Translating “-omics” Biomarkers from Bench to Bedside

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Financial Disclosure

None related to this presentation

Acknowledgements

- University of Minnesota
  - Dr. Gary Nelsestuen
  - Dr. Christ Wendt
- Harvard Medical School
  - Dr. Nader Rifai
  - Dr. Mark Kellogg
- University of Rochester Medical Center
  - Dr. Bruce Smoller
  - Dr. Neil Blumberg
- NIH/NCI
  - Dr. Henry Rodriguez
Outline

- Biomarker Research is an Interdisciplinary Field and Has Great Challenges Moving from Bench to Bedside.
- Mass Spec Bridges Discovery Research and Clinical Applications.
- Clinical Chemists Can Make a Difference in Transitional "-omics" Biomarkers from Bench to Bedside.

Ping Pong Diplomacy

The Boom of Biomarker Era
Current Status: Big Investments Small Returns

2008 – 2009 NIH funding
$2.5 billion | 5,943 Grants

1993 – 2008 FDA approved protein markers
Avg. 1.5 per year
e.g., Troponin I/T, BNP, Cystatin C
None from proteomics study

Challenges?

- Complexity of human proteome
  1 million proteins, 21,000 genes
  Dynamic range

- Lack of coherent pipelines from discovery to validation and validation is a very expensive process.

- Lack of standardization in sample collection
  Rely on whatever specimens conveniently available

Inherent Challenges |
Validation Pipeline |
Sample Quality
Low Abundance Provides Inherent Challenges for Protein Analysis

- Na+ concentration: 140 mmol/L
- Testosterone: 300 ng/dL → 10 nmol/L
- Vitamin D: 20 ng/mL → 50 nmol/L
- Albumin: 65 kDa
- Tg: 660 kDa (two 330 kDa monomers)

Biomarker Research is a Multi-Step System

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<td>Qualification</td>
<td>Verification</td>
<td>Assay Optimization Clinical Validation</td>
<td>FDA Approval Commercialization</td>
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- Mass spectrometrists
- "-omics"
- Biochemists
- Mass spectrometrists
- Lab Medicine
- Clinical Chemists

Inherent Challenges Validation Pipeline Sample Quality
Different Approaches for Discovery

- Mass spectrometry is the major platform
  - MALDI Based
  - Electro-spray based

- Specimen from post transplant patients
  - BALF (Bronchoalveolar Lavage Fluid)
  - Control: collected >100 months before diagnosis
  - Rejection: diagnosed with biopsy

Marker Candidates from MALDI

![Graph showing mass to charge (m/z) vs. peak intensity for Control and Rejection samples.](Zhang, Y., Wendt, C., et.al., (2007) Clinical Proteomics 3: 3-12)

Marker Candidates from iTRAQ

- Total of 117 Proteins

![Bar chart showing fold change distribution of proteins from iTRAQ.](Zhang, Y., Wendt, C., et.al., (2007) Clinical Proteomics 3: 3-12)
Validation on iTRAQ results
Confirm Real Differences, Remove False Positives

Total Sample: 65 (48 controls and 17 rejection)


Sensitivity: 82%
Specificity: 92%

Sensitivity: 76%
Specificity: 92%

Further Step: Prediction

Total Sample: 142 (48 controls, 17 rejection, and 77 intermediates)

Statistical model based on multiple markers allows early detection **35 months sooner**


Challenges in Validation Phase

- How to multiplex 100s candidates for validation?
- Current Method of Choice: ELISA
  - Available for < 2000 proteins
  - Time consuming for new assay development
- 150,000 papers generated thousands of claimed biomarkers
  - <100 have been validated
Validation is an Expensive Process

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- # of Candidates: 1,000s
- # of Samples: 1,000s
- Plasma or other fluids
- ELISA (dominant)
- Multiple Reaction Monitoring (MRM)
- Preferably Plasma
- Sensitivity
- Specificity
- Optimization
- Sample Type
- Methodology
- Purposes


Transition from Discovery to Targeted Mass Spec

Small Molecules

Proteins Peptides

Discovery Mass Spec

Targeted Mass Spec

Discovery Mass Spec

Validation pipeline

A pipeline that integrates the discovery and verification of plasma protein biomarkers reveals candidate markers for cardiovascular disease

Jeffrey W Whitaker1, 2, Chaoqi Liu1, 2, Jacob Kennedy2, 3, Luining Hou2, 3, Mary Train2, 3, Izabela Skiba2, 3, Ping Sun1, 2, Regina N Schonhofer1, Li Zhao1, 2, Osama I Vierheilig1, bacon S Kelly SPYRIN1, 2, Albert Koenigsberger3, Philip R Gotlieb1, Jason M Hager1, Lisa A Brown1, Peter Wang1, Ivan Amore1, Lewis A Caddock1, Peter S Nelson1, Martin W Mcintosh1, Christopher J Kemp1, B Amanda G Paskett1.


Targeted Mass Spec

Small Molecules

Proteins Peptides

Discovery Mass Spec

Targeted Mass Spec

Discovery Mass Spec

A targeted proteomics-based pipeline for verification of biomarkers in plasma

$4 million for 4 years

Discovery vs. Targeted MS

Balance in Discovery vs. Validation

CPTAC Assay Portal
Inherent Challenges

Sample Quality
- Pre-analytical concerns
- Sample type
- Sample collection process
- Compound Stability
- Storage
- Freeze thaw cycles
- Inter/Intra-individual variability
- Et al

Big variations among individuals
Biomarker Research shall Begin with the End in Mind

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Clinical Chemists Plays an Important Role in Translating Biomarkers from Bench to Bedside

Conclusions

- Biomarker Research is a complex system, which requires multiple disciplines to work together.
- Mass Spectrometry is a powerful platform to bridge discovery and validation process.
- Clinical chemists can play an important role in moving biomarkers from the bench to bedside.