Genomics of Prostate Cancer Health Disparity-Beyond the Gene List

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Florida A&M University
Tallahassee, Florida
29 July, 2010 Uof M
Yes, I brought the band: FAMU Marching 100
Prostate Cancer

• Most commonly diagnosed male cancer
• African American Males have highest morbidity and mortality rates than any other racial and ethnic groups
• Annual CaP incidence for AA was 59% higher than that of White Males (2001-2005).
• Overall CaP incidence down, but CaP decline in AA lags behind that of White males
• Hence, CaP is a health disparity for AA men

• Cause of this disparity still unclear…
Factors thought to Contribute to CaP Disparity

CAG repeats
Inadequate knowledge
Lower socioeconomics
Limited access to health care
Less likely to ID early symptoms
Poor CaP survival
Present at late stage
High Grade CaP
More aggressive CaP tumors
Different Disease Biology
Higher testosterone
Age, Race (non-modifiable factors)

Prostate Cancer

We favor the Hypothesis:

• Differences in Gene expression may explain CaP health disparity
Identified 97 Candidate Genes

- Reams RR, Agrawal D, Davis MB, Yoder S, Odedina FT, Kumar N, Higginbotham JM, Akinremi T, Suther S, Soliman KF.
- Infect Agent Cancer. 2009 Feb 10;4 Suppl 1:S3.PMID: 19208208
Identification of differentially expressed genes

• Filtering criteria of at least +/- 2.0 fold change AND p< 0.00001
Description of PCa Tumor Tissue

• This pilot study made use of (three) 3+3 CaP tumor & non-tumor (normal) matched control samples from African American Males and (three) 3+3 PCa tumor & matched control samples from White American males.

• Samples were obtained from Dr. Deepak Agrawal, a collaborator from H. Lee Moffitt Center via IRB approval. The limitation of this study is the small number of PCa samples available.
Microarray Platform

- Gene Expression data were measured using the Affymetrix GeneChip HG-U133 plus 2 arrays containing 54,000 probe sets.

- Total of 12 samples were used 3 tumor & 3 normal from AAM; 3 tumor and 3 normal from WAM.

- Data from cel files was uploaded to R-bioconductor (LIMMA package or SAM used)
Experimental Design II

Case matched ratios

Caucasian

Tumor

Normal

African-American

Tumor

Normal

Comparison of ratios
Case-matched ratios ~ test for race specific expression trends

- chromosome 9 open reading frame 93
- helicase (DNA) B
- cholinergic receptor, muscarinic 3
- zyg-11 homolog A (C. elegans)
- ring finger protein 187
- histone deacetylase 11
- hypothetical protein LOC440295
- interferon regulatory factor 4
- paired box gene 5 (B-cell lineage specific activator)
- homeobox D4
- alanine-glyoxylate aminotransferase
- contactin 4
- transcription factor 3 (E2A immunoglobulin enhancer)
- potassium voltage-gated channel, KQT-like subfamily
- zinc finger protein 650
- WD repeat domain 32
- inscuteable homolog (Drosophila)
- WD repeat domain 15 open reading frame 26
- transient receptor potential cation channel, subfamily
- DEAD (Asp-Glu-Ala-As) box polypeptide 19B
- cholinergic receptor, nicotinic, alpha 10
- paired-like homeodomain transcription factor 1
- chromosome 9 open reading frame 62
- chromosome 21 open reading frame 88
- tubulin tyrosine ligase-like family, member 5
- transcription factor 12 (HTF4, helix-loop-helix transcri
- progesterone receptor
- myeloid/lymphoid or mixed-lineage leukemia 4
- NSFL1 (p97) cofactor (p47)
- ring finger protein 168
- hypothetical protein FLJ32658
- glypican 5
- Friend leukemia virus integration 1
- heterogeneous nuclear ribonucleoprotein D (AU-rich e
- ADAM metallopeptidase with thrombospondin type 1
- myelin-associated oligodendrocyte basic protein
- Ran GTPase activating protein 1
- delta-like 3 (Drosophila)
- chemokine (C-X-C motif) ligand 2
- G protein-coupled receptor 98
- BUB3 budding uninhibited by benzimidazoles 3 homo
Beyond the Gene List
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<th>Pvalue</th>
<th>OddsRat</th>
<th>ExpCoun</th>
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</table>
Beyond the Gene List

New Bioinformatics Collaborator

Krishna Rani Kalari (Mayo Rochester):
Performed genotype-phenotype, SNP and expression transcript levels, correlations using HapMap Yorubi Population on the 97 genes previously revealed to be differentially expressed in BM tumors compared to WM tumors
**Results**

26527 genotype-phenotype associations were obtained with a p-value < 10^-3, of which 17,542/26,527 associations has a p-value < 10^-4 (table with association results is attached below).

Genome-wide associations with a p-value < 10^-3 were obtained for making Manhattan plots. Association and map files were created as input files for the Haploview software to generate Manhattan plots.

![Genome-wide association of SNPs and candidate prostate cancer genes](image.png)

**Figure 1:** Results of genome-wide association SNPs with candidate prostate cancer genes using HapMap lymphoblastoid cell lines in YRI population. X-axis consists of different chromosomes and Y-axis consists of negative log p-value for association.

Association results in lymphoblastoid cell lines show that RanGAP1 gene which is a key regulator of the RAN GTP/GDP cycle that is located on chromosome 22 may be involved with...
Association Results in YRI lymphoblastoid cell lines

RanGAP1 gene is located on chromosome 22 and may be involved with ABCD3 gene located on Chromosome 1.

***************

RanGAP1 gene, key regulator of the RAN GTP/GDP cycle.

*****************

ABCD3 gene which is ATP-Binding cassette, subfamily member.
Association Results in YRI lymphoblastoid cell lines

STXBP2 gene is located on chromosome 19 and may be involved with TMTC2 gene located on Chromosome 12

********************

STXBP2 gene, syntaxin binding protein

********************

TMTC2 gene a transmembrane tetraccapeptide repeat containing 2 gene
Association Results in YRI lymphoblastoid cell lines

Genome-wide results show 1167 cis interactions, where expression gene and SNP are located on the same chromosome (p < 0.03). Most cis interactions in coding region; possible functional role in gene expression hence
Ingenuity Pathway Analysis
Revealed 3 top Networks Hubs

1. ERK
2. MAPK
3. NFkB
ABCD3 gene which is ATP-Binding cassette, subfamily member that is located on chromosome 1 (Figure 1). In addition, STXBP2 gene which is a syntaxin-binding protein that is located on chromosome 19 may be involved with a region on chromosome 12. The chromosome 12 region consists of transmembrane and tetra-ricopeptide repeat containing 2 (TMTC2) gene that is approximately 400 kb away from the region where STXBP2 gene is associated (Figure 1).

Genome-wide results also show that there are 1167 cis interactions (where expression gene and SNP are located on the same chromosome) out of 26527 associations with a p-value < 10^-3. Most of the cis interaction associations are found in protein coding regions. Pathway analysis was performed using the genes that are involved with the downstream effects of SNPs along with candidate prostate cancer genes that are involved in cis regulation. Ingenuity software was used to perform the pathway analysis. This software consists of a curated database and several analysis tools to obtain pathways associated with a set of genes. Results of the Ingenuity Pathway Analysis showed that network hubs are centered on ERK, Mapk and NFkB pathways.
Attendance at This NCIBI

Much needed, to gain knowledge in current state of the art bioinformatic tools, Metabolomics, Proteomics

Collaborations to use NCIBI Tools to further probe this 97 candidate gene list

....
ACKNOWLEDGEMENTS

Florida A&M Univ
Folakemi Odedina
J. Mark Higginbotham, PhD Candidate
Univ of Chicago
Melissa B. Davis (bioinformatics)

Mayo Rochester
Krishna Rani Kalari (bioinformatics)

Moffitt Cancer Center
Nagi, Kumar
Deepak Agrawal
Sean Yoder
Microarray Core Facility
Tissue Bank

DoD FAMU Moffitt Prostate Cancer Training Center Grant
YOU ARE INVITED TO ATTEND!!
NCI Sponsored/Univ of Florida
Hosted
The Science of Global Prostate Cancer Disparities in Black Men
Aug. 27-29, 2010
Crowne Plaza
Jacksonville, FL
Welcome
The Science of Global Prostate Cancer Disparities in Black Men
"Bridging Gaps Through Research, Education, and Outreach Worldwide"

http://conferences.dce.ufl.edu/docs/GPC/UF_GPCD_Agenda.pdf
1st Biennial Conference

Highlights

• Global Collaborations Among Cancer Scientists from UK, Bahamas, Africa and USA, clinicians, survivors, and advocates
• To better understand the etiology of CaP among at-risk Black Men, develop interventions to address CaP disparities
• Prof Folake Odedina won an NCI grant award to host conference and bring in international scientists
• CME credits
Experimental Design I

Case paired tests

Random pairs

Caucasian

Normal

Tumor

African-American
CA tumor vs. normal
Case Paired t-test analysis

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<tr>
<th>Gene Name</th>
<th>Log2 Fold Change</th>
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Volcano plot
African American tumor vs. normal
Case Paired t-test analysis

leukemia inhibitory factor receptor alpha 1.612491
aldehyde oxidase 1 -1.582018
acyl-CoA synthetase medium-chain family member 3 1.462871
natriuretic peptide receptor C/guanylate cyclase C (a histone cluster 1, H2bg 1.198831
1.245253
1.148056
RAD23 homolog B (S. cerevisiae) -1.22417
insulin-like growth factor 1 (somatomedin C) 1.025239
paired related homeobox 1 -0.966881
prostate-specific non-coding gene 0.918045
1.024488

Volcano plot

average MA plot
Overlaps case paired t-test Af Am. & Caucasian genelists

Top 100 genes from each t-test

transcription elongation factor A (SII)-like 7
Case-matched ratios ~ Race group test

Volcano plot

Modified MAplot