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TITLE: Genetic Alterations in Prostate Cancers among African-American Men and Comparisons with Cancers from European and Asian Patients

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14. ABSTRACT

A large and systematic evaluation of somatically acquired changes in the tumors of African American man is needed to identify race-specific signatures that may be associated with increased aggressive and poor outcome of prostate cancer (PCa) in this under-studied population. We have analyzed DNA copy number alterations (CNAs) in a subset of the tumors from African Americans with PCa and compared them to those in European American and Chinese PCa. Our data reveal that the most frequent CNAs include hemizygous deletions on chromosomal 8p and 13q represented by *BNIP3L* and *RB1*, respectively. To our surprise, no subjects in this subset of African American patients harbored the deletion between the 3' of *TMPRSS2* and 3' *ERG* (T_E) that creates the fusion of these two genes. Preliminary result analysis suggests that the tumor genome of African American PCa may harbor a distinct CNA landscape, though analyzing a large number of tumors from additional patients is warranted to confirm our findings. In addition, we have developed a multiplex ligation-dependent probe amplification (MLPA)-based method and a probemix panel for identifying *PTEN* deletions and *MYC* amplifications that have been shown to be associated with lethal PCa. Additional experiments are needed to demonstrate the utility of this probemix for the identification of patients with aggressive PCa in African Americans.

15. SUBJECT TERMS

Prostate cancer; genetics

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INTRODUCTION:

Prostate cancer (PCa) research is lacking in the area of genetic epidemiology, particularly as it relates to African American men, who are 50 percent more likely to develop PCa than white men. While extensive genome-wide analyses have been carried out using tumor DNA from European Americans, the landscape of the tumor genome in African American men has not been established. In addition, more research is needed to be able to distinguish aggressive from non-aggressive PCa, especially at early stages. This study seeks to identify somatically acquired genetic alterations in African American men that are associated with aggressive and lethal PCa. Our secondary goal is to develop a cost-effective genetic test to better identify men at heightened risk of developing progressive PCa, which will allow for earlier detection of disease and more effective treatment.

KEYWORDS: Prostate cancer, genetics

ACCOMPLISHMENTS:

- **What were the major goals of the project?**

  Aim 1: We will recruit 100 African American patients, and their tumor-specific DNA alterations will be identified using high resolution SNP (single nucleotide polymorphism) array and targeted next-generation sequencing technology.

  Aim 2: We will compare these alterations with those identified from 240 European Americans and 65 Chinese PCa patients in order to find race-specific alterations that are associated with lethal PCa, as well as other clinical outcomes, such as Gleason score and pathological stage of the tumors.

  Aim 3: We plan to develop a genetic test to translate these research findings to clinics.

- **What was accomplished under these goals?**

  Aim 1: ***A). Genomic DNA of fresh-frozen tumor and matched normal tissues from 9 African American patients has been analyzed*** using Affymetrix Genome-Wide Human SNP 6.0 Arrays. To characterize the landscape of genomic changes, we analyzed DNA copy number alterations (CNAs) of 31 genes that either represent significant CNAs in the PCa tumor genome of European Americans or have significant biological implications in cancer development. As shown in Table 1, the most frequent CNAs included hemizygous deletions on chromosomal 8p and 13q represented by *BNIP3L* and *RB1*, respectively, with ~ 56% and 44% of patients affected, respectively. To our surprise, none of these African American patients harbor the deletion between the 3' of *TMPRSS2* and *ERG* (T_E in Table 1) that creates the fusion of these two genes, as this fusion is one of the most common somatically acquired changes of genomic structure in European American PCa.

Table 1. Frequency of DNA copy number alterations (CNAs) in the tumor genome of African American patients with PCa

CNAs*	*ADAR*	*DISC1*	*LRP1B*	*FOXP1*	*RYBP*	*SHQ1*	*ATP1B3*	*PDE4D*	*CHD1*	*TMEM157*	*MAP3K7*	*COL1A2*	*BNIP3L*	*TPDS2*	*MYC*	*CDKN2A*	*PTEN*	*HTR3A*	*ETV6*	*CDKN1B*	*MDM2*	*RB1*	*DIAPH3*	*WWOX*	*USP10*	*TP53*	*SMAD4*	*PhLPP*	*BCL2*	*SERPINB5*	*T_E*
n	89	67	78	89	89	100	89	56	89	78	67	89	44	67	67	100	67	89	78	89	100	56	78	89	89	100	89	89	89	89	100
l	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
g	11	0	11	0	0	0	11	11	0	0	0	11	0	33	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d	0	33	11	11	11	0	0	33	11	22	33	0	56	0	0	0	33	11	22	11	0	44	22	11	11	0	11	11	11	11	0

* 'd' and 'dd' denote hemizygous and homozygous deletion, respectively; 'g' and 'a' denote one and > 1 additional copy gain of DNA, respectively;
n' denotes no change; 'l' denotes loss of heterozygosity without change in DNA copy number.

2). We have requested tumor and matched normal FFPE tissues from an additional 100 African American subjects from the Prostate Cancer Biorepository Network (PCBN) at Johns Hopkins. The work at PCBN is supported by the prostate cancer research program of the DOD. Because FFPE tissues represent the most common types of specimens in clinical settings, we aim to expand our landscape analysis of the tumor genome among African American PCa using DNA from this type of clinical sample. The FFPE tissues collected at PCBN from the 1980s to present represent a large set

of specimens linked to clinical, pathological and outcome data, which will enable us to identify a subset of DNA-based molecular markers for predication of cancer progression.

To evaluate the feasibility of using DNA from FFPE samples, we performed exploratory assays using both SNP array 6.0 and OncoScan FFPE array from Affymetrix. The results reveal that there are large variations and noises among the data generated from SNP array 6.0. On the other hand, we were able to clearly identify CNAs in the tumor genome using OncoScan FFPE assay kit. With ~ 80 ng DNA starting material, this assay using the molecular inversion probe has been shown to perform well with highly degraded DNA, such as that derived from FFPE-preserved tumor samples of various ages, which are the most common specimens in clinical settings. Using this assay, individual CNAs across the whole genome of each tumor can be clearly identified based on log2 ratio and B allele frequency. As shown in Figure 1, tumor D15-1342-9 harbors 3 major regions of CNAs, including amplifications of 3q affecting 284 genes and deletions of 8p and 13q affecting 227 genes and 196 genes, respectively.

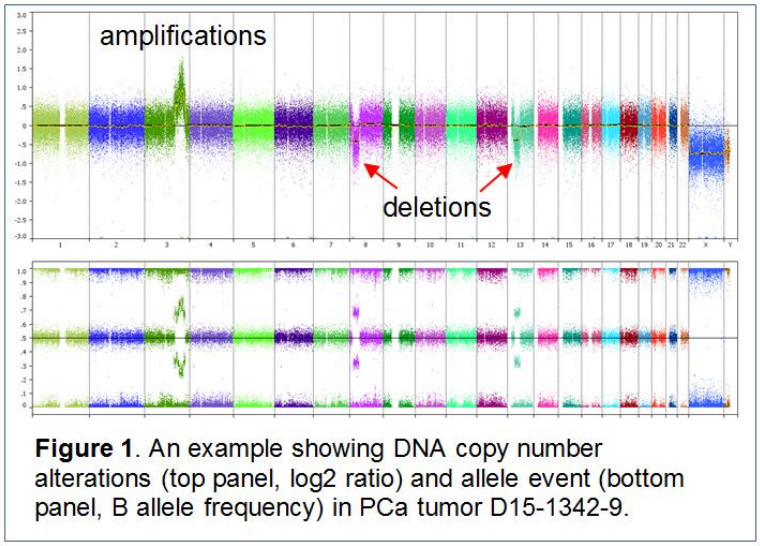

Figure 1. An example showing DNA copy number alterations (top panel, log2 ratio) and allele event (bottom panel, B allele frequency) in PCa tumor D15-1342-9.

Aim 2: ***Preliminary results suggest distinct tumor CNA landscape of African American PCa*** in comparison to those of European American and Chinese PCa. Although tumor CNA landscape of European American has been well established, CNAs across the tumor genome in Chinese PCa have not been revealed using a large tumor cohort. To uncover the CNA landscape of Chinese PCa, we have started with FFPE samples of tumors from 30 Chinese patients treated at Hua Shan Hospital using an Affymetrix OncoScan FFPE assay. The preliminary CNA landscape of Chinese PCa presented in Figure 2 is characterized by apparent amplifications of 3q and 8q and deletions at 8p and 13q among these patients. These include well known cancer genes such as *PIK3CB*, *MLF1* and *PIK3CA* at 3q; *MYC* at 8q; *TUSC3*, *MSR1*, *NKX3-1*, and *BNIP3L* at 8p; *BRCA2*, *FOXO1* and *RB1* at 13q. With a limited number of tumors analyzed to-date, we are unable to identify high frequencies of *PTEN* deletion or *TMPRSS2-ERG* fusion as commonly observed in Caucasian men. Therefore, it is apparent that the frequency of *TMPRSS2-ERG* fusion is much lower in African American and Chinese PCa than that in European Americans. In addition, the frequency of *RB1* loss seems higher in African American PCa cases than in European and Chinese PCa cases, while a large number of tumors from both African American and Chinese men is needed to validate these preliminary findings in subsequent studies.

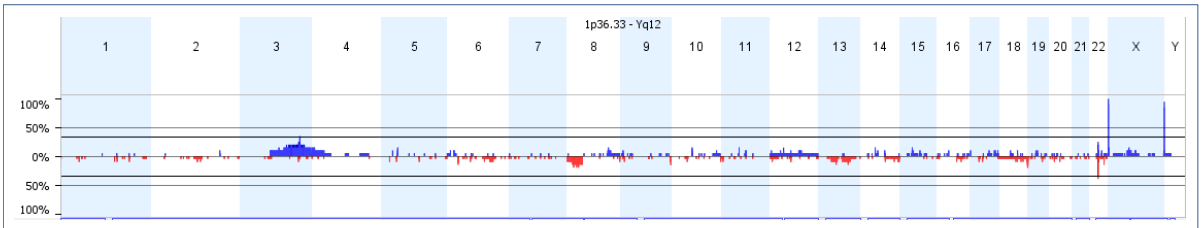

Figure 2. Frequency plot showing the preliminary landscape of Chinese PCa revealed by OncoScan PPFE assay. Dark blue represents DNA amplifications. Red depictures genomic deletions.

Aim 3: ***1). Design and synthesize MLPA probemix***. As we have reported previously, deletion of *PTEN* and/or amplification of *MYC* are significantly associated lethal PCa. Using current genomic information from a UCSC genome browser (hg38), we designed a total of 29 pairs of synthetic

MLPA (Multiplex Ligation-dependent Probe Amplification) test probes for *PTEN* and *MYC*, as well as reference probes on chromosomes 1, 2, 6, 9, 11, and 15 for controls. All probes were synthesized by IDT Integrated DNA Technologies Inc. (Coralville, Iowa). After vigorous testing using DNA with known CNAs at these locations, 16 of the probes, including 5 pairs for *PTEN*, 5 pairs for *MYC* and 6 pairs for reference controls, were selected to use in our primary panel of probemix to detect CNAs of *PTEN* and *MYC*.

2). Probe test and analytical validity. To test these probe mixes under various conditions with different amounts of DNA, we first used DNA from prostate cancer and normal cell lines with 50 ng per reaction as recommended by MRC-Holland (Amsterdam, Netherlands). We then created FFPE-equivalent DNA from prostate cell lines RWPE (normal control) and PC3 (cancer cell with *PTEN* deletion and *MYC* amplification) by fixing these cells using formalin. DNA isolated from these formalin-fixed cells of 100% RWPE, 100% PC3, 25% RWPE plus 75% PC3, 50% RWPE plus 50% PC3, or 75% RWPE plus 25% PC3 was used to test the detection limits with different amount of normal DNA "contamination". We next tested the detection limits with reduced amounts of input DNA from 50 ng to 0.2 ng per reaction using these types of DNA.

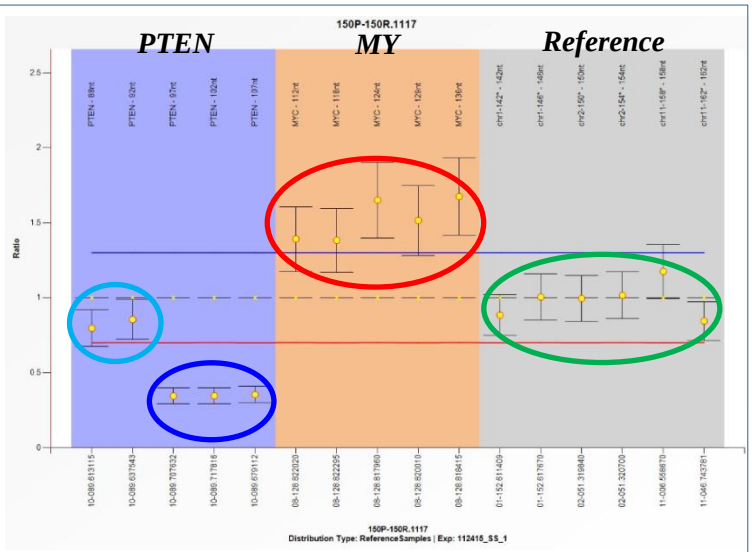

Figure 3. An example of detecting CNAs of *PTEN* and *MYC* in DNA isolated 50% PC3 plus 50% RWPE cells fixed with formalin. Light blue, heterozygous deletion, dark blue, homozygous deletion; red, amplification; green, normal DNA.

We found that 1) the assay required a pair of tumor and matched normal samples to reduce analytical variations; 2) the fragment sizes of the DNA isolated from formalin-fixed cell lines was very similar to those isolated from FFPE tissues; 3) the minimum amount of DNA from each of the samples in the current protocol was 1 ng to control analytical variability, with a working range from 1 to 2 ng per reaction for routine assays; 4) the minimum amount of cancer cells (with CNAs of *PTEN* and/or *MYC*) required in the test samples was ≥ 50% to assure the sensitivity of detection; 5) under these conditions, the analytical specificity and reproducibility was 100% for *PTEN* heterozygous/homozygous deletions and *MYC* amplification using the DNA isolated from formalin fixed cells. The analytical validity of our probemix panel and MLPA assay method will be further assessed using DNA isolated from fresh-frozen and FFPE prostate tumor and matched normal tissues.

- **What opportunities for training and professional development has the project provided?**
  - Not applicable
- **How were the results disseminated to communities of interest?**
  - Not applicable at this stage
- **What do you plan to do during the next reporting period to accomplish the goals?**

  During the next reporting period, which will be our second active period for this study, we will complete the patient recruitment portion of this project, obtain additional tumor and matched normal tissues, and complete CNA analysis and next-generation sequencing to identify DNA-based biomarkers associated with risk of aggressive PCa in African American men.

IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Nothing to report
- **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?**
  - Nothing to report

CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
  - Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - Nothing to report
- **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
- **Significant changes in use or care of human subjects.** N/a
- **Significant changes in use or care of vertebrate animals.** N/a
- **Significant changes in use of biohazards and/or select agents** N/a

PRODUCTS:

- **Publications, conference papers, and presentations**
  - **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

- **Other Products**
    - Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**
    - Wennuan Liu, William B Isaacs, Siqun Lilly Zheng, Jianfeng Xu

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
    - New award to J. Xu (W81XWH-16-1-0765)
        - PCRP Impact Award - Partnering PI
        - Performance Period 09/30/2016 - 09/29/2019
        - $982,801

- **What other organizations were involved as partners?**
    - **Organization Name:** Johns Hopkins Hospital, Department of Urology
    - **Location of Organization:** Baltimore, MD
    - **Partner's contribution to the projec**t
        - **Financial support** N/a
          In-kind support N/a
          Facilities N/a
        - **Collaboration** Yes – Dr. William Isaacs
        - **Personnel exchanges** N/a
        - **Other.** 100 prostate biopsy tissue samples

SPECIAL REPORTING REQUIREMENTS N/a

COLLABORATIVE AWARDS: N/a

QUAD CHARTS: N/a

APPENDICES: N/a