GENE EXPRESSION ANALYSIS OF PROSTATE TUMORS FROM AFRICAN-AMERICAN AND EUROPEAN-AMERICAN MEN

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Prostate Cancer (PCa) disproportionately affects African-American men in terms of incidence, morbidity, and mortality.

The incidence of PCa is 60% higher in African-American men than in European-American men.

African-American men have over two times the mortality rate from PCa than European-American men.

Socioeconomic factors do not fully explain the differences in PCa incidence, aggressiveness, and mortality amongst different ethnic groups.
Hypothesis for Study

Significant differences in tumor biology exist between African-American and European-American men that contribute to the health disparity associated with prostate cancer.
Clinical Characteristics of the Study Population

<table>
<thead>
<tr>
<th></th>
<th>All Cases (n = 69)</th>
<th>African-American (n = 33)</th>
<th>European-American (n = 36)</th>
<th>P value‡ t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at prostatectomy [median (range)] n = 69</td>
<td>60 (44 – 73)</td>
<td>61 (46 – 72)</td>
<td>60 (44 – 73)</td>
<td>0.77</td>
</tr>
<tr>
<td>PSA at diagnosis [median (range)] n = 50*</td>
<td>6.1 (1.3 – 47.7)</td>
<td>6.1 (1.3 – 47.7)</td>
<td>6.1 (4.0 – 20.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Largest nodule (grams) [median (range)] n = 59*</td>
<td>1.6 (0.2 – 2.9)</td>
<td>1.5 (0.8 – 2.9)</td>
<td>1.6 (0.2 – 2.8)</td>
<td>0.97</td>
</tr>
<tr>
<td>Source of Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCI CPCTR</td>
<td>59 (86)</td>
<td>30 (90)</td>
<td>29 (81)</td>
<td>0.31</td>
</tr>
<tr>
<td>University of Maryland</td>
<td>10 (14)</td>
<td>3 (10)</td>
<td>7 (19)</td>
<td></td>
</tr>
<tr>
<td>Pathological stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>38 (55)</td>
<td>18 (55)</td>
<td>20 (55)</td>
<td>1.0</td>
</tr>
<tr>
<td>≥ pT3†</td>
<td>31 (44)</td>
<td>15 (45)</td>
<td>16 (42)</td>
<td></td>
</tr>
<tr>
<td>Gleason sum score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7 (5-6)</td>
<td>18 (26)</td>
<td>9 (27)</td>
<td>9 (25)</td>
<td>1.0</td>
</tr>
<tr>
<td>≥ 7 (7-9)</td>
<td>51 (74)</td>
<td>24 (73)</td>
<td>27 (75)</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle invasion*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>49 (83)</td>
<td>22 (73)</td>
<td>27 (93)</td>
<td>0.08</td>
</tr>
<tr>
<td>Yes</td>
<td>10 (17)</td>
<td>8 (27)</td>
<td>2 (7)</td>
<td></td>
</tr>
<tr>
<td>Surgical margin status*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>35 (59)</td>
<td>18 (60)</td>
<td>17 (59)</td>
<td>1.0</td>
</tr>
<tr>
<td>Positive</td>
<td>24 (41)</td>
<td>12 (40)</td>
<td>12 (41)</td>
<td></td>
</tr>
</tbody>
</table>

* Cases with unknown status are not included. †One European-American patient was staged pT4
Cross-Validation of Tumor vs. Non-Tumor Signature

**Upregulated Genes**
- NME1 non-metastatic cells 1
- ST14 suppression of tumorigenicity 14
- PEPS3C protein phosphatase 3
- p100 EBNA-2 co-activator
- PESS8 protease, serine, 8 (prostatin)
- FASN fatty acid synthase
- ARMIC arg-rich, mutated in tumors
- ANACR alpha-methylacyl-CoA racemase
- LIM LIM protein
- DKFZP664E167
- BDH 3-hydroxybutyrate dehydrogenase
- HPN hepsin (transmembrane protease)
- ISTAS tissue specific transmembrane Ag
- APRT adenine phosphoribosyltransferase
- LG6 t cell receptor gamma locus
- PLAB prostate differentiation factor
- LG3 ligase I, III, DNA, AT-P-dependent
- HST-2 putative transmembrane protein
- TRAP1 heat shock protein 75
- TDP2 tumor protein D52
- ANCT 5-adenosylhomocysteine hydrolase NRB nuclear FGFR3 binding protein
- EIF3S2 translation initiation factor 3
- POLE2 polymerase delta 2
- TFF3 trefoil factor 3
- ADE2H1 similar to SCA14 synthetase
- UAP1 UDP-N-acetylglucosamine phos- tase
- RCL putative c-Myc-responsive
- S0X4 SRY-box 4
- NULL Human D9 splice variant B
- ATP6V1 ATPase H+ translocating
- SLC25A6 solute carrier family 25
- ACO372 thioredoxin peroxidase
- KRT18 keratin 18
- ODC1 ornithine decarboxylase 1
- FAAH fatty acid amidohydrolase
- DAP death-associated protein
- TRIM14 tripartite motif-containing 14
- KRT8 keratin 8
- MYC v-my c avian myelocytomatosis

**Downregulated Genes**
- 0.01
- 0.02
- 0.05
- 0.10
- 0.04

**Tumor vs. Non-tumor Signature**
- Upregulated Genes 70%
- Downregulated Genes 12.5%

Microarray Results Comparing Prostate Tumors from Different Ethnic Populations

African-American tumors vs. European-American tumors

FDR ≤ 5%: 162 differentially expressed genes
- 134 increased in African-American tumors (~83%)
- 28 decreased in African-American tumors (~17%)

80 well-described PCa marker genes were identified in this meta-analysis

Cancer Res. 2002, 62, 4427-4433

None of these genes overlap with genes identified in the ethnicity contrast
Pathway Analysis of Genes Differentially Expressed by Ethnicity

GOBP Pathway Analysis

Disease Association Analysis

GOBP: Gene Ontology Biological Processes

Immune response
Defense response
Response to biotic stimulus
Organismal physiological process
Response to stimulus
Response to pest, pathogen, or parasite
Humoral immune response
Response to external biotic stimulus
Humoral defense mechanism
Response to stress
Antigen processing
Antigen processing via MHC class 1
Antigen presentation
Antimicrobial humoral response
Antimicrobial humoral response
Antigen presentation
Cellular defense response
Signal transduction
Cell communication
Apoptosis

Tumor (AA) vs. tumor (EA)
Non-tumor (AA) vs. non-tumor (EA)

**Genes Regulated in the Immune Response Pathway**

GOBP Pathway Analysis

### Tumor (AA) vs. tumor (EA)

### Non-tumor (AA) vs. non-tumor (EA)

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Fold Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-C</td>
<td>MHC, class 1, C</td>
<td>1.41</td>
<td>7.75E-05</td>
</tr>
<tr>
<td>HLA-B</td>
<td>MHC, class 1, B</td>
<td>1.34</td>
<td>3.19E-04</td>
</tr>
<tr>
<td>HLA-E</td>
<td>MHC, class I, E</td>
<td>1.41</td>
<td>4.93E-04</td>
</tr>
<tr>
<td>CD28</td>
<td>CD28 antigen</td>
<td>1.38</td>
<td>8.11E-04</td>
</tr>
<tr>
<td>TRBC1</td>
<td>T cell receptor β constant 1</td>
<td>1.64</td>
<td>1.02E-03</td>
</tr>
<tr>
<td>TAP2</td>
<td>Transporter 2, ATP-binding</td>
<td>1.31</td>
<td>1.07E-03</td>
</tr>
<tr>
<td>CCL5</td>
<td>Chemokine(C-C motif)ligand 5</td>
<td>1.61</td>
<td>1.36E-03</td>
</tr>
<tr>
<td>LSP1</td>
<td>Lymphocyte-specific protein 1</td>
<td>1.24</td>
<td>1.57E-03</td>
</tr>
<tr>
<td>CD1C</td>
<td>CD1C antigen</td>
<td>1.3</td>
<td>1.79E-03</td>
</tr>
<tr>
<td>HLA-G</td>
<td>MHC, class 1, G</td>
<td>1.22</td>
<td>2.34E-03</td>
</tr>
<tr>
<td>IGHG3</td>
<td>Immunoglobulin heavy constant 3</td>
<td>1.78</td>
<td>2.46E-03</td>
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<tr>
<td>HLA-DQB1</td>
<td>MHC, class II, DQ beta 1</td>
<td>1.48</td>
<td>2.48E-03</td>
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<tr>
<td>IL7R</td>
<td>Interleukin 7 receptor</td>
<td>1.61</td>
<td>2.88E-03</td>
</tr>
<tr>
<td>INDO</td>
<td>Indoleamine 2,3-dioxygenase</td>
<td>1.64</td>
<td>3.03E-03</td>
</tr>
<tr>
<td>HLA-F</td>
<td>MHC, class I, F</td>
<td>1.36</td>
<td>3.58E-03</td>
</tr>
</tbody>
</table>

**Contributors of immune tolerance**
What is Indoleamine-2,3-dioxygenase?

- IDO is a rate-limiting enzyme that promotes “tumor escape”.

- Overexpression of IDO has been correlated with poor prognosis in numerous types of cancer.

- IDO induces its immunosuppressive effects by catalyzing the degradation of tryptophan.

- IDO transcription and enzyme activation is dependent upon numerous inflammatory mediators (such as IFN-γ).
IDO expression is increased in tumors from African-American patients.

Values on graph are shown as Relative Expression (18s Ct - INDO Ct) + 20.
Tryptophan levels are decreased in the serum from African-American PCa patients

African-American Men
European-American Men

Controls n=50
Cases n=50
Controls n=49
Cases n=50

Tryptophan, uM

p=0.03
Summary of Study Results

- There are significant differences in tumor immunobiology between African-American and European-American PCa patients.

- Indoleamine-2,3-dioxygenase is upregulated in prostate tumors from African-American men, when compared to European-Americans.

- Systemic levels of tryptophan are decreased in African-American PCa patients, suggesting increased activity of IDO.
Future Course of Study

ENVIRONMENTAL FACTORS
(Infectious Agents)

Viruses

INTERFERON-γ SIGNATURE

Chronic Infection

Chronic Inflammation

Prostate tumor progression and aggressiveness
Implications of Research

While preliminary, these data identify differences in tumor biology between African-American and European-American men. These differences may have important consequences in disease aggressiveness and may affect response to therapy, particularly in the African-American population.
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**Cooperative Prostate Cancer Tissue Resource**