Moving Biomarkers from Discovery through Translation

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Outline

- Introduction
  - An integrated approach for biomarker discovery, validation and translation – Johns Hopkins Clinical Chemistry Division.
- The road map for biomarker translation: The 4Bs, 4Gs & 4Ps case studies.
- Conclusion.

An Integrated Approach for Biomarker Discovery, Validation and Translation – Johns Hopkins Clinical Chemistry Division
Our Strategies
Cancer biomarker discovery, validation & translation

- Select the right technologies: Protein/Lectin array and/or mass spectrometry.
- Use well characterized clinical specimens – plasma, serum, urine, body fluid, tissue, cell: Pathology.
- Develop bioinformatics tools for data analysis and multiplexing of biomarkers: Engineering.
- Design multi-center case control study with extensive clinical validation to minimize the impact of possible confounding variables: Statistics.
- Discover and identify biomarkers (profile is not sufficient) with biological (clinical) significance: Cancer Biology.
- Translation of biomarker into multiplex clinical diagnostics: Clinical Chemistry.

Why are we interested in glycoproteomics?

- Glycoproteins modified by complex carbohydrates are extracellular proteins - membrane proteins, cell surface proteins, and secreted proteins.
- Most likely detected as surrogate serum biomarkers.
- Glycosylation are associated with disease progression.
- Most FDA approved serum cancer biomarkers are glycoproteins.


PSA Screening ?

The U.S. Preventive Services Task Force (USPSTF) recommends against PSA-based prostate cancer screening and gave the guidance a Grade D recommendation (Annals of Internal Medicine July 17, 2012).
Clinical unmet needs: Prostate cancer

- Opportunity for biomarkers to detect aggressive prostate cancer.
- One of the current definitions for aggressive prostate cancer is based on tissue diagnosis using Gleason score = 7 and above. In addition, the assessment of prostate cancer recurrence (5-10 years after radical prostatectomy) is useful.
- Better biomarkers are needed for aggressive prostate cancer.

Solid phase extraction of glycopeptides (SPEG) and quantitative glycoproteomics


Simultaneous Analysis of Glycosylated and Sialylated PSA in Prostate Cancer Tissues
94 lectins were immobilized on the array surface.
Lectin-antibody immunosorbent assay
Fluorescent detection

Rabbit anti-mouse IgG
Alexa Fluor 647 conjugate
Mouse anti-human PSA antibody
Human PSA protein extract from clinical specimens

Lectin 1
Lectin 2

Glass slide with N-Hydroxysuccinimide (NHS) esters coating

Lectin microarrays: Identification of lectins interacting with PSA and MME for aggressive prostate cancer.

(A) Lectin that interact with PSA from pooled tissue samples of different groups
(B) Lectin that interact with MME (membrane metallo-endopeptidase) from pooled tissue samples of different groups.

Yan Li; Sheng-Ce Tao; G. Steven Bova; Alvin Y. Liu; Daniel W. Chan; Heng Zhu; Hui Zhang; Anal. Chem. 2011, 83, 8509-8516.

Lectin-based immunosorbent assays

(A) A lectin-based immunosorbent assay
(B) PSA_SNA & PSA_Jacalin
(C) MME_GS-II & MME_MAL-I

Yan Li; Sheng-Ce Tao; G. Steven Bova; Alvin Y. Liu; Daniel W. Chan; Heng Zhu; Hui Zhang; Anal. Chem. 2011, 83, 8509-8516.
Glycosylated PSA and MME in tissue specimens for aggressive prostate cancer.

The 4Bs (bridges) for Biomarker Translation

1. To define clearly a specific clinical “intended use” for unmet needs.
2. To generate sufficient evidence in preliminary studies to support the investment for a large-scale validation study.
3. To select/develop assays with analytical performance suitable for clinical use.
4. To conduct clinical trial to demonstrate clinical utility to obtain regulatory approval and gain acceptance by the clinical community.

The road from discovery to clinical diagnostics: Lessons learned from the first FDA-cleared in vitro diagnostic multivariate index assay of proteomic biomarkers.

Ovarian Cancer
Wall Street Journal 3-9-2010

Deadly Cancer
The five-year survival rates for ovarian cancer have lagged behind the overall cancer survival rate.
Case Study: Ovarian Cancer

- A 50-year-old woman presented at the Johns Hopkins Hospital clinic. Physical examination revealed masses in the pelvic area. An ultrasonography was performed, however, the result was not diagnostic. Her serum CA125 was 105 U/mL. What was the diagnosis?

OVA1 – Wall Street Journal 3-9-2010

OVA1 – Intended Use (FDA)

- The OVA1 test is a qualitative serum test that combines the results of 5 immunoassays into a single numerical score.
- It is indicated for women who meet the following criteria: over age 18, ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist.
- The OVA1 test is an aid to further assess the likelihood that malignancy is present when the physician’s independent clinical and radiological evaluation does not indicate malignancy.
- The test is not intended as a screening or stand-alone diagnostic assay.
Test to Help Determine If Ovarian Masses Are Cancer - 3/9/2010

- "Doctors and hospitals are getting a new test that many think will help fight ovarian cancer... by helping them to more quickly distinguish cancers from benign growths."
- The test, which is called OVA1..., was shown to correctly flag 92% of cancers, when used along with radiological imaging and a standard patient work-up, in a study of 27 hospitals, doctors' offices and clinics. physicians using their usual detection methods but not OVA1 had previously found 72% of the cancers."
Bioinformatics:
UMSA for Nonlinear Classification

UMSA: Unified Maximum Separation Analysis

Model Evaluation and Comparison:
ROC Curves

Independent validation set: stages I/II epithelial ovarian cancer vs. healthy controls.

OVA 1: Choice of Assay
- Discovery assay: SELDI mass spectrometry
- Clinical assay: ELISA
Translation into clinical diagnostics

- Vermillion Inc. licensed the invention, conducted clinical trial and cleared by the FDA for clinical use on September 11, 2009, as the OVA1 test - the 1st proteomics IVDMIA (in vitro diagnostic multivariate index assays) cleared by the US FDA.

Lesson learned: Do we have the right approach for biomarker discovery and validation?

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<tr>
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<th>Traditional Approach</th>
<th>New Approach</th>
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<tbody>
<tr>
<td>Discovery Study</td>
<td>Small convenient set of specimens (n=10-100)</td>
<td>Large well defined set of specimens (n=100-1000)</td>
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<tr>
<td>Validation Study</td>
<td>Large, well defined set of specimens (n=100-1000)</td>
<td>Large, well defined set of specimens (n=100-1000)</td>
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Do you have the right specimens for biomarker discovery?

- To obtain the right specimens from the right targeted population for biomarker discovery.
- For example, if the goal is to discover biomarkers for aggressive prostate cancer, specimens should be obtained from patients with and without aggressive prostate cancer.
Lesson learned: The intended use define clinical performance and the target population.

The 4Bs & 4Gs for Successful Biomarker Translation

Moving Forward: 4Ps (Partnership)
Case Study – Prostate cancer biomarkers decision gate

- In 2005, 14 biomarkers were submitted to EDRN for consideration (Chair of prostate cancer group – Daniel W. Chan).
- Based on preliminary data from the investigators, the top 5 biomarkers were selected for pre-validation using the same prostate clinical specimen reference set (blinded): ProPSA, human Kallikreins, EPCA2, PCA3 and TSP1.
- Three biomarkers (human kallikreins, TSP1 and EPCA2) failed pre-validation. Pro-PSA and PCA3 were recommended for clinical validation.

4Ps: Public-Private Partnership

- Early Detection Research Network
- BECKMAN COULTER
- UT HEALTH SCIENCE CENTER
- Becton Dickinson
- Biostatistics
- Informatics Center
**ROC Analysis: PSA 2.0-10 ng/mL Range**

Non-Cancer: \( n=234 \); Cancer: \( n=195 \)


AUC

Base model: age, race, DRE, and prostate cancer family history

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**Prostate Health Index (phi) – Beckman Coulter**

Prostate Health Index –

\[ \phi = \left( -2 \right)^{\text{proPSA/free PSA}} \times \sqrt{\text{PSA}} \]

Multi-Center study showed 31% reduction in unnecessary biopsies.

*Approved by FDA on June 25, 2012*

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**PCA3 Molecular Urine Test – Non-coding mRNA**

Low expression in normal prostate cells and highly expressed in prostate cancer cells

Quantitative ratio of PCA3/PSA mRNA = PCA3 Score

Digital Rectal Exam (3 strokes per lobe)

Urine Specimen

PCA3 and PSA mRNA concentrations measured in separate tubes

PCA3 Score < cutoff

PCA3 Score ≥ cutoff

Lower risk of positive biopsy

Higher risk of positive biopsy
Intended Use for PCA3 (PROGENSA)

- To determine the need for repeat prostate biopsies in men who have had a previous negative biopsy: Gen-Probe, Inc.
- FDA approved 2/15/2012.
- High risk Cut-off score > 35

Conclusion

- The future of cancer diagnostics will be more individualized with a specific clinical-intended use.
- It will most likely be a panel of multiple biomarkers.
- The 4Bs (bridge), 4Gs (gate) and 4Ps (partnership): To translate cancer biomarkers into clinical diagnostics, we need to construct a roadmap for the development of cancer diagnostics and with close collaboration between researchers, industry, clinicians and clinical chemists.
- We learned that the road from biomarker discovery, validation to clinical diagnostics could be long and winding, sometimes frustrating, however, we know that at the end of the road...