cat number: 00-3009). The sections were rinsed with PBS solution (pH7.0) for 5 min. The sections were dehydrated with serial dilutions of ethanol (70%, 80%, 95% and 100%). The air-dried sections were combined with FISH probes (10 µl Cytotest LSP CHD1 CytoOrange and chromosome 5centromeric probe: CCP 5 CytoGreen Cocktail). The sections applied in FISH assay were covered with glass cover slips and sealed with rubber cement. The sealed sections were denatured at 94°C hot plate for 10 min. Then, the sections were incubated at 37°C overnight then washed with the 2X Saline Sodium Solution (SSC) (pH7.0) at 73°C for 5 min. The sections were washed with 0.5X SSC (pH7.0) at room temperature for 5 min for 3 times and rinsed with deionized water for 1 min. Finally, the air-dried sections were covered with DAPI Mount (ProLong Gold antifade reagent with DAPI, Invitrogen, Cat Number: P36935). The FISH probe signals were observed under fluorescence microscope with 60X magnification objective. The excitation peaks of CytoOrange and CytoGreen labels were 551 and 495 nm respectively. Tumor cells with at least two centromeres were counted (Figure 1.). Numbers of centromeres and CHD1 signals were compared to determine whether cells were homozygous or heterozygous for this locus. Minimal 100 cells from each tissue core were evaluated. Deletions were called when more than 75% of evaluable tumor cells showed loss of allele. Focal deletions were called when more than 25% of evaluable tumor cells showed loss of allele or when more than 50% evaluable tumor cells in each gland of a cluster of two or three tumor glands showed loss of allele. Benign prostatic glands and stroma served as built in controls.

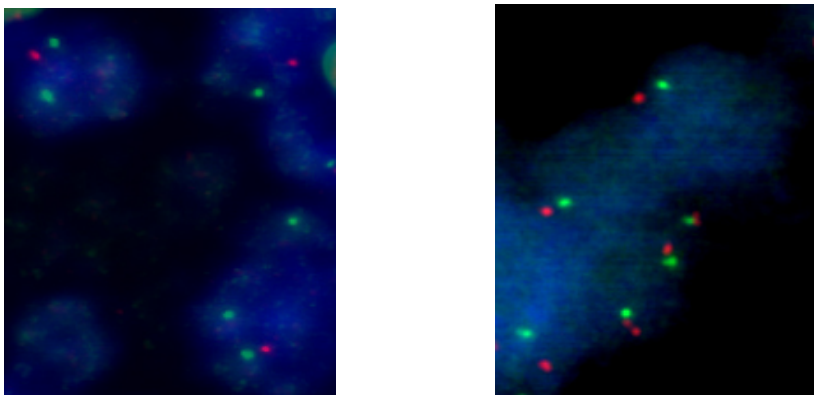


Figure 1. Prostate cancer cells harboring mono-allelic deletion for CHD1 (left) and prostate tumor cells with wild type (diploid) CHD1 (right). Orange signal: CHD1 probe; green signal: human chromosome 5 centromeric probe; blue color: DAPI nuclear stain. Representative view fields capture 3-3 cell nuclei at 60X magnification.

Identified the AA patient derived CPDR RC165 cell line for CHD1 deletion: Encouraged by the high quality of CHD1 detection we have performed FISH assay for CHD1 on selected AA and CA patient-derived cell lines. Reviewing our previous data on the transcriptome of the AA prostate derived CPDR RC165 hTERT immortalized cell line (Kim, Dobi et al., PCPD 2007) we have noted gene expression signatures of prostate cancer progression resembling the consequences of homologous recombination defects. Thus, we evaluated the AA patient derived RC165 cell line for CHD1 deletion by FISH assay (Figure 2.). The experiment result indicates the mono-allelic deletion of CHD1 gene in this cell line.

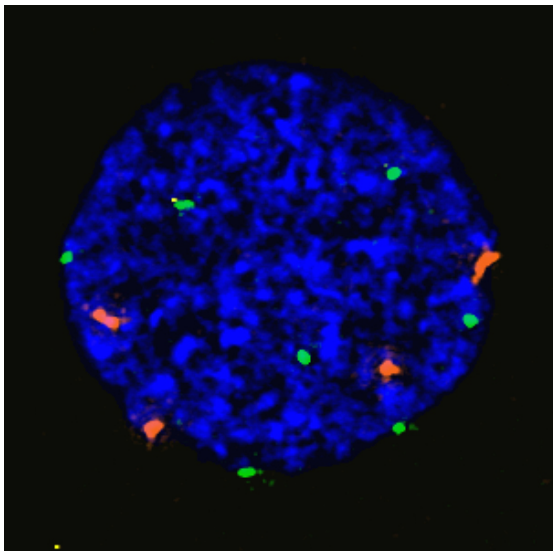


Figure 2. Allelic loss of CHD1 in the African American patient-derived CPDR cell line, RC165. The RC165 cell line is aneuploid for chromosome 5 (seven centromeric signals (green), whereas, only four CHD1 copy was detected in the cell nucleus (orange probe). Representative view of cell nucleus shown at 60X magnification.

In summary, in year #1 we have obtained regulatory approvals, completed the quality control of the CHD1 detection reagents and completed the CHD1 FISH experiment on TMAs derived from the prostate tumors of 50 AA and 50 CA patients.

Major developments mainly associated with the Dana Farer Cancer Institute site:

Specific Aim 3: Functional evaluation whether HRD mutational signatures can be directly induced in prostate cancer cell lines**.**

Major Task 1: To functionally evaluate if the HRD mutational signature can be induced in prostate cancer cell lines by deleting the CHD1 gene using clustered regularly interspaced short palindromic repeats (CRISPR) technology**.**

Subtask 1: In order to increase CRISPR based cleavage efficiency, we will create stable Cas9 protein expressing cell lines the PC-3 cell line.

Subtask 2: the CHD1 gene will be deleted using CRISPR technology both in a heterozygous and homozygous fashion. Single cell colonies will be isolated, validated and then grown for 50-100 generations to induce sufficient number of genomic aberrations to be detected by whole genome sequencing

Major activity

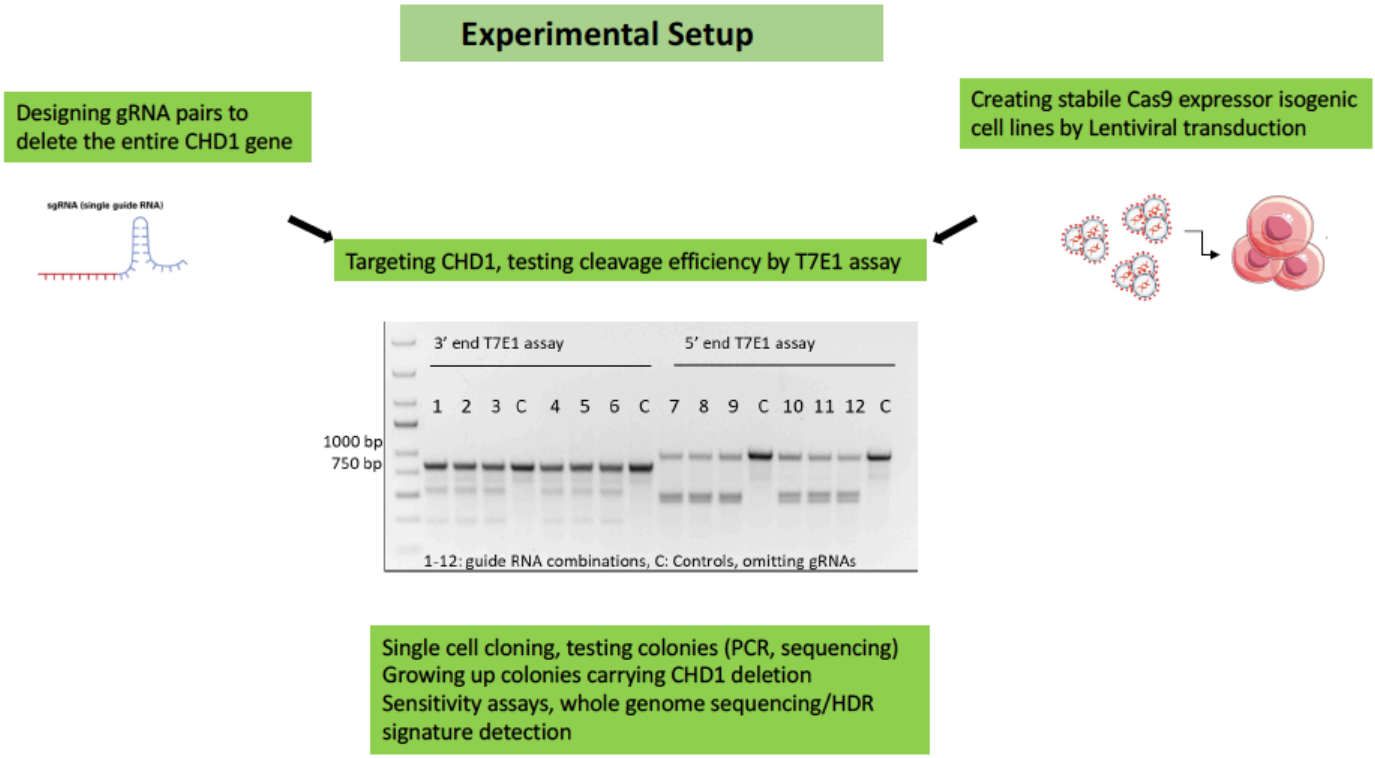
A stable Cas9 expressing version of the PC3 cell line was created by Lentiviral transduction.

After sufficient propagation, the stable Cas9 cell population was single cell cloned, and isogenic cell lines tested for Cas9 activity.

CHD1 deletion was induced by transfection using guide RNA pairs targeting the entire CHD1 gene. T7E1 assay protocol was optimized for testing Cas9 cleavage efficiency.

Single cell cloning: Cells were filtrated and plated 3 days after transfection into 20% FBS containing media with 1000, or 2000 cells per 10 cm dish (Corning) After 14-28 days, the formed colonies were picked up and growing in 96 well tissue culture plate (Corning).

The experimental system is outlined on the figure below.



The process of creating homozygous and heterozygous deleted CHD1 mutant cells is outlined below

PC-3 wt
Stable Cas9
expressor by
Lentivirus

Single cell cloning

Single cell clone derived,
isogenic stable Cas9
expressors:
Clone1
Clone2
Clone3
Clone4
Clone5
Clone6
All of them has functional
Cas9, **Clone1** was used for
further experiments

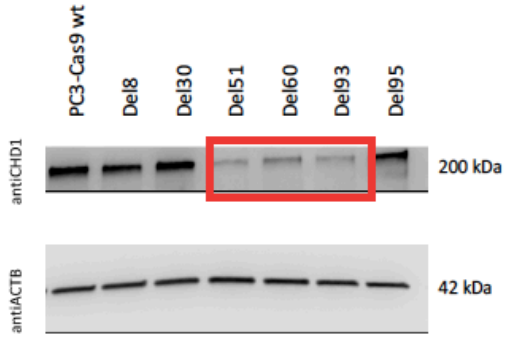
CRISPR based inducing CHD1
ko in Clone 1
Whole deletion of CHD1 was
induced, resulting **del51**
isogenic cell line, which is
carrying ***heterozygous***
deletion of CHD1, resulting
functional protein residue
verified by Western
Hibrydization.

Creating null mutants, by
using del51 isogenic cell line
for CRISPR. Frameshift
mutation of CHD1 was
induced by targeting exon2.
Complete abscence of CHD1
protein was verified in
several **isogenic clones** by
Western Hibrydization.

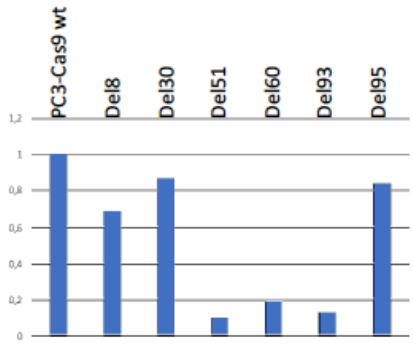
The figure below shows the significantly diminished expression of CHD1 in the heterozygous deleted mutant cell lines.

Induction of chd1 heterozygous mutation

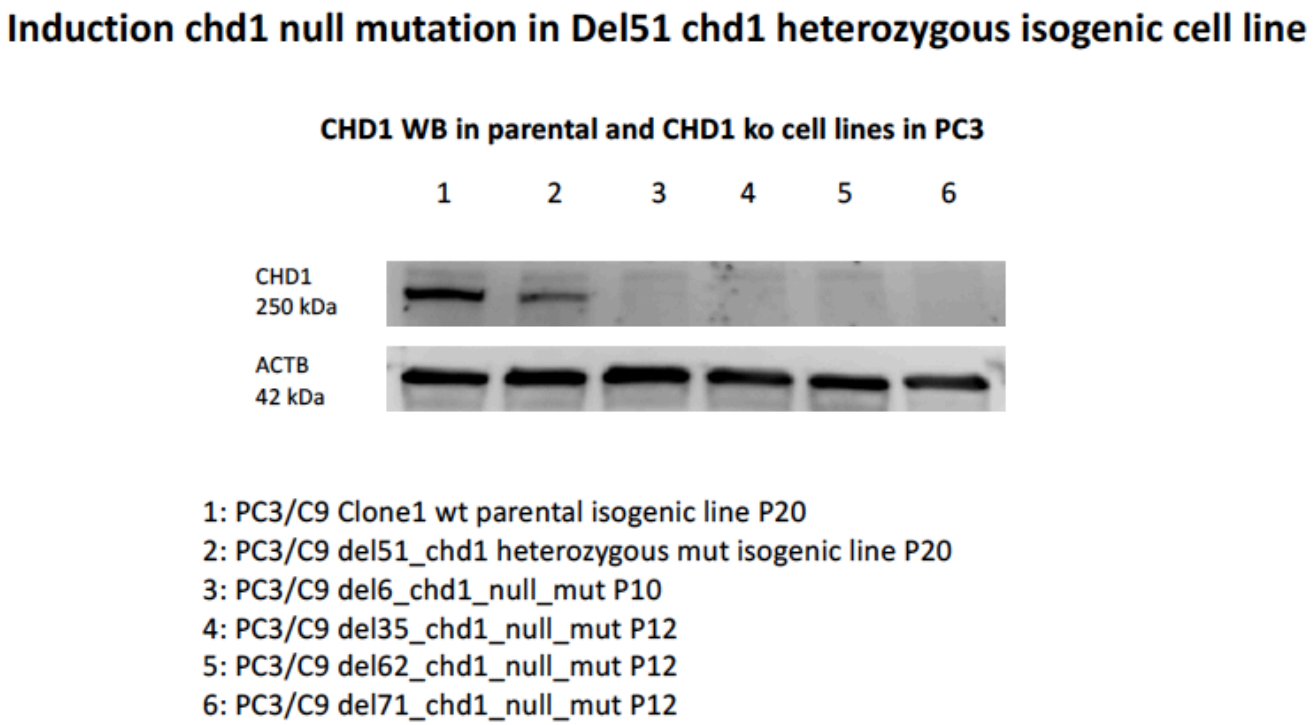
Western hybridization results of parental PC3-Cas9 wt and CHD1 deleted clones



CHD1 intensities normalized to parental PC3-Cas9 wt cell line.



The CHD1 homozygous deletion required repeating the entire process described above starting with a homozygous deleted clone, (clone del51)



What opportunities for training and professional development has the project provided? Nothing to report

How were the results disseminated to communities of interest? As outlined above, we needed to perform a detailed analysis on all the detectable mutational profiles induced by the various causes of homologous recombination deficiency in prostate cancer. This is our benchmark, starting point to understand the effects of CHD1 loss in prostate cancer. This work is being completed and summarized in a manuscript entitled "Genomic aberration based molecular signatures efficiently characterize homologous recombination deficiency in prostate cancer".

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

Children's Hospital, Boston:

Specific Aim #2: To analyze association between CHD1 loss and HR deficiency induced mutational signatures in clinical samples from prostate tumors using whole genome sequencing data.

With a robust computational pipeline and all benchmark analysis completed we are beginning to process all WGS data produced for African American prostate cancer cases. This will further strengthen our understanding about the loss of CHD1 in African American PRAD cases and will allow us to investigate the link between CHD1 loss and HRD induced mutational signatures.

Henry M. Jackson Foundation for the Advancement of Military Medicine (CPDR):

Towards Task #1:

- complete FISH and IHC assays of the existing 50AA-50CA TMA for other prevalent cancer gene defects (ERG, PTEN, LSAMP)
- perform association analyses with clinical-pathological features

Towards Task #2:

- generate a new TMA from the Dana Farber Cancer Institute carboplatin treated cohort of castration resistant PCa cases
- perform FISH assay for CHD1 deletion on the DFCI TMAs

Towards Task #3:

- obtain data sharing agreement to provide the existing Whole Genome Sequencing data of 35 CPDR AA PCa patients to DFCI

Dana Farer Cancer Institute:

We will start characterizing the CHD1 null PC3 cell lines for drug sensitivity (olaparib, talazoparib, niraparib, platinum agents. etc.)
We will expand the cultures and prepare them for whole genome sequencing.

What was the impact on the development of the principal discipline(s) of the project?
In our benchmarking work for the HR deficiency induced mutational signature analysis we found that the number of HR deficiency cases (by robust display of HR deficiency induced mutational signatures) is about twice as high (5-8%) than expected by BRCA1/2 mutational analysis. This strongly suggests that proportion of patients significantly benefiting from PARP inhibitor/platinum treatment is higher than initially thought and careful analysis of HR deficiency in prostate cancer will likely become and important diagnostic decision point for more personalized therapy.
The design, selection and careful quality control of CHD1 FISH probe and the establishment of hybridization condition in prostate tissues will assure the rapid adaptation of this prognostic tool from research to clinical settings. The rapid adaptation for standard pathology has been a key consideration through our project

recognizing the urgent need for prognostic tools and predictors of therapeutic response for African American prostate cancer patients.
All these results are important milestones towards a more targeted therapy of African American prostate cancer. We are, in fact, discussing this specific demographic aspect with planned clinical trials for talazoparib.

What was the impact on other disciplines? Nothing to report.
What was the impact on technology transfer? Nothing to report.
What was the impact on society beyond science and technology?
Our results constitute one of the few instances when a robust biological difference can be detected underlying the well-known disparities in cancer outcome. By the successful completion of the proposal we will have a significant advancement in determining which African American prostate cancer patient will benefit from platinum-based or PARP inhibitor treatment. Precise detection of CHD1 deletion will likely become a clinical tool aiding therapeutic stratification for therapies targeting homologous recombination.

CHANGES/PROBLEMS:
Changes in approach and reasons for change: Within the reporting period there was a change of Sub-award PI for the Henry M Jackson Foundation site that has been approved by the awarding agency. The project or its direction was not affected by the PI change.

Actual or anticipated problems or delays and actions or plans to resolve them:
An unexpected challenge was to obtain IRB approvals, originally planned for months 1-4. After 9 months in review, our protocol was determined as “research not involving human subjects as defined by 32 CFR 219.102(e) because the research involves the use of de-identified specimens and data not collected specifically for this study”. The initial and secondary reviews and approval by the USAMRMC, Office of Research Protections, Human Research Protection Office took an additional 3 month.

To resolve these delays, we may request a one year no-cost extension.

An anticipated challenge is the analyses of Whole Genome Sequences (WGS). One genome set of WGS at 110X depth is 3 Terabyte of uncompressed data. For 35 patients and their matched germ line DNA (total 70 WGS) it will be 210 Terabyte. The current secured file system for data transfer allows only 2 Gigabyte (that is 100,000 times smaller that the transfer capacity we need) while the use of portable data storage devises that could solve this issue is prohibited.

A potential solution for this problem is to obtain authorization/clearance for the DFCI PI to perform the bioinformatic analyses within our system, or to obtain a special permission for data transfer on portable device.

Changes that had a significant impact on expenditures: Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

PRODUCTS:

Journal publications: Nothing to report

Books or other non-periodical, one-time publications: Nothing to report

Other publications, conference papers and presentations: Nothing to report

Website(s) or other Internet site(s): Nothing to report

Technologies or techniques: Nothing to report

Inventions, patent applications, and/or licenses: Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name: Zoltan Szallasi, MD

Project Role: PI

Researcher Identifier (e.g. ORCID ID): 0000-0001-5395-7509

Nearest person month worked: 1month

Contribution to Project: Dr. Szallasi provides overall supervision for the project and supervises and performs some of the bioinformatic analysis.

Funding Support:

Name: Viktoria Tisza, PhD

Project Role: Research Associate

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 5 months

Contribution to Project: Dr. Tisza works on preparing the CRISPR edited cell lines, she propagates the clones and prepares those for next generation sequencing.

Funding Support:

Name: Albert Dobi, PhD

Project Role: Subaward PI

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr. Dobi provided directions under this subaward, wrote the IRB protocol, corresponded with the IRB, coordinated and oversaw the generation of TMA, directed the selection and quality control of CHD1 FISH probe.

Funding Support:

Name: Hua Li, MD, PhD

Project Role: Co-Investigator

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 2

Contribution to Project: Dr. Li contributed to the IRB protocol writing, generated the new TMA, performed FISH assay for CHD1, performed pathology and reading of FISH results.

Funding Support:

Name: Matthew Freedman, MD, PhD

Project Role: Subaward PI

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr. Freedman is supervising most of the CRISPR editing of the cell lines.

Funding Support:

Name: Ji-Heui Seo PhD

Project Role: research fellow

Researcher Identifier (e.g. ORCID ID):

Nearest person month worked: 1

Contribution to Project: Dr. Spisak is supporting the CRISPR editing of the cell lines as a technical expert in the field.

Funding Support: