The Biology of Prostate Cancer and the Search for New Molecular Targets: Does Race Matter?

Duke Urologic Assembly
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Duke Cancer Institute
Prostate cancer (PC) health disparities among racial groups

Number of New Cases per 100,000 Persons

MALE

- All Races: 147.8
- White (W): 139.9
- Black (AA): 223.9
- Asian / Pacific Islander (AS): 79.3
- American Indian / Alaska Native: 71.5
- Hispanic: 122.6
- Non-Hispanic: 151.5

Number of Deaths per 100,000 Persons

MALE

- All Races: 23.0
- White: 21.2
- Black: 50.9
- Asian / Pacific Islander: 10.1
- American Indian / Alaska Native: 20.7
- Hispanic: 19.2
- Non-Hispanic: 23.2

PC health disparity (AA vs W) after adjustment for social determinants of health

Adopted from Robbins et al., Am J Epidemiol, 2000, 151(4), p.409-16
RACE IS NOT A BIOLOGICAL CONSTRUCT

RACE/ETHNICITY ARE SOCIO-CULTURAL CONSTRUCTS

But, RACIAL ANCESTRY, AS A FUNCTION OF THE HUMAN DIASPORA, AFFECTS GENETIC, PHENOTYPIC & CULTURAL DIVERSITY AND THEREFORE DISEASE RISK AND OUTCOMES
RACE IS NOT A BIOLOGICAL CONSTRUCT

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But, RACIAL ANCESTRY, INCLUDING AS A FUNCTION OF FORCED MIGRATION, AFFECTS GENETIC, PHENOTYPIC & CULTURAL DIVERSITY AND THEREFORE DISEASE RISK AND OUTCOMES
Translational research program:
molecular mechanisms of tumor aggressiveness
using population-level differences to identify genes
AA and W prostate normal and tumor specimens (varying Gleason grade)  

AA and W patient blood specimens

- **Duke University: sample collection**
  - Michael N. Ferrandino MD, Brant A. Inman MD, Judd W. Moul MD, Thomas J. Polascik MD, Edward N. Rampersaud Jr. MD, Cary N. Robertson MD, Wen-Chi Foo MD, John F. Madden MD PhD, CCRL and BRPC

- **George Washington University: sample collection**
  - Norman Lee PhD, Ramez Andrawis MD
First Clue: Differential Gene vs Exome Level Analysis Between AA and EA Prostate Cancer Biopsy Specimens; circa 2010

A  gene-level analysis

B  exon-level analysis
Central Dogma of Molecular Biology

**DNA**

Exon 1  intron 1  Exon 2  intron 2  Exon 3  intron 3  Exon 4

**transcription**

**pre-mRNA**

Exon 1  intron 1  Exon 2  intron 2  Exon 3  intron 3  Exon 4

**Alternative RNA splicing**

**mRNAs**

Exon 1  Exon 2  Exon 3

Exon 1  Exon 3  Exon 4

Exon 1  Exon 2  Exon 4

Exon 2  Exon 3  Exon 4

**translation**

Protein A with Function A

Protein B with Function B

Protein C with Function C

Protein D with Function D
Exome Level Analysis: CA and NP
(Nature Communications, June 2017)
PI3Kδ-L is sensitive to small molecule inhibition of xenograft growth and metastasis but PI3Kδ-S is not.

Supplementary Fig. 2. Heatmaps of alternative splicing differences in EA PCa vs. EA NP and AA PCa vs. AA NP specimens. (a) PCA plot and 2D-clustergram depicting 1,604 significant differentially expressed exons in 15 independent EA PCa vs. 15 independent EA NP specimens. (b) PCA plot and 2D-clustergram depicting 1,669 significant differentially expressed exons in 20 AA PCa vs. 20 AA NP specimens. PCa and NP specimens are represented by red and blue circles/bars, respectively. Rows represent specimens and columns represent exons in hierarchical clustergrams. Log2 expression values of exons were subjected to 2D hierarchical clustering using average linkage method and Euclidean distance.
Challenging the “DNA Mutation” dogma on what drives the tumor phenotype

Prostate Cancer Driver Mutations vs Alternatively Spliced RNA Burden

\[ \sim 11 : \text{>2500 including > 600 from GL} \]
What drives race-related Alternative RNA Splicing? What if it pre-exists in the germ line and carries into PC? Does it affect PC biology? Does it contribute to clinical aggressiveness of PC in different races? AND HOW? Does it predict risk or survival? Is it targetable?
Supplementary Fig. 3. Oncogenic signaling pathways with an over-representation of differential splicing events in AA PCa vs. EA PCa. Pathways with statistical over-representation of genes exhibiting DS events (red outlined circles) were identified by a Fisher’s exact test implemented using the Ingenuity Pathway Analysis (IPA) program.
Affymetrix exon array profiling and Alternative Splice Modeling

**a)**
- Exon expression of *PIK3CD*
- Exon 8: 1p36.22
- Exon 20: 1p36.22
- Chromosome 1: 9,711,803-9,788,977

**b)**
- Exon expression of *FGFR3*
- Exon 14: 4p13.4
- Chromosome 4: 1,795,034-1,810,598

**c)**
- Exon expression of *TSC2*
- Exon 20: 16p13.3
- Chromosome 16: 2,097,466-2,138,721

**d)**
- Exon expression of *RASGRP2*
- Exon 12: 11q13.1
- Exon 11: 11q13.1
- Chromosome 11: 64,494,383-64,512,928

**e)**
- Exon expression of *MET*
- Exon 15: 7q31.2
- Exon 28: 7q31.2
- Chromosome 7: 116,312,248-116,438,440

**f)**
- Exon expression of *NF1*
- Exon 8: 17q11.2
- Chromosome 17: 29,421,945-29,705,949

**g)**
- Exon expression of *ITGA4*
- Exon 13: 2q31.3
- Exon 23: 2q31.3
- Chromosome 2: 182,321,619-182,400,914

**h)**
- Exon expression of *BAK1*
- Exon 2: 6p21.31
- Chromosome 6: 33,540,324-33,548,070

Legend:
- **AA PCa**
- **EA PCa**
Supplementary Fig. 4. Quantification of qRT-PCR results of race-specific/enriched oncogene and tumor suppressor gene variants in AA and EA PCa specimens. Quantitative real time RT-PCR was performed on samples depicted in Fig. 3b. RNA from n= 22-25 AA PCa and n= 21-24 EA PCa specimens were analysed. Shown are the plots for the AA-specific/enriched variants FGFR3-S, TSC2-S, ITGA4-L, MET-L, NF1-L, BAK1-L and RASGRP2-b; and plots for the EA-specific/enriched variants FGFR3-L, TSC2-L, ITGA4-S, MET-S, NF1-S, BAK1-S and RASGRP2-a. EIF1AX and PPA1 transcripts served as internal normalization controls. * P < 0.05 using Student t-test.
Supplementary Fig. 6. Enrichment of AA-specific/-enriched variants of FGFR3, TSC2 or RASGRP2 enhances proliferation and/or invasion of AA PCA cell line MDA PCA 2b. (a) Exon 14-specific siRNA (siF) was designed to target the EA-enriched FGFR3-L variant. (b) Exon 20-specific siRNA (siT) was designed to target the EA-enriched TSC2-L variant. (c) Exon 11-specific siRNA (siR) was designed to target the race-independent RASGRP2-a variant. Knockdown efficiency was determined by the variant ratio (-S/-L or -b/-a ratio) derived from the RT-PCR reactions in EA PCA cell line VCaP and AA PCA cell line MDA PCA 2b (upper panels). RT-PCR representative images of n = 3-5 independent knockdown experiments. Enrichment of the AA-specific/-enriched FGFR3-S, TSC2-S or RASGRP2-b variants augmented proliferation and/or invasion of AA PCA cell line MDA PCA 2b (bottom panels). Data presented as the mean ± SEM of n=3-5 independent experiments for each treatment group. *P < 0.05 by ANOVA with Tukey’s post-hoc test.
qRT-PCR Validation of Differential Splicing

[Diagram showing exon exclusion in different genes]
What to know about PI3K’s

• PI3K family: 4 classes; 1-4
• Class 1 PI3K’s produce PI(3)P, PI(3,4)P2, and PI(3,4,5)-P3.
• PI3K’s are activated by G-protein coupled receptors and tyrosine kinase receptors
• Heterodimeric, composed of catalytic (p110) and regulatory (p85) subunits
• There are 5 variants of p85, produced by alternative splicing of Pik3r1 gene.
• There are 3 variants of p110, each produced by 3 different genes (Pik3ca, Pik3cb, Pik3cd)
• p110 A and B are thought to be expressed in all cells
• p110 D ”was” thought to only be produced in leukocytes

• We discovered that p110 D is expressed in malignant prostate tissue
• We also discovered that the Pik3cd gene undergoes alternative RNA splicing to produce at least 2 different splice—isoforms of D
Are PI3K’s important in cancer?
Biological significance of race-related alternative RNA splicing: PIK3D Isoform Knockdown
Biological significance of race-related alternative RNA splicing: PIK3D Isoform Cloning and Overexpression

**PI3KCD long variant:**

- p85 binding: K46, K210
- RAS binding: S315, S312
- C2: S411, S406
- Helical: Y485, Y524, T935
- Catalytic: Y936, T935

**PI3KCD short variants:**

1. **i)**
   - Δ aa810-865

2. **ii)**
   - Δ aa311-340

3. **iii)**
   - Δ aa311-340
   - Δ aa810-865

4. **iv)**
   - Δ aa443-876

*Supplementary Fig. 5. Molecular cloning of the long and short variants of PI3KCD.* Schematic representation of the cloned PI3KCD variants. The long variant contains 24 exons based on sequence alignment to the PI3KCD genomic sequence described in the UCSC (genome.ucsc.edu) or Ensembl Genome Browser (www.ensembl.org). The short variants relative to the long variant are as follows: i) variant excluding exon 8 (encoding amino acids 311 to 340), variant excluding exon 20 (encoding amino acids 810 to 865), variant excluding both exons 8 and 20 and a large deletion variant excluding 1,299 nucleotides (encoding amino acids 443 to 876).
Biological significance of race-related alternative RNA splicing: PIK3D Isoform Overexpression – Cell Proliferation

Supplementary Fig. 7. Baseline proliferative activity (BrdU labeling) of PC-3 and VCaP cell over-expressing PI3Kδ-S and PI3Kδ-L. Proliferation was assessed using a BrdU labeling assay. Data presented as the mean ± SEM of n=3-6 independent experiments for each treatment group. *P < 0.05 by ANOVA with Dunnett’s post-hoc test.
Overexpressed PI3Kδ-L is sensitive to small molecule inhibition of PI3K/AKT/mTOR signaling and proliferation, but PI3Kδ-S is not. (CAL-101, Idelalisib, Zydelig for CLL: Gilead)

No difference between L and S with downstream AKT inhibitor, MK-2206
Overexpressed PI3Kδ-L is sensitive to small molecule inhibition of xenograft growth and metastasis but PI3Kδ-S is not.
Cell-free kinase assay of PI3Kδ-L and –S isoforms and small-molecule inhibition
The figure illustrates the domain structure of PI3Kδ and examines the effects of CAL-101 on PI3Kδ-L and PI3Kδ-S isoforms. (c) shows the relative PI3K activity of PI3Kδ-L and PI3Kδ-S with and without CAL-101 treatment, with vehicle and Wortmannin as controls. The data is represented as a bar graph with error bars. (d) displays the molecular structures of PI3Kδ-L and PI3Kδ-S with CAL-101 binding, highlighting specific residues and interactions.
Supplementary Fig. 8. Knockdown of p85α increases invasive activity of EA PCa cell lines. Invasion was assessed using Matrigel assay following siRNA-mediated knockdown of p85α (PIK3R1). SiRNA-SMART pool for targeting PIK3R1 was purchased from Dharmaco (Lafayette, CO). Data presented as the mean ± SEM of n=3-4 independent experiments for each treatment group. *P < 0.05 by ANOVA with Dunnett’s post-hoc test. siNS, Nonsense siRNA control.
Biological significance of race-related alternative RNA splicing: Clinical Significance: Survival Plots as function of S/L ratio

Supplementary Fig. 9. Survival plots for breast, colon and prostate cancer patients with high and low PIK3CD-S/PIK3CD-L expression ratios. RNA-Seq and disease free survival data for breast (n = 1,068 patients), colon (n = 277 patients) and prostate cancers (n = 494 patients) were obtained from The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/tcga/). P-values for survival curves were determined by the log-rank test.
Supplementary Fig. 10. PI3Kδ expression in patient specimens and PCa cell lines. (a) Western blot analysis of cell lysates from PCa cell lines (VCaP, PC-3, LNCaP, E006AA and MDA PCa 2b), breast cancer (BCa) cell lines (MCF7 and HCC1428) and colon cancer cell lines (HT29 and SW620). β-actin served as loading control. Representative images from n = 3-4 independent western blot experiments. (b) Immunohistochemistry (IHC) analysis of PI3Kδ expression in formalin-fixed, paraffin-embedded (FFPE) tissue samples derived from AA and EA PCa patients. Representative images from n = 3 independent IHC assays.
Alternative RNA splicing events: current and future directions

- Expand cohort and extend to other populations
- Expand gene targets
- Associate With Tumor Aggressiveness or Stemness?
- Genetic and/or Epi-genetic drivers of events?
- Biochemical mechanisms: cis and/or trans factors
- Biological/therapeutic significance to more aggressive PC biology in AA and less aggressive PC biology in Asian men
- Targeting with Splice Switching Oligonucleotides
- Clinical trials Stratified by Race
Cis-acting splicing elements: biomarkers?

Splicing regulatory regions

ESE/ESS: exonic splicing enhancer/silencer
ISE/ISS: intronic splicing enhancer/silencer

SNP in a splicing regulatory region
regulating inclusion/exclusion of exon 2

DNA

Pre-mRNA

RNA splice variant 1
RNA splice variant 2

Protein A
Function A
Protein B
Function B

Translation

Agarose gel electrophoresis

G/G   A/A

RNA 1
RNA 2
Snail promotes resistance to enzalutamide through regulation of androgen receptor activity in prostate cancer

Kathryn E. Ware,1,2 Jason A. Somarelli,1,2 Daneen Schaeffer,3 Jing Li,4 Tian Zhang,1,2 Sally Park,2 Steven R. Patierno,1,2 Jennifer Freedman,1,2 Wen-Chi Foo,8 Mariano A. Garcia,5,6 and Andrew J. Armstrong1,2,7

*Snail is a stemness gene at the nexus of enzalutamide resistance and prostate cancer metastasis both in preclinical models of prostate cancer and in patients.

*Snail expression is associated with Gleason 9-10 high-risk disease and is strongly overexpressed in metastases as compared to localized prostate cancer.

*Snail expression is also elevated in enzalutamide-resistant prostate cancer cells compared to enzalutamide-sensitive cells.

*Downregulation of Snail re-sensitizes enzalutamide-resistant cells to enzalutamide, increases migration and invasion, and promotes enzalutamide resistance in enzalutamide-sensitive cells.

*Snail-mediated enzalutamide resistance is a consequence of increased full-length AR and AR-V7 expression and nuclear localization.

*Downregulation of either full-length AR or AR-V7 re-sensitizes cells to enzalutamide in the presence of Snail.

Snail is capable of mediating-resistance through AR even in the absence of AR-V7.
Associations of SNPs in race-related alternatively spliced genes with PC

- **MEC GWAS (AA PC cases and controls)**
  - 11,073 SNPs
  - Risk: 670 cases and 658 controls
  - Aggressiveness: 234 aggressive and 436 non-aggressive

- **PLCO GWAS (white PC cases and controls)**
  - 10,385 SNPs
  - Risk: 1150 cases and 1101 controls
  - Aggressiveness: 237 aggressive and 843 non-aggressive
  - Survival: 1150 overall, 237 aggressive, 843 non-aggressive
Associations between RNA splicing regulatory variants of stemness-related genes and racial disparities in susceptibility to prostate cancer

Authors

• Yanru Wang, Jennifer A. Freedman, Hongliang Liu, Patricia G. Moorman, Terry Hyslop, Daniel J. George, Norman H. Lee, Steven R. Patierno, Qingyi Wei.

• Identified 32 SNPs in five genes (TP63, ALDH1A1, WNT1, MET and EGFR) that were significantly associated with prostate cancer risk.
• Of these, six SNPs in three genes (TP63, ALDH1A1 and WNT1) and eight EGFR SNPs showed heterogeneity in susceptibility between these two racial groups.
• 13 SNPs in MET and one in ALDH1A1 were found only in African descendants.
• In silico bioinformatics analyses revealed that EGFR rs2072454 and SNPs in linkage with the identified SNPs in MET and ALDH1A1 ($r^2 > 0.6$) were predicted to regulate RNA splicing.

* These splice—regulatory variants may serve as novel biomarkers for racial disparities in prostate cancer risk.
Single nucleotide polymorphisms of stemness pathway genes predicted to regulate RNA splicing, micro RNA and oncogenic signaling are associated with prostate cancer survival

• Jennifer Freedman, Yanru Wang, Hongliang Liu, Patricia G. Moorman, Terry Hyslop, Daniel J. George, Norman H. Lee, Qingyi Wei, Steven Patierno

• Identified SNPs in CD44, ABCC1, and GDF15 that associate with PC survival.

• In silico bioinformatics analyses revealed that CD44 rs9666607 and ABCC1 rs35605 were functional and involved in RNA splicing regulation

• ABCC1 rs212091 affects miRNA binding site activity (Transmembrane transporter)

• GDF15 rs105857 causes an amino acid change (TGF-B family member)

• These variants may serve as novel biomarkers for prostate cancer survival.
### Choosing Targets: The Beauty of Genomic Approaches:

#### Race-related Alternatively Spliced Genes in common to Prostate, Breast, Lung and Liver Cancer

- **Prioritized Targets**
  - **INSR**: Insulin receptor
  - **CD44**: Receptor for hyaluronic acid
  - **ITGA6**: Integrin, alpha 6
  - **RELN**: Extracellular matrix serine protease
  - **ABLIM3**: Actin binding LIM protein family, member 3
  - **BPTF**: Bromodomain PHD finger transcription factor
  - **COL6A3**: Collagen, type VI, alpha 3
  - **EHBP1**: EH domain binding protein 1
  - **EPB41L2**: Erythrocyte membrane protein band 4.1-like 2
  - **EXOC1**: Exocyst complex component 1
  - **FN1**: Fibronectin 1
  - **FXR1**: Fragile X mental retardation syndrome-related protein 1
  - **LMO7**: LIM domain 7
  - **NCOR2**: Nuclear receptor co-repressor 2
  - **RECQL4**: RecQ protein-like 4
  - **SPAG5**: Sperm associated antigen 5
  - **THRB**: Thyroid hormone receptor, beta
  - **WARS**: Tryptophanyl-tRNA synthetase
  - **WDR4**: WD repeat domain 4

- **Gene Description**
  - **INSR**: Skip of exon 11
  - **CD44**: Skip of exon v1-v10
  - **ITGA6**: Skip of exon 5
  - **RELN**: Skip of second to the last exon
  - **ABLIM3**: Skip of exon 14
  - **BPTF**: Skip of exon 5
  - **COL6A3**: Skip of exon 4
  - **EHBP1**: Skip of exon 15
  - **EPB41L2**: Skip of exon 14
  - **EXOC1**: Skip of exon 11
  - **FN1**: Skip of exon 40
  - **FXR1**: Skip of exon 2
  - **LMO7**: Skip of exon 11
  - **NCOR2**: Alternative 5’ splice site in exon 46
  - **RECQL4**: Skip of exon 15
  - **SPAG5**: Alternative 5’ splice site in exon 2
  - **THRB**: Skip of exon 5 (first coding exon)
  - **WARS**: Alternative first exon
  - **WDR4**: Alternative 5’ splice site in exon 3

- **Targets are prioritized for further study based on:**
  - Alternative splicing events involving differential exon inclusion/skipping
  - Gene involvement in control of cell adhesion and migration (highlighted in red)
  - Gene involvement in pathways relevant to health disparities among racial groups
Function of Alternatively Spliced Genes with race-related SNPs that Associate with PC Aggressiveness and/or Survival

ACACA, ADH1C, EHBP1, FASN, HPGD, INSR, LAT2, SREBF2, STEAP4

BPTF, CD44, COL6A3, FGFR3, FN1, INSR, MET, NCOR2, NF1, PIK3CD, RHOU, SPAG5, THRB, WDR4

COL6A3, FGFR3, MET, SEMA3C, WARS

BAK1, BPTF, CD44, COL6A3, FGFR3, INSR, MET, NF1, PIK3CD, RHOU, SPAG5, THRB, ZNF385B

RECQL4

TSC2, RASGRP2

CD44, COL6A3, FXR1, HPGD, SEMA3C, STEAP4

ABLIM3, CD44, EHBP1, EPB41L2, EXOC1, FGFR3, FN1, FXR1, ITGA4/6, LMO7, MET, MYBPC1, RELN, RHOU, SEMA3C, NCOR2, PIK3CD, RELN, SPAG5, THRB
Therapeutic Targeting of Alternative RNA Splicing

pre-mRNA

Splicing regulatory regions can be targeted with splice-switching oligonucleotides

Simultaneously limit pathogenic RNA splice variants and maintain/induce RNA splice variants with therapeutic value

Indolent Prostate Cancer
No Agents in Clinical Use That Target AR-V7 in PCa

No Agents in Clinical Use That Target AR-V7 in PC

Some current and future directions

- Interrogate biological effects of splice variants
- Evaluation in PC xenografts and patient-derived explant models
Some current and future directions

- Interrogate biological effects of SNPs in RNA splice-regulatory site

**Aim 1:** Evaluate associations of RNA splicing regulatory SNPs in 1,393 candidate genes with PCa aggressiveness and mortality in AAs.
  - Genotype RNA splicing regulatory SNPs on an Illumina fully customized genotyping array in AA men in NC-LA PCaP cohort.
  - Evaluate associations of SNPs with PCa aggressiveness and mortality among AA men in NC-LA PCaP.

**Aim 2:** Determine the function of candidate RNA splicing regulatory SNPs in driving the alternative RNA splicing mechanism and PCa aggressiveness.
  - Evaluate functionality of top SNPs identified in preliminary data and Aim 1 at the cellular-level by expressing genes containing these SNPs in PCa cell lines and assessing resulting alterations in expression of RNA splice variants and PCa cell biology.

**Aim 3:** Determine the biological-clinical association of functional SNPs with expression of RNA splice variants and PCa aggressiveness.
  - Evaluate biological-clinical association of functional SNPs at the tissue-level by examining RNA splice variant expression in NC-LA PCaP participant-matched prostatectomy specimens of varying aggressiveness.
- AA and White patients
- Adenocarcinomas of the prostate (Gleason 9 &10)
- Core prostate samples
  - Very poor differentiation
  - Prominent eosinophilic nucleoli
  - Pale chromatin
Establishment of AA and W Prostate Cancer patient-derived primary cell lines

- Pathological, biological and molecular characterization underway
Functional Validation of cis-acting Splice Factors Associated with PCa Aggressiveness

SNPs associated with PCa Aggressiveness

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MAF = minor allele frequency; Agg = patients with aggressive prostate cancer; Non-Agg = patients with non-aggressive prostate cancer

CRISPR to introduce SNPs into target genes

Functional Studies

- Proliferation
- Colony Formation
- Invasion
- Senescence
- Migration
Functional Studies on Race-related Splice Variants and PCa Aggressiveness

Identification of ARS in PCa

163 ARS events were found in breast, lung, liver cancers using TCGA data. Tsai YS et al, Oncotarget. 2015 Mar


Prioritized genes

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* Skip, exon skipping
** AS, alternative splicing site

Demonstrating ARS events of prioritized genes using VCaP (white) and MDA PCa (AA) cell lines by PCR

Enforcing or suppressing ARS in cells

CRISPR

Studying ARS impact on Prostate cancer cell biology

- Proliferation
- Invasiveness
- Migration
- Drug Resistance

Dr. Muthana Al Abo, PhD – Postdoctoral fellow

Functional Studies on Race-related Splice Variants and PCa Aggressiveness
Nanoplasmonnic molecular sentinels with Tuan Vo-Dihn in Biomedical Engineering.

- To sense and therapeutically target oncogenic RNA isoforms with SSOs
- Stem-loop nucleic acid probe labeled with Raman reporter
- Plasmonic-active nanoparticle
- Unlabeled capture placeholder strand
Small molecule targeting of RNA splice variants

with Amanda Hargrove in Chemistry (DoD Funded)

potential small molecule binding targets

secondary structure prediction of PIK3CD pre-mRNA fragment

A. small molecule microarray labeled RNA

B. dynamic ligand assembly
- AMPK signaling
- AMPK inhibits AR signaling
- Screening a library for novel small molecule activators of AMPK
- 2 direct AMPK targets (FASN and ACACA), differentially alternatively spliced between AA and white PCa

- Do different splice variants differentially affect AMPK signaling?
- Do AMPK activators affect alternative RNA splicing and PCa cell biology?
- Is AMPK signaling important for response to Abi among and between racial groups?
Race-Stratified Trials: Abi & Apa Race

- Baseline, during, end of treatment (whole blood, plasma, serum)
- Clinical
  - PSA response, duration of response, objective response, radiographic disease progression
- Genetic
  - Race- and splicing-related SNPs, angiome, AMPK targets, steroid levels

Registration

Self-reported & Ancestry-validation
- AA
- W

Abiraterone + Prednisone

Disease progression or Adverse event

- Metastatic, CRPC
- No history of chemotherapy
- Karnofsky performance status ≥ 70
- Adenocarcinoma of the prostate
- No evidence of neuroendocrine cancer

- Self-reported & Ancestry-validation
Race-Stratified Trials: *Idelalisib in PC?*

- **Clinical**
  - What to do after hormone resistance is insurmountable and standard chemotherapy has failed?
- **Genetic**
  - Race- and splicing-related expression of PIK3D in refractory PC
  - Will PC tumors respond to anti-PIK3D therapy?

**Metastatic, CRPC**
- Hormone and Chemotherapy
- Refractory?

Registration

Self-reported & Ancestry-validated AA

Self-reported & Ancestry-validated W

Idelalisib?

Disease progression or Adverse event
Summary

• Alternative RNA Splicing is a dominant source of genomic and phenotypic heterogeneity in cancer
• SNPs in coding and non-coding splice regulatory regions of DNA may serve as biomarkers of risk for aggressive disease
• ARS produces a broad array of novel targetable protein isoforms and variants
• ARS itself is a target for therapeutic intervention

• MAIN MESSAGE
• In addition to sequencing patient tumors for DNA mutations we should be performing deep RNA sequencing for ARS profile. It will be a richer source of “actionable” molecular targets.
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