http://proteomics.cancer.gov

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Experimental Design and Biospecimens

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NATIONAL CANCER INSTITUTE

A Functioning Pipeline for Cancer Biomarker Development Requires Both Discovery and Directed Assay Components

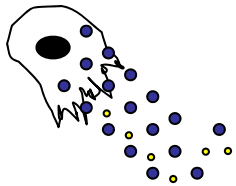
CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Bio-Specimens
- Plasma
- Tissue
- Proximal fluids

Discovery
- Tissue
- Proximal fluids

Verification
- Blood
- Population

Clinical Validation
- Blood
- Population



- untargeted proteomics
- genomics

"hypotheses"

biomarker candidates

Found in blood? higher in cancer?

Biomarkers worth evaluating

Experimental design and biospecimens

Problem

- In biomarker research, rate-limiting step is faulty study design, when bias (systematic difference between compared groups) makes results wrong and misleading.

Approach

- (to be described)

Problem: Bias – Example 1

Mechanisms of disease

Use of proteomic patterns in serum to identify ovarian cancer

Emanuel F Petricoin III, Ali M Ardekani, Ben A Hitt, Peter J Levine, Vincent A Fusaro, Seth M Steinberg, Gordon B Mills, Charles Simone, David A Fishman, Elise C Kohn, Lance A Liotta

Summary

Background New technologies for the detection of early-stage ovarian cancer are urgently needed. Pathological changes within an organ might be reflected in proteomic patterns in serum. We developed a bioinformatics tool and used it to identify proteomic patterns in serum that distinguish neoplastic from non-neoplastic disease within the ovary.

Methods Proteomic spectra were generated by mass spectroscopy (surface-enhanced laser desorption and ionisation). A preliminary "training" set of spectra derived from analysis of serum from 50 unaffected women and 50 patients with ovarian cancer were analysed by an iterative searching algorithm that identified a proteomic pattern that completely discriminated cancer from non-cancer. The discovered pattern was then used to classify an independent set of 116 masked serum samples: 50 from women with ovarian cancer, and 66 from unaffected women or those with non-malignant disorders.

Introduction

Application of new technologies for detection of ovarian cancer could have an important effect on public health,[1] but to achieve this goal, specific and sensitive molecular markers are essential.[1–3] This need is especially urgent in women who have a high risk of ovarian cancer due to family or personal history of cancer, and for women with a genetic predisposition to cancer due to abnormalities in predisposition genes such as *BRCA1* and *BRCA2*. There are no effective screening options for this population.

Ovarian cancer presents at a late clinical stage in more than 80% of patients,[1] and is associated with a 5-year survival of 35% in this population. By contrast, the 5-year survival for patients with stage I ovarian cancer exceeds 90%, and most patients are cured of their disease by surgery alone.[1–6] Therefore, increasing the number of women diagnosed with stage I disease should have a direct effect on the mortality and economics of this cancer without the need to change surgical or

Lancet 2002; 359: 572-577

Bias may explain 'discrimination'

Claim

- ~100% sensitivity, specificity for ovarian cancer

Problem: Compared groups: *different,* not due to cancer

- Mass spectrometry measurements done on different days in cancer specimens vs controls
- Spectrometer drifts over time; 'signal' or 'discrimination' is hardwired into results.

(Baggerly. *Bioinformatics* 2004)

Problem: Bias – Example 2

CLINICAL PROTEOMIC TECHNOLOGIES FOR CANCER

Imaging, Diagnosis, Prognosis

Diagnostic Markers for Early Detection of Ovarian Cancer

Irene Visintin,[1] Ziding Feng,[2] Gary Longton,[2] David C. Ward,[3] Ayesha B. Alvero,[1] Yinglei Lai,[4] Jeannette Tenthorey,[1] Aliza Leiser,[1] Ruben Flores-Saaib,[5] Herbert Yu,[6] Masoud Azori,[1] Thomas Rutherford,[1] Peter E. Schwartz,[1] and Gil Mor[1]

Abstract **Purpose:** Early detection would significantly decrease the mortality rate of ovarian cancer. In this study, we characterize and validate the combination of six serum biomarkers that discriminate between disease-free and ovarian cancer patients with high efficiency.

Experimental Design: We analyzed 362 healthy controls and 156 newly diagnosed ovarian cancer patients. Concentrations of leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, and CA-125 were determined using a multiplex, bead-based, immunoassay system. All six markers were evaluated in a training set (181 samples from the control group and 113 samples from OC patients) and a test set (181 sample control group and 43 ovarian cancer).

Results: Multiplex and ELISA exhibited the same pattern of expression for all the biomarkers. None of the biomarkers by themselves were good enough to differentiate healthy versus cancer cells. However, the combination of the six markers provided a better differentiation than CA-125. Four models with <2% classification error in training sets all had significant improvement (sensitivity 84%-98% at specificity 95%) over CA-125 (sensitivity 72% at specificity 95%) in the test set. The chosen model correctly classified 221 out of 224 specimens in the test set, with a classification accuracy of 98.7%.

Conclusions: We describe the first blood biomarker test with a sensitivity of 95.3% and a specificity of 99.4% for the detection of ovarian cancer. Six markers provided a significant improvement over CA-125 alone for ovarian cancer detection. Validation was performed with a blinded cohort. This novel multiplex platform has the potential for efficient screening in patients who are at high risk for ovarian cancer.

Clin Cancer Res 2008;14:1065

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Problem: Compared groups: *different,* not due to cancer

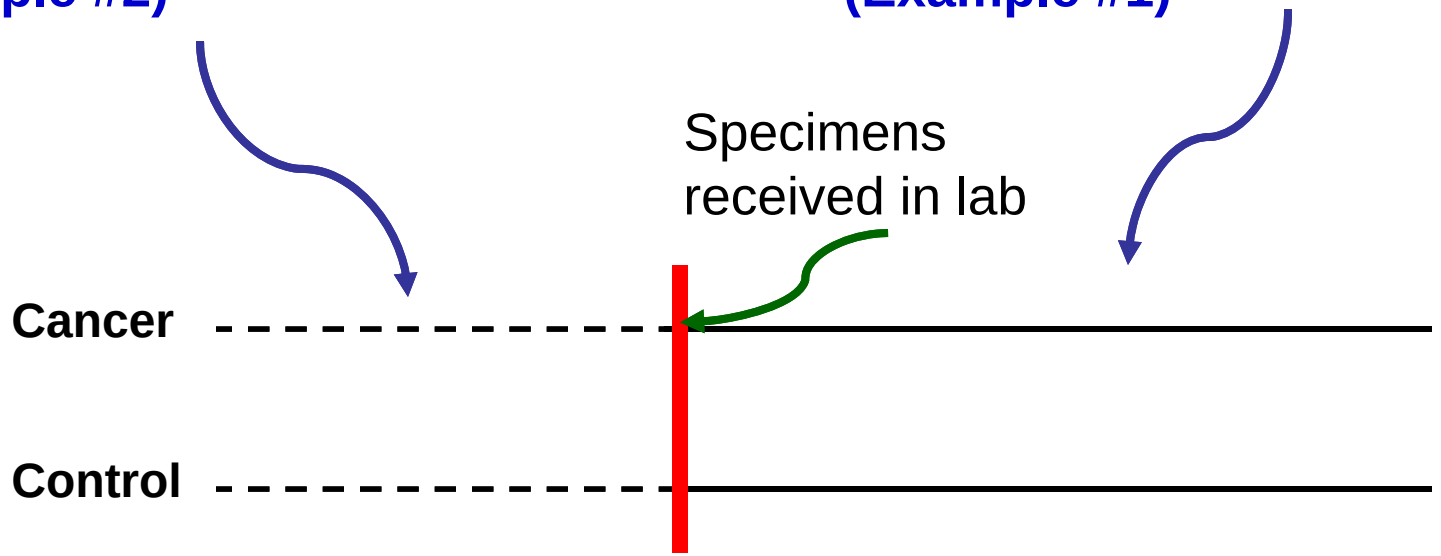
- Cancers from 'high-risk clinic' (pelvic mass)
- Controls from screening clinic
- "Stress" protein markers may differ in compared groups; bias may explain results; interpretation should be moderated.

(McIntosh. *CCR*, 2008;14:7574)

Bias may occur in different 'locations' in observational study design

Before specimens are received in lab, differences occur in demographics, collection methods, etc.
(Example #2)

After specimens are received in lab, differences occur in handling: time, place, etc.
(Example #1)



Experimental design and biospecimens

Problem

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Approach

- Understand specimens are *product of* ***a study***. *Specimen collection* must be *designed* to avoid bias.

RFA focused on technology, not discovery

RFA said "no discovery"

Request For Applications (RFA) Number: RFA-CA-07-005

"This funding opportunity will **not support research addressing discovery of**... proteins and peptides (biomarker discovery)...."

RFA also said "collect specimens"

Request For Applications (RFA) Number: RFA-CA-07-012

"(2a) Availability of Human Clinical Samples.
...The application must include **explicit plans for procuring prospectively collected samples...."**

CPTAC approach to experimental design and biospecimens

Initial proposal from CPTAC sites

- Collect blood specimens from Breast Ca vs not, *after* diagnosis is made

Decision of CPTAC Biospecimen Working Gp (S. Skates)

- Collect before diagnosis, to avoid bias of baseline inequality

CPTAC approach to experimental design and biospecimens

Source

- BrCa screening clinics at 4 CPTAC sites, 500 patients/site
- Patients with breast masses on x-ray, *before* biopsy

Patients (accrual goal: 2000 patients with breast masses)

- Expected cases: 500 BrCa (250 invasive, 250 DCIS)
- Expected controls: 1500

Comment: Design of 'prospective collection' (before diagnosis) avoids bias occurring before specimens reach lab (Example 2).

CPTAC approach to experimental design and biospecimens

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Is 'PRoBE design' (Pepe, JNCI 2008; 100:1432)

CPTAC approach to experimental design and biospecimens

Future direction

- High-quality specimens: resource for ‘discovery’ questions, to assess technology
- Positive result (technology discriminates cancer vs not, or different kinds of cancer), demonstrates ‘proof of principle’ that *‘protein signal exists and can be detected’* (i.e., not due to bias).

Caveat

- Negative result does not say “technology doesn’t work”
- RFA focus: *not* to design process for ‘discovery’.

But perhaps CPTAC approach may be useful in future efforts.

Accomplishments

Problem

- In biomarker research, ***rate-limiting step*** is faulty study design, when bias (systematic difference between compared groups) makes results wrong and misleading

Accomplishments provide direction for future:

- High quality specimens
- PRoBE study design
- Advanced technology