Generation of Function Based Biomarkers in Prostate Cancer

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What is a biomarker?

- Substance used as an indicator of a biological state
- Can be used to monitor a normal physiologic state, a pathologic process, a pharmacologic response to therapeutic intervention, or a toxic exposure
- Examples: antibodies, radioactive isotopes, specific DNA sequences, PSA
## Phases of Biomarker Development

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical Exploratory</td>
<td><strong>PHASE 1</strong> Promising directions identified</td>
</tr>
<tr>
<td>Clinical Assay and Validation</td>
<td><strong>PHASE 2</strong> Clinical assay detects established disease</td>
</tr>
<tr>
<td>Retrospective Longitudinal</td>
<td><strong>PHASE 3</strong> Biomarker detects disease early before it becomes clinical and a “screen positive” rule is defined</td>
</tr>
<tr>
<td>Prospective Screening</td>
<td><strong>PHASE 4</strong> Extent and characteristics of disease detected by the test and the false referral rate are identified</td>
</tr>
<tr>
<td>Cancer Control</td>
<td><strong>PHASE 5</strong> Impact of screening on reducing the burden of disease on the population is quantified</td>
</tr>
</tbody>
</table>
Prostate Specific Antigen (PSA) as a Biomarker in Prostate Cancer

Fig. 1 PSA clinical course and biomarker uses.

## United States Cancer statistics, 2012

### Estimated New Cases

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>241,740</td>
<td>26%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>116,470</td>
<td>14%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>73,420</td>
<td>9%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>55,600</td>
<td>7%</td>
</tr>
<tr>
<td>Melanoma of the skin</td>
<td>44,250</td>
<td>5%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,250</td>
<td>5%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>38,160</td>
<td>4%</td>
</tr>
<tr>
<td>Oral cavity &amp; pharynx</td>
<td>28,540</td>
<td>3%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>26,830</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>22,090</td>
<td>3%</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td>848,170</td>
<td>100%</td>
</tr>
</tbody>
</table>

### Estimated Deaths

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>87,750</td>
<td>28%</td>
</tr>
<tr>
<td>Prostate</td>
<td>28,170</td>
<td>9%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>26,470</td>
<td>9%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>18,850</td>
<td>6%</td>
</tr>
<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>13,980</td>
<td>5%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>13,560</td>
<td>4%</td>
</tr>
<tr>
<td>Esophagus</td>
<td>12,040</td>
<td>4%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>10,510</td>
<td>3%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,320</td>
<td>3%</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,650</td>
<td>3%</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td>301,820</td>
<td>100%</td>
</tr>
</tbody>
</table>
Cumulative Hazard of Death from Prostate Cancer among Men 55 to 69 Years of Age.

Number of Diagnoses of All Prostate Cancers (Panel A) and Number of Prostate-Cancer Deaths (Panel B).

Why is PSA Screening for Prostate Cancer Controversial?

For starters ....... PSA is NOT a rationale based biomarker
Prostate Specific Antigen (hK3)

- PSA made by prostate tissue – benign and malignant tissue
- Sink effect – performance to monitor malignant progression improves when prostate tissue is minimal
- Serine protease of the human kallikrein family
- Liquefies the coagulum by acting on semenogellin I & II and Fibronectin
- Produced as a precursor molecule- the pro-PSA
HYPOTHESIS

Function based biomarkers may demonstrate improved clinical performance characteristics.
Cancer Phenotype

Benign Cells → Proliferation → Migration → Invasion → Cancer Cells
Cancer Biology - Hallmarks of Cancer

Hanahan D, Weinberg RA. 2011
OCCASIONS IN PROSTATE CANCER

Unmet need: Biomarkers to distinguish cancers with low or gray-zone PSA from BPH

Unmet need: Biomarkers to distinguish indolent from aggressive disease earlier

Unmet need: Biomarkers to identify metastatic disease

Metastatic Cascade

- Prostate
  - Brain
  - Lungs
  - Liver
  - Bone marrow

- Pancreas
- Breast
- Colon

- Genetically heterogeneous primary tumor
  - Dissemination of metastatic cells
    - Subsequent removal of primary tumor
  - Micrometastases scattered throughout the body
    - Minimal residual disease
  - Acquisition of ability to colonize
  - Macrometastasis
    - New, secondary wave of metastatic dissemination
  - New micrometastases
  - Multiple macrometastases and disease relapse

Source: Expert Rev Proteomics © 2007 Future Drugs Ltd
Castration Resistant Prostate Cancer (CRPC)

Schulman et al., European Urology, Vol 58, 1, pages e1 – e18, July 2010
Differential gene expression
The model

LNCaP cells
- Derived from human prostate cancer lymph nodal metastasis
- Androgen dependent
- Produce PSA

C4-2 cells
- Derived from LNCaP cells grown in presence of bone stromal cells
- Low steady state of androgen receptor
- Androgen independent
- Highly metastatic and produces osteoblastic lesions / produce PSA

Wu et al., Int J Ca, 57, 406-12, 1994
Differential gene expression
Experimental design

LNCaP tRNA

mRNA

C4-2 tRNA

mRNA

MICROARRAY
1: RPA Data

LNCaP C4-2

2: Western Blotting

3: Kinase activity

4: PKD1 - down regulated in advanced human prostate cancer

Varambally et al. Cancer Cell 2005

**Protein Kinase D1 (PKD1)**

**Phosphatidylserine, Ca^{2+}, Diacylglycerol/phorbol ester,**

**ACTIVATORS:**
- Regulatory peptides (neurotensin, bombesin)
- Lysophosphatidic acid,
- Thrombin that act through Gq, G12, Gi, and Rho,
- Growth factors, such as PDGF and IGF
- B/T cell antigen engagement,
- Oxidative stress

**Tissues and Cells:**
- Fibroblasts
- Intestinal and kidney epithelial cells
- Smooth muscle cells
- Cardiac myocytes
- Neuronal cells
- Osteoblasts
- B and T lymphocytes,
- Mast cells and platelets,
- **Several human cancers**
LNCaP cells were stained with antibody specific for PKD1 (A) is DIC image (B) is showing immunolocalization of PKD1. (C) is merge of image (A) and (B) showing perinuclear and junctional staining (Jaggi et al., 2003).
Epithelial cells

- microvilli
- tight junction
- adherens junction
- desmosome
- keratin filaments
- gap junction
- hemi-desmosome
- basal lamina
- band of actin filaments

Molecular Biology of the Cell 3rd Edition, Figure 19-18
Cadherin–Catenin Protein Complex

PKD1 interacts and phosphorylates E-cadherin
PKD-1 and E-Cadherin regulate PC cells proliferation and soft agar colony formation
PKD-1 and E-Cadherin regulate PC cells proliferation and soft agar colony formation – Corroboration with molecular markers
β-Catenin mediates the PKD1 and E-cadherin functions in PC cells
Cadherin–Catenin Protein Complex

PKD1 promotes β-catenin membrane trafficking.
PKD1 is associated with membrane trafficking of β-catenin
PKD1 phosphorylates β-catenin at Thr120

active PKD1 - + -
dead PKD1 - - +

autoradiograph
Staining

Raamfp etldegmqip
sTqfdaahpI nvqR

PKD1 phosphorylates β-catenin at Thr120

β-catenin

α-catenin

β-cat H102 Ab
p230

β-cat pT120 Ab
p230

pT120 Ab only
pT120 Ab+ phospho-peptide
pT120 Ab+ nonphospho-peptide
Active Beta-catenin (unphosphorylated S37/T41) in the Nucleus

PKD1 represses generation of ABC

<table>
<thead>
<tr>
<th>C4-2</th>
<th>C4-2/PKD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>32</td>
<td>11</td>
</tr>
<tr>
<td>56</td>
<td>26</td>
</tr>
<tr>
<td>37</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C4-2</th>
<th>C4-2/PKD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>1.0</td>
<td>4.2</td>
</tr>
<tr>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C4-2</th>
<th>C4-2/PKD1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>1.0</td>
<td>4.2</td>
</tr>
<tr>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

- **pT120**: 
  - C4-2: 11, 3, 86
  - C4-2/PKD1: 28, 58%

- **ABC**: 
  - C4-2: 32, 11, 57
  - C4-2/PKD1: 0, 55%

- **pS333/S37/T41**: 
  - C4-2: 56, 26, 18
  - C4-2/PKD1: 25, 52%

- **total β-cat**: 
  - C4-2: 37, 20, 43
  - C4-2/PKD1: 26, 54%

- **β-act**: 
  - C4-2: 1.0, 1.2, 1.0
  - C4-2/PKD1: 0.8, 0.9

- **E-cadherin**: 
  - C4-2: 1.0, 1.1
  - C4-2/PKD1: 1.1, 0.8

- **Lamin A/C**: 
  - C4-2: 1.0, 1.0
  - C4-2/PKD1: 0.8, 0.7

- **exo endo PKD1**: 
  - C4-2: **red**
  - C4-2/PKD1: **green**

- **pT120 ab**: 
  - C4-2: **red**
  - C4-2/PKD1: **green**

- **TGN and Nuc**: 
  - C4-2: **green**
  - C4-2/PKD1: **green**
### Table 1. Analysis of pT120 antibody staining in prostate cancer

<table>
<thead>
<tr>
<th></th>
<th>TransGolgi network staining</th>
<th>Two-sample t test (P value)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal vs. tumor</td>
<td>Gleason 3-6 vs. 7-10</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>positive (%)</td>
<td>negative (%)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>22</td>
<td>16 (72.7%)</td>
<td>6 (27.3%)</td>
<td></td>
</tr>
<tr>
<td>Total tumor</td>
<td>200</td>
<td>15 (7.5%)</td>
<td>185 (92.5%)</td>
<td></td>
</tr>
<tr>
<td>Gleason 3-6</td>
<td>27</td>
<td>4 (14.8%)</td>
<td>23 (85.2%)</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>Gleason 7-10</td>
<td>173</td>
<td>11 (6.3%)</td>
<td>162 (93.7%)</td>
<td></td>
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</tbody>
</table>

### Table 2. Comparison of H102 and pT120 staining patterns

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>H102 staining</th>
<th>pT120 staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>membranous β-catenin</td>
<td>9</td>
<td>8 (88.9%)</td>
<td></td>
</tr>
<tr>
<td>TGN β-catenin</td>
<td>9</td>
<td></td>
<td>8 (88.9%)</td>
</tr>
<tr>
<td>Tumor tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>increased expression</td>
<td>56</td>
<td>18 (32.1%)</td>
<td>9 (16.1%)</td>
</tr>
<tr>
<td>decreased expression</td>
<td>56</td>
<td>5 (8.9%)</td>
<td>5 (8.9%)</td>
</tr>
<tr>
<td>membranous</td>
<td>54</td>
<td>42 (77.8%)</td>
<td>8 (14.3%)</td>
</tr>
<tr>
<td>cytoplasm/nuclear</td>
<td>54</td>
<td>12 (22.2%)</td>
<td>6 (10.7%)</td>
</tr>
</tbody>
</table>
E-cadherin and Beta-catenin Expression are Down Regulated in High Risk Prostate Cancer

(6F9 monoclonal anti-beta catenin COOH terminal antibody)
TGN staining of pT120 Beta-catenin antibody
OPPORTUNITIES

- Phospho-specific Beta-catenin characterize functional changes in cells
- Understanding post-translational modifications and implications on biological functions can facilitate development of rationale based biomarkers
- **HYPOTHESIS**: Generation of function based biomarkers will improve performance characteristics of biomarkers in clinical setting
Acknowledgements

Mentors, Staff and Colleagues

- University of Nebraska Medical Center, Omaha, NE
- University of Massachusetts Medical School, Worcester, MA
- Wake Forest University School of Medicine, Winston Salem, NC

Funding: DoD, VA, Prostate Cancer Foundation – Univ of Neb Med Ctr, UMass Med Sch, Wake Forest Univ Sch of Med Cancer Center, WFU Institute of Regen Med
References


