EXOME SEQUENCING REVEALS NOVEL SOMATIC MUTATIONS UNIQUE TO AGGRESSIVE PROSTATE CANCER IN AFRICAN AMERICANS

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INTRODUCTION

- Prostate cancer (PCa) is the most common male cancer and a leading cause of cancer-related death in the United States. Incidence of PCa is higher in African Americans than any other race. African-American men are diagnosed with PCa at a younger age and are more frequently diagnosed with high grade tumors, resulting in greater mortality than other races. The contribution of genetic factors to the aggressiveness of PCa in African Americans is largely unknown.
- Exome sequencing technology allows complete sequencing of the coding portion of the genome, thus providing an opportunity to detect somatic mutations. We hypothesize that exome sequencing of African-American prostate tumors and matched blood samples may reveal unique mutations that may contribute to the aggressiveness of PCa in African Americans. Identification of such mutations may provide the basis for improved prognostic tests and personalized treatment options, ultimately reducing the disparity of PCa in African Americans.
- In this study, we performed exome sequencing on nine aggressive-phenotype prostate tumors as well as the blood samples from the corresponding African-American patients in order to identify somatic mutations associated with aggressive PCa. Bioinformatics analysis was then performed to identify novel putative somatic mutations. Mutation frequency in the nine samples was compared to the reported mutation frequency in Caucasians in order to select those mutations unique to African Americans. A total of seventy-four genes containing recurrent, novel somatic mutations were identified.
- As a result, the mutations within the MLL3 gene were selected for validation. In this process, the mutations were re-sequenced in the original samples through Sanger sequencing. Large sample set validation of the mutation frequency using data from aggressive and non-aggressive African-American PCa groups is still required in order to determine if these MLL3 somatic mutations are associated with aggressive PCa in African Americans.

EXOME SEQUENCING

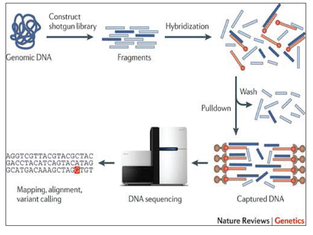
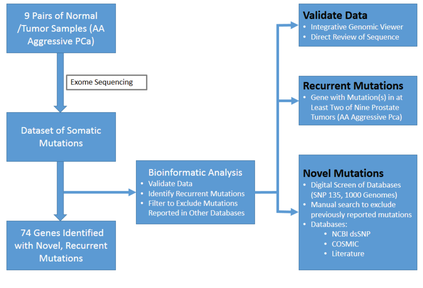


Figure 1. Genomic DNA is fragmented, and adapters are ligated to the fragments. The fragments which contain exons are "captured" via hybridization to an exome library. The exome DNA library is retained by magnetic beads. The intron DNA is then removed, and the exome DNA is sequenced on a microarray chip.

- Exome sequencing technology was used to sequence tumor/ matched normal DNA.
- Seven pairs of prostate tumors/blood samples and two additional prostate tumor samples were used.

BIOINFORMATICS ANALYSIS



SOMATIC MUTATION DATA

Table 1

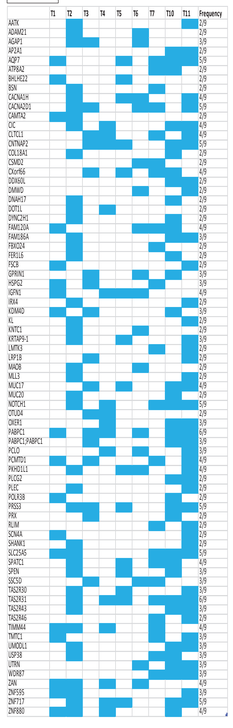


Table 1. Seventy-four genes contain recurrent mutations within nine tumor samples from African Americans. Blue cells indicate the presence of at least one mutation within the tumor sample for each gene.

Table 2

Tumor Sample	Age	Gleason	PSA
1T	53	4+5	18.8
2T	58	4+3	170.4
3T	57	3+4	21.4
4T	53	3+4	NR
5T	54	3+4	6
6T	52	4+3	NR
7T	60	3+5	17.9
10T	56	4+3	6.9
11T	53	4+3	NR

Table 2. Chart of clinical characteristics for each tumor sample. NR = not recorded.

Figure 2

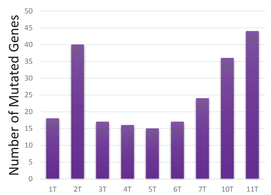


Figure 2. Histogram shows the total number of genes with mutations per tumor sample.

SELECT EXAMPLES

Table 3. Examples of Somatic Mutation Identification in AA Pca*

ID	Gene	Mutations	Mutation Type	Depth	M-allele	COSMIC	CA-AA
11T	AATK	NM_004920:c.G2363A:p.R788H	1	34	18	1/482 (0.21%)	0/112
11T	AATK	NM_004920:c.G1756A:p.G586S	2	54	28		
2T	AATK	NM_004920:c.C3121T:p.R1041C	3+	51	26		
1T	DNAH12	NM_178504:c.G3577T:p.A1193S	1	45	13	1/424 (0.24%)	0/112
10T	DNAH12	NM_178504:c.C3972G:p.C1324W	2	81	45		
3T	DNAH12	NM_178504:c.G3577T:p.A1193S	1	21	9		
6T	DNAH12	NM_178504:c.G8423A:p.G2808E	3	10	4		
11T	MLL3	NM_170606:c.G8180A:p.G2727D	1	113	14	24/424 (5.66%)	2/112
2T	MLL3	NM_170606:c.A2495C:p.K832T	2	253	26		

**These mutations are neither present in normal samples nor reported in the SNP 135 Database, the 1000 Genomes Project, or the NCBI SNP Database.*

MLL3

- MLL3 is a histone methyltransferase involved in epigenetic regulation of transcription.
- MLL3 somatic mutations have a role in acute myeloid leukemia (AML).

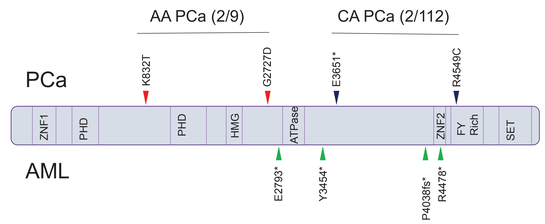


Figure 3. Mixed-Lineage Leukemia 3 (MLL3) is a 4911 amino acid protein. The red arrowheads indicate the somatic mutations identified by exome sequencing of nine aggressive prostate tumors from African Americans. The blue arrowheads show the mutations from a Caucasian population. The green arrowheads show somatic mutation found in AML.

PCR AMPLIFICATION

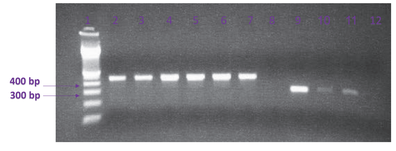


Figure 4. PCR amplification of genomic DNA using primers designed to flank the mutated regions of MLL3. Lane 1 contains a 100 bp DNA ladder. Lanes 2-8 contain DNA samples 2T, 2N, 4T, 4N, 11T, 3T, and a negative control amplified with a primer pair flanking a 466 bp product. Lanes 9-12 contain DNA samples 11T, 3T, 3N, and a negative control amplified with a primer pair flanking a 306 bp product.

MLL3 SEQUENCE CHROMATOGRAM

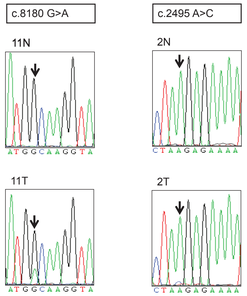


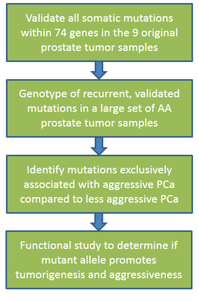
Figure 5. Validation of MLL3 mutations with Sanger sequencing.

- Black arrow indicates location of mutation.
- Gene mutation is recurrent (present in PCa samples 2T and 11T).

CONCLUSION

- Exome sequencing and bioinformatics analysis revealed seventy-four genes with novel, recurrent somatic mutations among nine aggressive AA PCa.
- Recurrent somatic mutations in MLL3 were validated by Sanger sequencing.
- MLL3 somatic mutations identified in AA PCa are novel and have a higher frequency (2/9) than in CA PCa (2/112).
- Exome sequencing technology has the potential to identify somatic mutations unique to aggressive AA PCa which may lead to improve diagnosis and treatment of aggressive PCa in African Americans, ultimately reducing the disparity.

WHAT'S NEXT?



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