Validation of biomarker candidates and molecular diagnostics of tumours adapted for analysis of fine-needle biopsies (FNA)

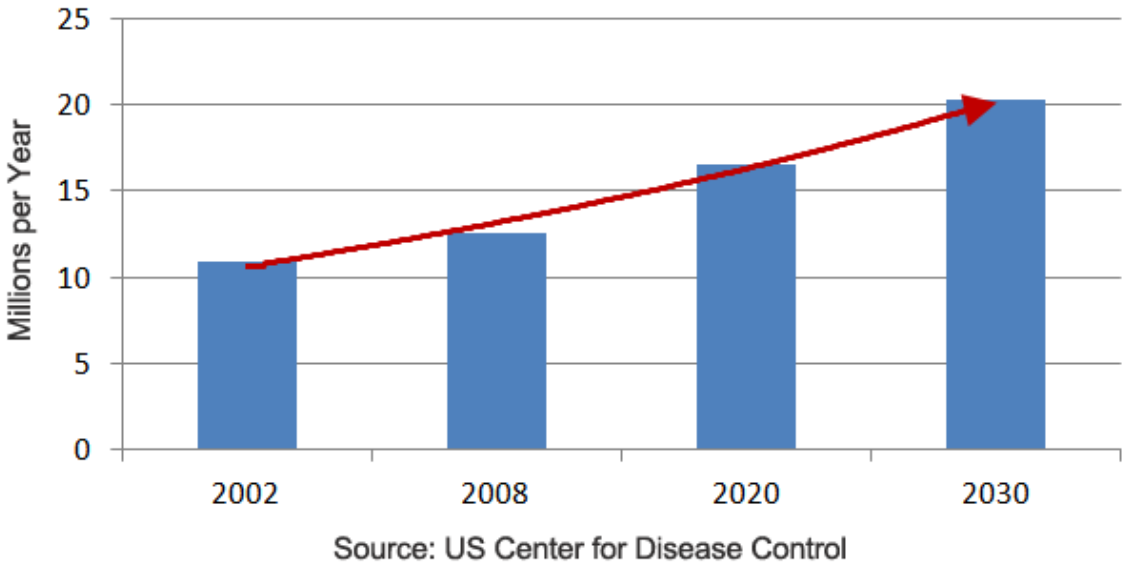
Bo Franzén and Gert Auer
Susanne Becker, Christina Björklund, Marta Lomnytska

Department of Oncology and Pathology

CancerCenter Karolinska

Karolinska Institutet and University Hospital
Stockholm

Incidence of New Cancers Worldwide Will DOUBLE Between 2002 and 2030



Source: US Center for Disease Control

From:
A review of the Swedish pathology
Government commission S2011/5140/FS
Marie Beckman Suurküla
(March 2012)

Immediate needs: **- 275 specialists**

… pathology weaknesses threaten the care of cancer patients…

…Treatment options requires that each tissue **sample is analyzed more detail**...

This means increased capacity requirements and the **application of new techniques in molecular pathology**…

…methodological developments in the pathology is **slow**...

*…and the **implementation** of new technology is slow*
(Note: my comment)

Socialdepartementet
REGERINGSKANSLIET

This is needed to meet future demands

- Increased number of experienced pathologists and cytopathologists
- Increased level of diagnostic objectivity through improved molecular tools
- Increased analytical sensitivity and precision (*i.e.* fine-needle aspiration/FNA and PTM specific)
- Multiplexicity – define combination of markers

Start by asking basic questions:

1. Is the lesion benign or malignant?
2. If malignant: High or low metastatic potential?

(Research focus: *epithelial tumours*)

Tissue/cell samples for analysis: Different types of biopsies

Fine-Needle Aspiration



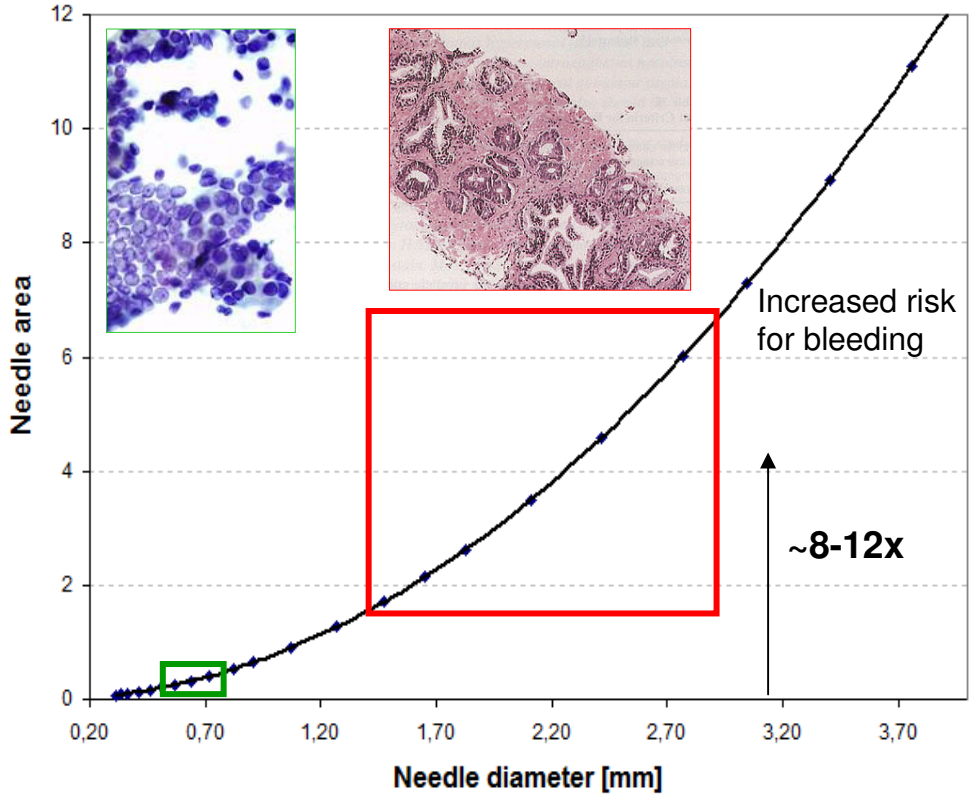
Core-Needle Biopsy



Surgical Biopsy



www.nationalbreastcancer.org



FNA is more patient friendly but require experienced cytopathologist and/or sensitive analytical methods.

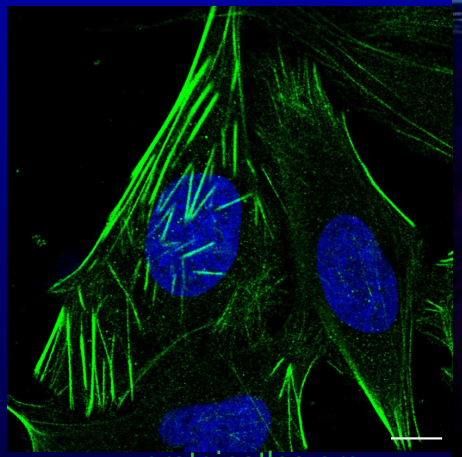
If this can be fulfilled:
- earlier diagnosis may be possible
- same day diagnosis may be possible

Can 2D-gel based proteomics improve diagnosis?

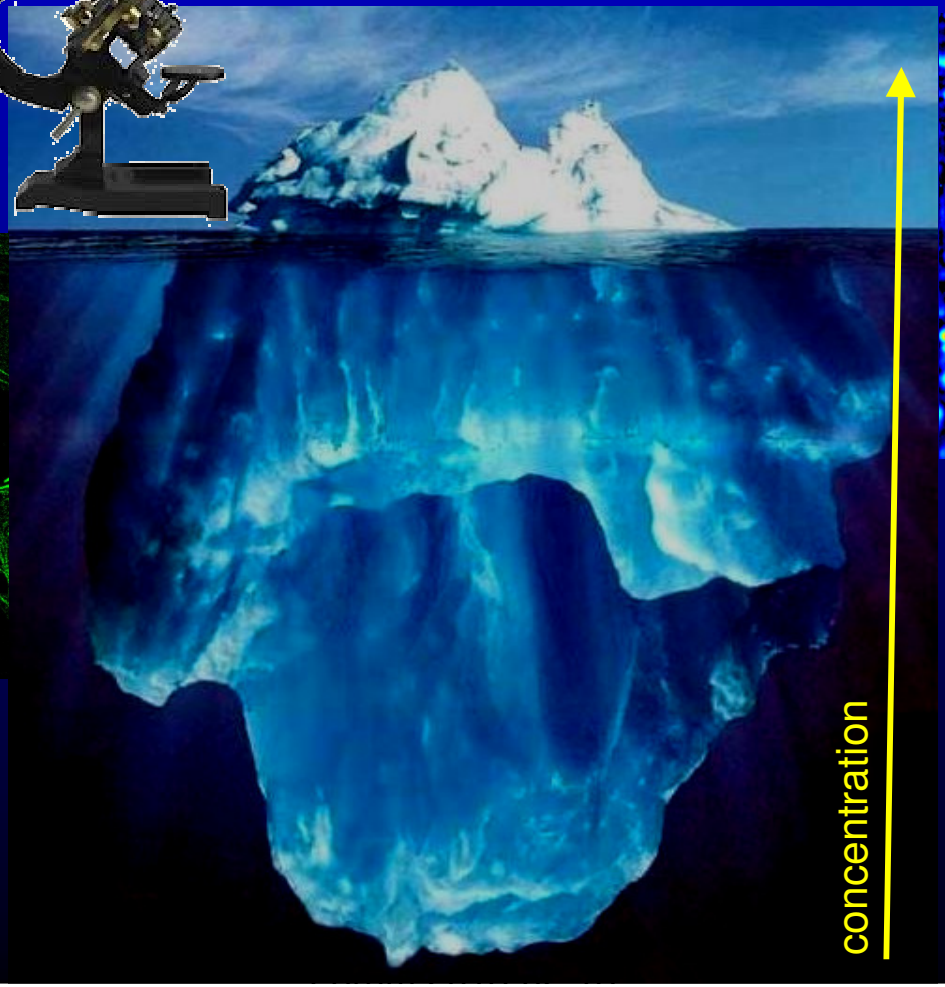


Microscopy:
– we observe
”high tips of the iceberg”

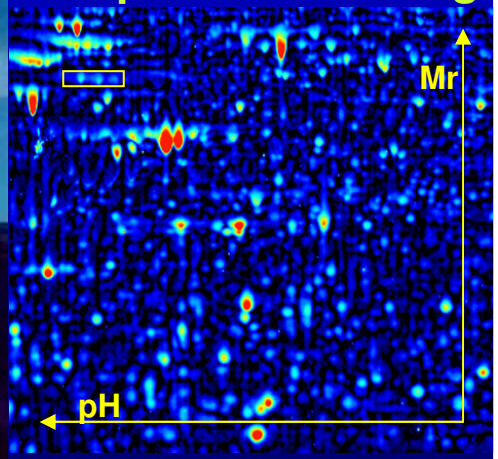




www.proteinatlas.org

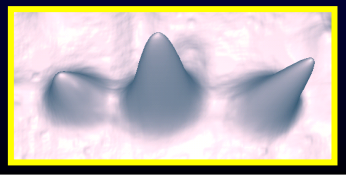


2D-gels:
– we observe
”low tips of the iceberg”



=
”Molecular morphology”

…with PTM resolution:



Tumour tissue analysis: What we learned 20 years ago

***Sample preparation* is critical for high 2D-gel quality!**

-Use **fresh**, not **frozen** tissue

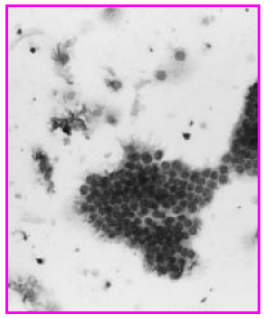
-Control of **representativity**

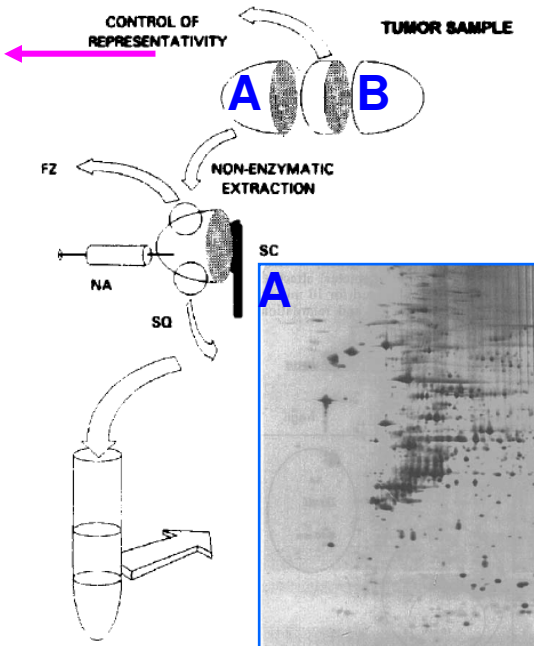
-Monitor tumour heterogeneity

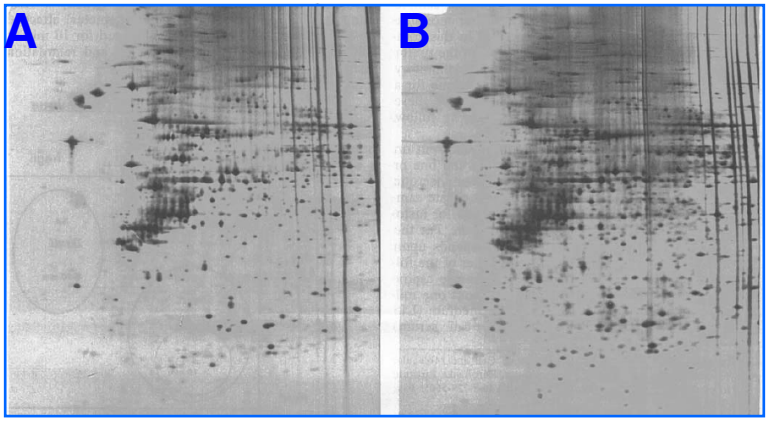
Prep. of **frozen** *vs* **fresh** tissue











Electrophoresis 1993, *14*, 1045–1053

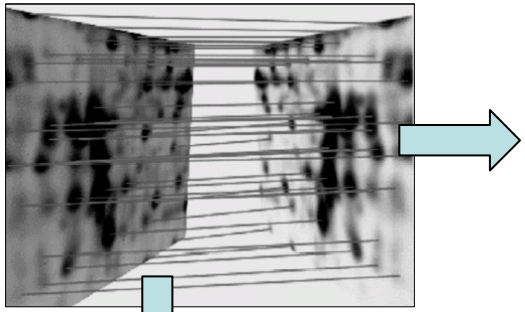
Preparation of human tumors for analysis by 2-D electrophoresis

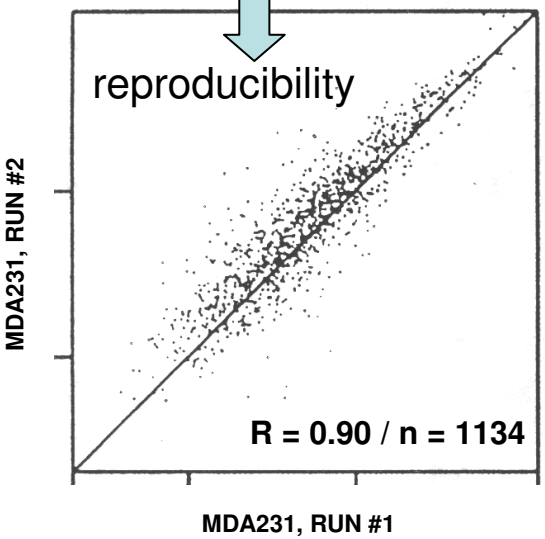
Bo Franzén[1]
Stig Linder[3]
Ken Okuzawa[2]
Harabumi Kato[2]
Gert Auer[1]

Nonenzymatic extraction of cells from clinical tumor material for analysis of gene expression by two-dimensional polyacrylamide gel electrophoresis

Results revealed major challange:

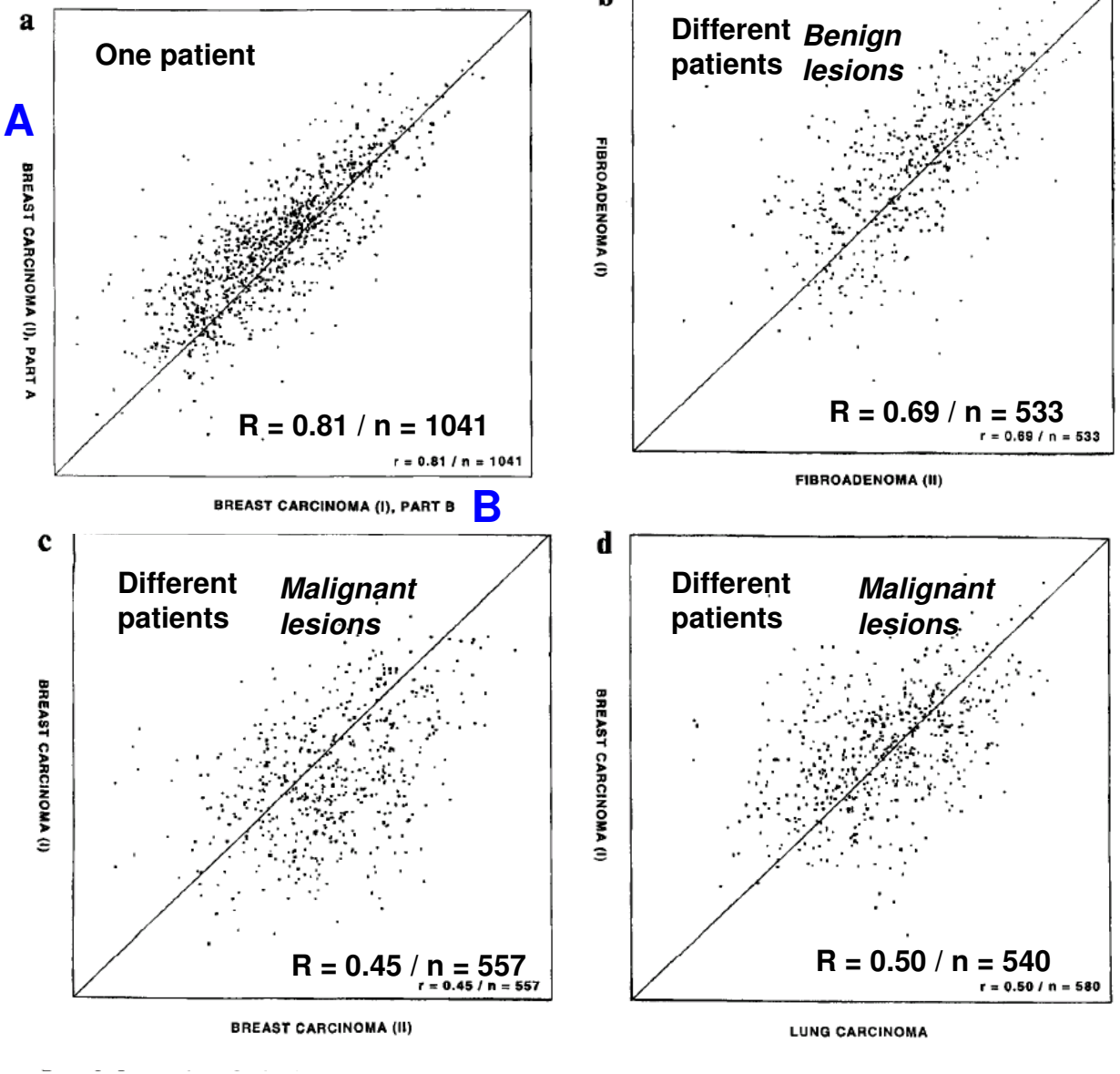
Large *biological* variability at proteome level





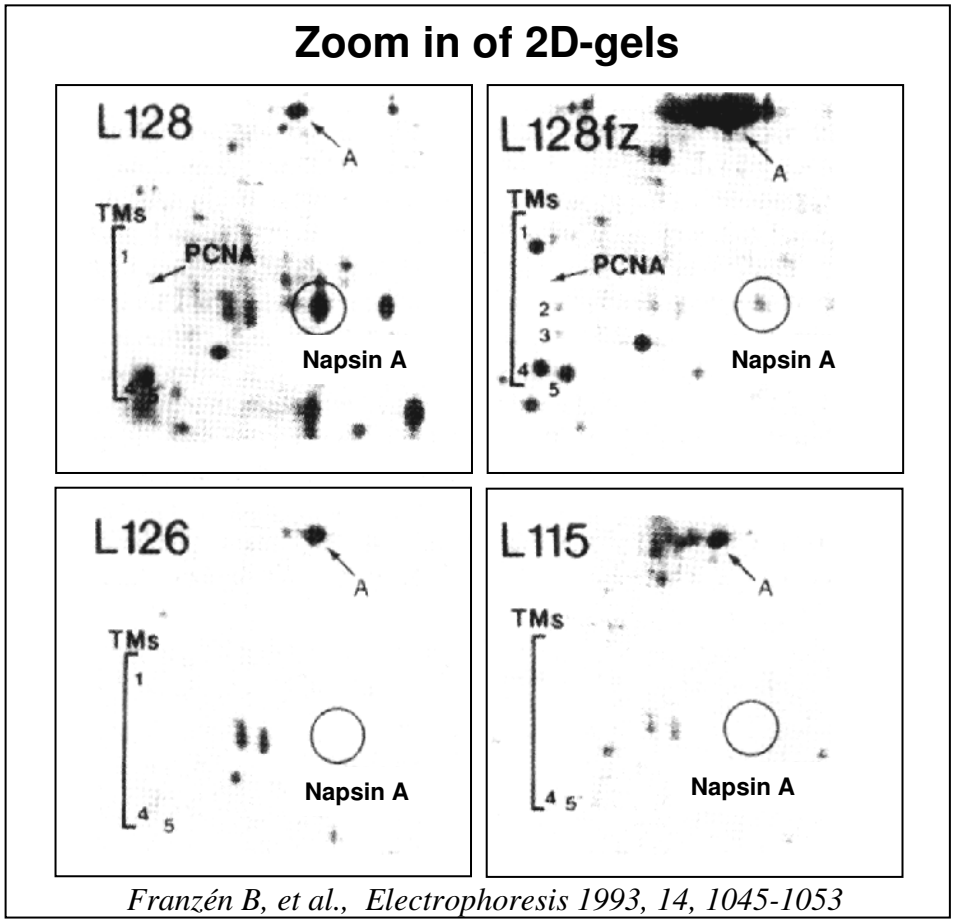
Franzén et al., Electrophoresis, 1997, 18, 582-587.

Variability in breast tumors



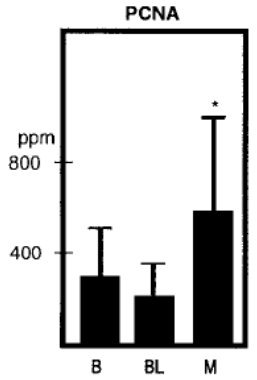
For diagnostics: it will be nesessary to define *combinations* of biomarkers

Identification of diagnostic candidate biomarker in clinical tumor material

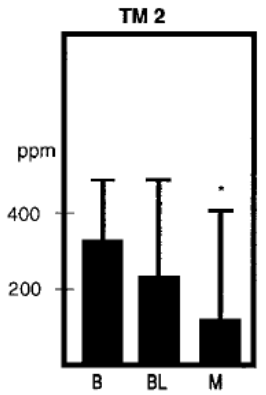


Franzén B, et al., Electrophoresis 1993, 14, 1045-1053

- Progression marker
  PCNA,
  increased in cancer



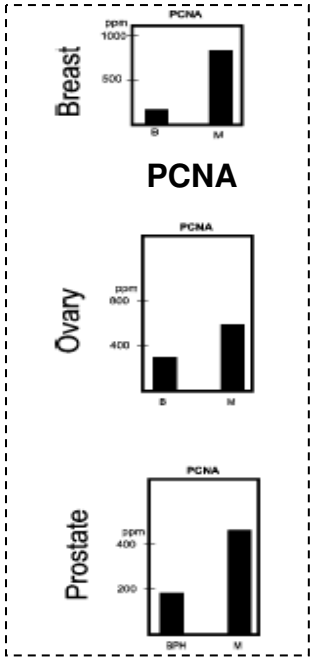
- Early diagnosis markers
  High Mw tropomyosins decreased in cancer



- Differential diagnosis marker
  Napsin A, specific for primary lung adenocarcinoma
  ⇒ therapy selection

Several biomarker candidates show similar a expression pattern in tumours from breast, ovary and prostate

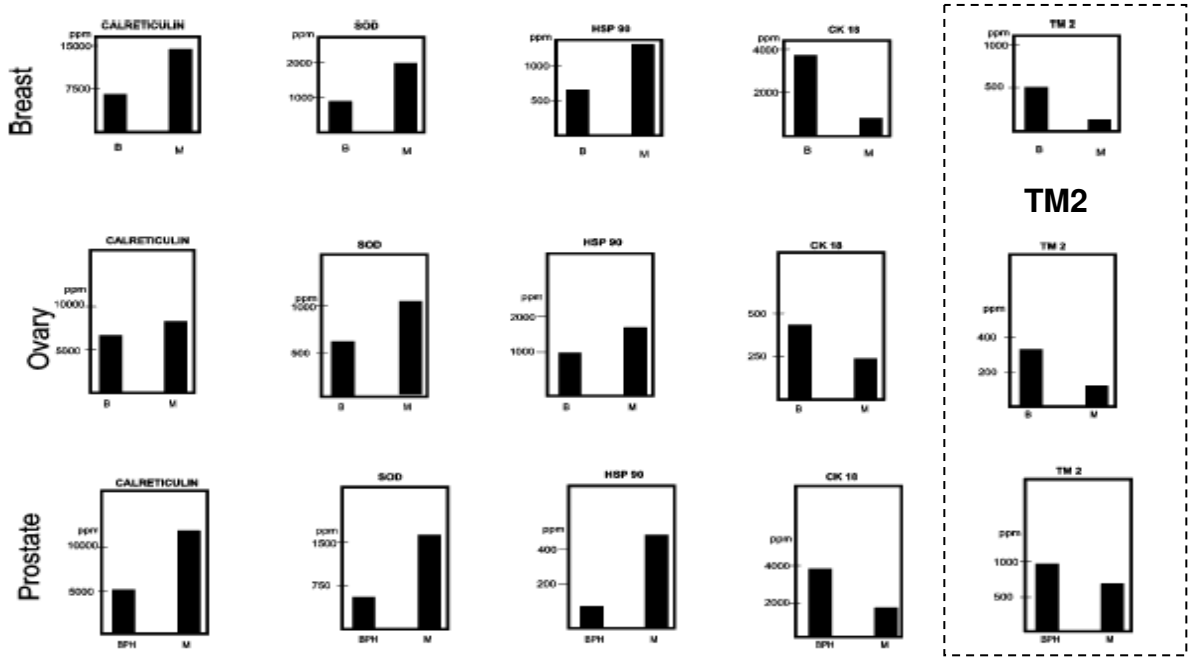
Benign
vs
malignant



PCNA

A. Alaiya et al. / Polypeptide expression in human prostate tumors

2. Expression of 10 polypeptides previously identified to differ in expression between benign and malignant human breast and ovarian tumors [1,10]. Shown is the expression of each ker as a fraction of the total loading on the gel (in ppm).



TM2

A. Alaiya et al. / Polypeptide expression in human prostate tumors

Analytical Cellular Pathology 21 (2000) 1–9

From more than a decade of comprehensive biomarker discovery efforts

Thesis by:

- **Franzén**, Bo, A two-dimensional gel electrophoretic study of proteins in tumours of the **lung** and **breast**. **1996-04-26**.
- **Alaiya**, Ayodele A. Cancer proteomics. Characterisation of protein expression in human **epithelial** tumours. **1999-06-04**.
- **Roblick**, Uwe Johannes. **Colon** cancer. Disease related proteins in tumor tissue and serum. **2004-12-10**.
- **Lundgren**, Caroline. **Endometrial** carcinoma. Prognostic factors and protein expression profiling. **2004-10-15**.
- **Hellman**, Kristina. **Vaginal** carcinoma. Studies on etiology and prognostic factors. 2005-11-25.
- **Habermann**, Jens Karsten. **Colorectal** cancer. Genome, transcriptone and proteome dynamics. **2005-06-17**.
- **Kronenwett**, Ulrike. Genome instability, gene expression and prognosis in **breast** cancer. **2005-03-11**.
- **Lexander**, Helena. Protein Expression In **Prostate** Cancer. **2006-01-27**.
- **Gemoll**, Timo. Identification of protein signatures of genomic stability/instability in **epithelial** cancers. **2011-12-16**.

And other publications, *e.g.*

- **Lomnytska**, MI, et al. Diagnostic protein marker patterns in squamous **cervical** cancer. PROTEOMICS - Clinical Applications. 2010, 4 (1): 17–31.
- **Hellman**, K, et a. Differential tissue-specific protein markers of **vaginal** carcinoma. Br J Cancer. 2009; 100(8): 1303–1314.
- **Lomnytska** MI, et al., Impact of genomic stability on protein expression in endometrioid **endometrial** cancer. Br J Cancer. 2012, 106 (7):1297-305.

***Current status*:**

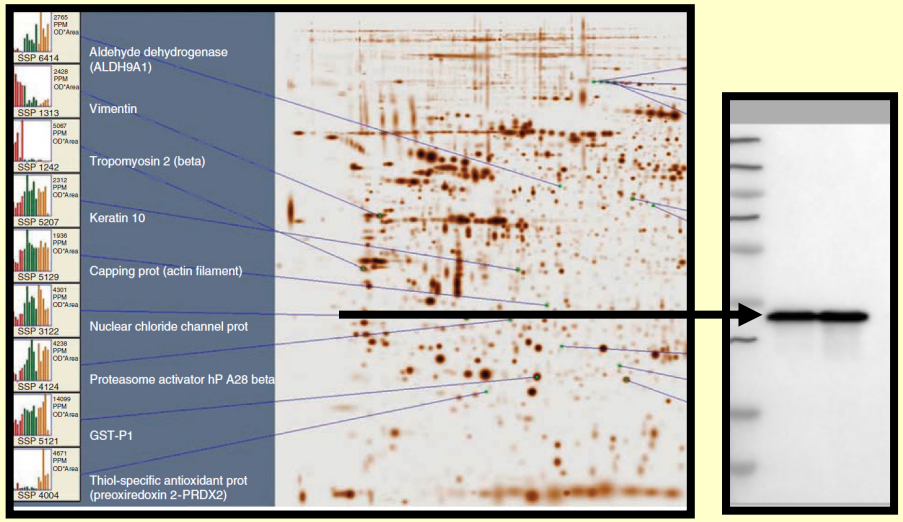
Review (ongoing) of >10 years of research,

> 1000 2D-gels and >500 gel-plug identifications by MS

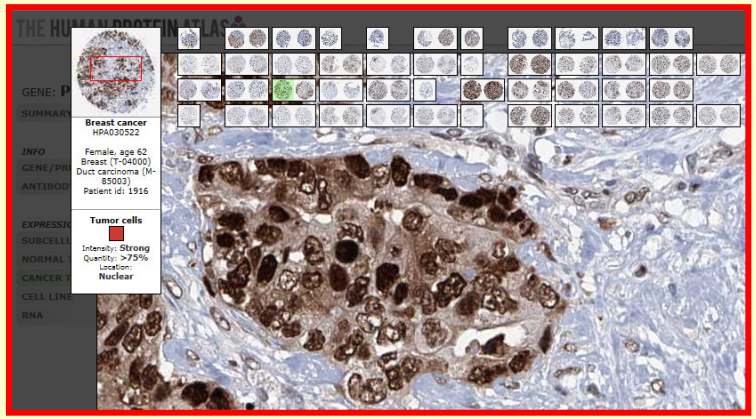
Validation of biomarker candidates

Several options

Check PTM´s by Western blot validation: 1D and/or 2DE

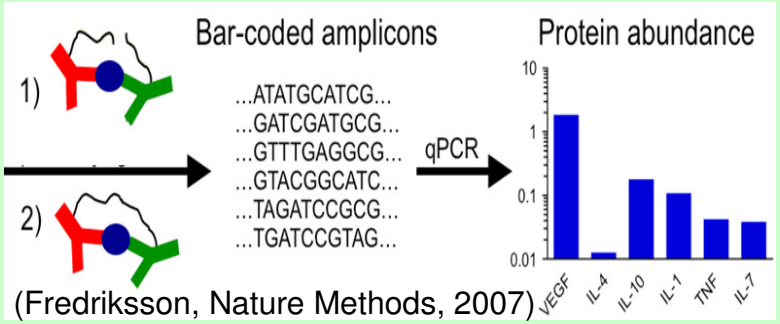


Check IHC data



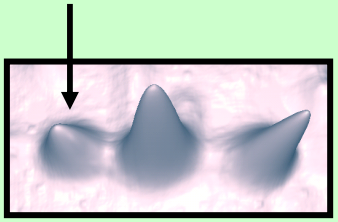
www.proteinatlas.org

PLA based technology



(Fredriksson, Nature Methods, 2007)

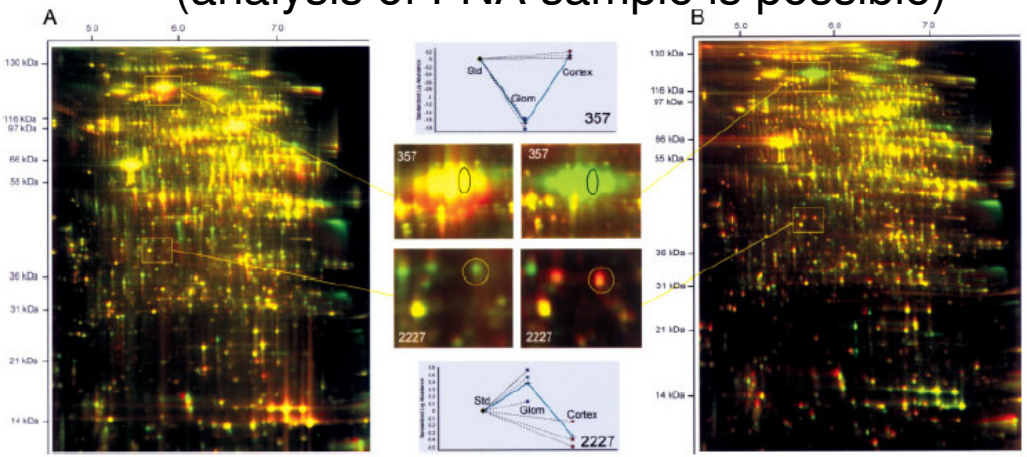
…to obtain **PTM** information



Other options….?

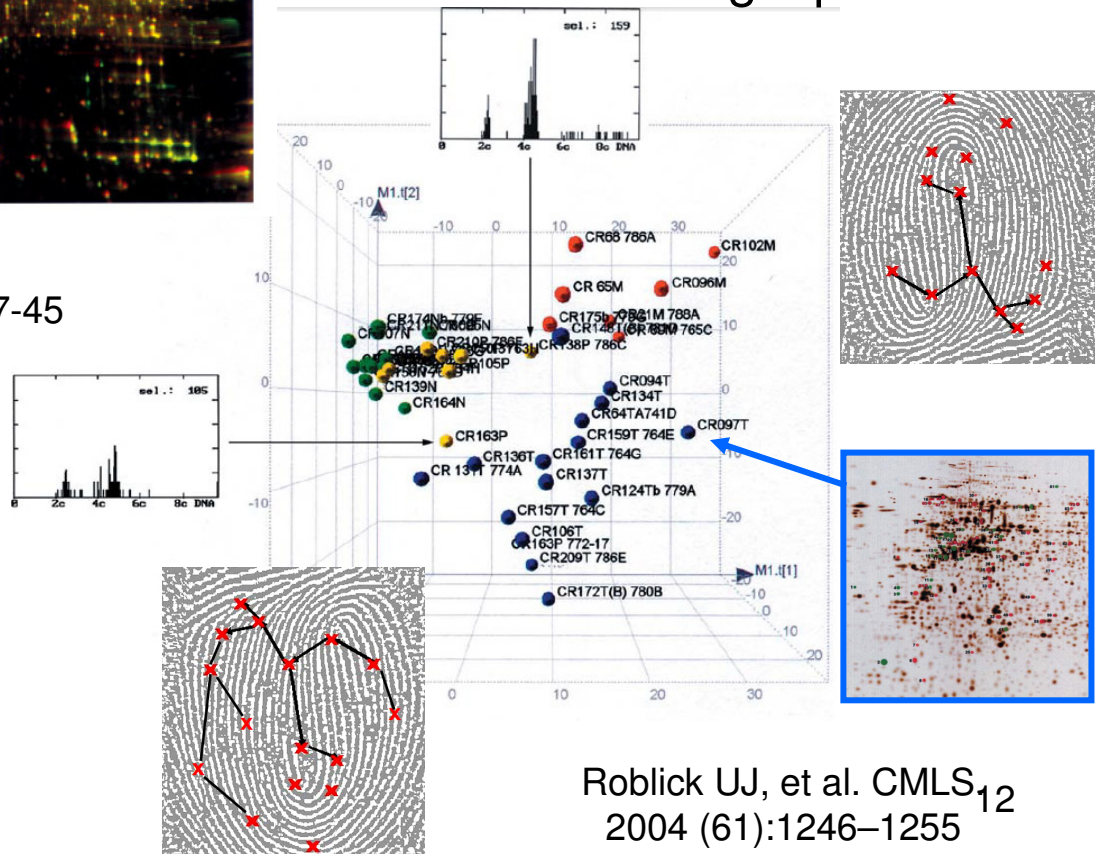
Alternative possibilities….

DIGE saturation labelling technology
require only 1000-10.000 cells
(analysis of FNA sample is possible)



Sitek B, et al. Proteomics. 2006 (15):4337-45

A database of 2D-gel patterns



Roblick UJ, et al. CMLS 2004 (61):1246–1255

Summary

- Promising marker candidates
  - *validation* needed
- Define *combination* of markers
- Define high sensitivity,
  PTM specific technology platforms
- Funding needed

Identification of technology platform for cost-effective multiplex analysis of diagnostic markers in minute amounts of tissue/cell samples (e.g. FNA)