Reverse-Phase Protein Microarrays: Biomarker Validation and Discovery

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BIOMARKERS: the clinical need

• $210 million on cancer drugs (Canada): 30% efficacy

• E.g. Herceptin $40000/year with approx 30% clinical benefit in metastatic breast cancer

• Global revenues biomarker industry expected $21 Billion by 2012

• Biomarker development part of the Critical Path of drug development (FDA)
GENOMICS TO THE CLINIC

- Multiple “secreted” markers

- High-throughput screening of diagnostic potential missing!
BLOOD BIOMARKERS

- Accessible

- Patients very willing to undergo blood test: least invasive procedure

- Proven biomarkers (CEA, PSA) that anticipate disease recurrence in cancers

- Host-factor: e.g. SDF-1 in breast cancer

- Endothelial bathes pathological tissues
PLASMA BIOMARKERS: THE HOST FACTORS/RESPONSE

Hassan S et al. Clinical Ca Res  2008
PROTEOMICS AND BIOMARKERS

- POST-TRANSLATIONAL MODIFICATIONS
- UNBIASED
- CLINICALLY VALIDATED?
WHY?

- **DISCOVERY**: Dynamic range and high abundance proteins

- **VALIDATION**: The bottleneck
BIOMARKER VALIDATION

• OBJECTIVE:
  - VALIDATE INDIVIDUAL MARKERS IN LARGE COHORTS OF CLINICAL SAMPLES

• OBSTACLES
  - Well collected clinical samples
  - Well annotated clinical samples
  - Successive freeze/thaw cycles of samples
  - ELISA: large volumes (100-200 µl)
    expensive and low-throughput
SOLUTIONS

• High-throughput validation
  - Bead platform
  - Microfluidics
  - Microarray
    • Uniformity
    • Enhanced sample accessibility
Protein Microarrays

Modified from Liotta et al., Cancer Cell 2003
Figure 1. Our reverse phase protein microarrays consist of serum or plasma spotted onto glass slides. Thousands of clinical samples can be screened in a single experiment for a protein of interest using fluorescently labelled antibodies.
- Approx 3 nL of tissue/cell lysate
- Glass backed nitrocellulose slides
- 250-350 µm wide spots each containing the whole cellular repertoire
- Each slide probed with an antibody that can be detected by fluorescent, colorimetric, or chemiluminescent assays

Over 1000 individual cellular lysates can be accommodated on a 20 X 30 mm slide

USES OF LYSATE RPPMs

- Rapid validation of protein levels in tissue samples
- Quantification of post-translationally modified proteins in cohorts of tissue samples
PHOSPHO-PROTEIN PROFILING

- A map of known cell signaling networks or pathways for an individual patient
- Allows comparison across patients in a cohort
- Availability of reliable phospho-antibodies
SERUM/PLASMA RPPMs

• If tissues can be spotted, why not plasma/serum?
  - Surface and buffers?
  - Volumes?
  - Limit of detection?
  - Measureable change?
  - Reproducibility?
  - Shelf-life?
PROTEIN ARRAY TECHNOLOGY

Biomek 2000

Printing robot
Virtek Arrayer
Quill type pins

384 wells

Scan Array Lite (PerkinElmer)

Cy3 labeled secondary antibodies

Labeled secondary antibody
Primary antibody
Printed lysates, cells or serum
TRIAL OF DIFFERENT SURFACES and BUFFERS

A. Black plastic PPB
   Black plastic UREA

B. Nitro PPB
   Nitro UREA

C. Epoxy PPB
   Epoxy UREA

D. Buffer Comparison on Black Plastic Slides

E. Buffer Comparison on Nitrocellulose Slides

F. Buffer Comparison on Epoxy-Coated Slides
PLASMA CLUSTERIN: candidate cancer biomarker

- Mid-abundant plasma protein (μg/μl)
- Secreted sulfated glycoprotein
- Anti-apoptotic
- Oncogenic transformation
- Collaborators with antibodies and recombinant protein (Maureen O’Connor/André Nantel - BRI)
CLUSTERIN can be DETECTED in PLASMA

VOLUME = 0.7 nanoliter/spot
LIMIT OF DETECTION
780 ng/ml (550 femtograms/spot)

Initial plasma clusterin concentration was 50µg/mL
MINIMUM DIFFERENCE = 8 µg

Arrow points to the concentration of spiked clusterin that yielded a signal at least 2SD above the levels of endogenous plasma concentration.
CORRELATION OF RPPM WITH ELISA (spiked clusterin)

$r=0.98$
RPPM PLATFORM is HIGHLY REPRODUCIBLE

- Spot to spot variability:
  Average %CV was 5%

- Array to array variability:
  Average %CV was 2.2% following normalization
CLINICAL SAMPLES

- Samples from Pilot Study to investigate blood collection protocols:
  - CTAD first draw
  - Serum
  - CTAD second draw
  Processed at different time points after collection

- 135 clinical samples spotted in quadruplicate on 3 replicate arrays
DISTRIBUTION OF CLUSTERIN LEVELS (RPPM DATA)

Clusterin Detection Levels in RPPM

Median Fluorescence Intensity (Log10)

Sample Number

CTAD1
CTAD2
serum
CORRELATION OF RPPM WITH ELISA (CLINICAL SAMPLES)

98.6% of samples had a log2 ratio between 1 and -1 indicating a very good overall correlation across the range of clusterin concentrations.
CONCLUSION

• Serum/Plasma RPPM feasible and reproducible

• LOD ng/ml (780 ng/ml)

• Medium abundance proteins
LIMITATIONS OF SERUM/PLASMA RPPMs

• Not a sandwich technique (only one antibody):
  - requires much greater specificity of antibodies
  - AND greater sensitivity
FUTURE DIRECTIONS

• Increase sensitivity of detection
  - Planar wage guide technology
  - DNA/antibody conjugates
THE RPPM PLATFORM

• Genome Quebec Technology Consortium awarded Oct 2004
  - Batist, Schipper, Beliveau, Desrosiers, Gotlieb, O’Connor, Nantel

• Platform for larger projects, clinical trials

• Service to research community and to pharmaceutical industry
THANKS

- Adriana Aguilar
- Min Wu
- Marie Claude Huneau

BRI:
- Maureen O’Connor
- André Nantel
- Christiane Cantin
- Jean Sebastien Déneault