



Proteomics and Cancer Detection

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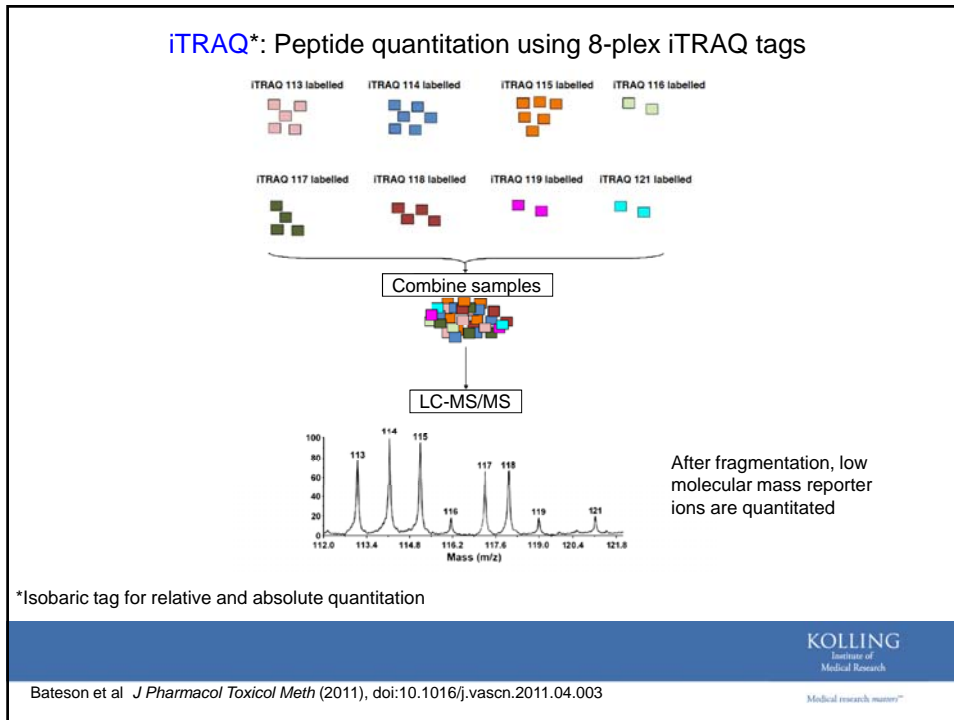
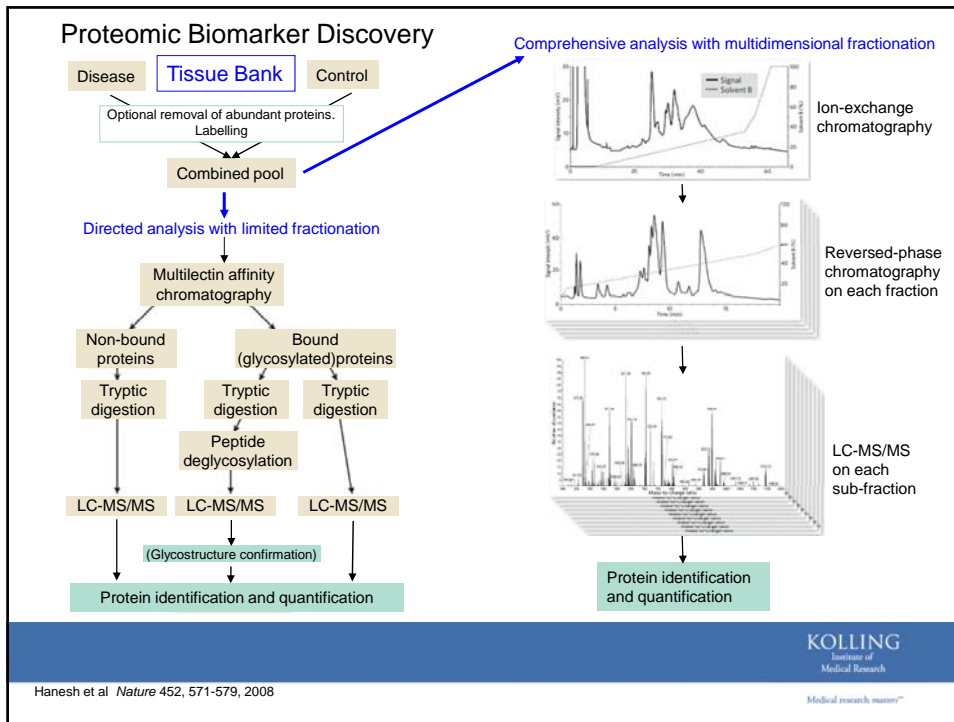
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- Quantitative approaches to biomarker discovery
- Diagnostic and predictive models
- MS Imaging
- Proteomics in cancer — present and future

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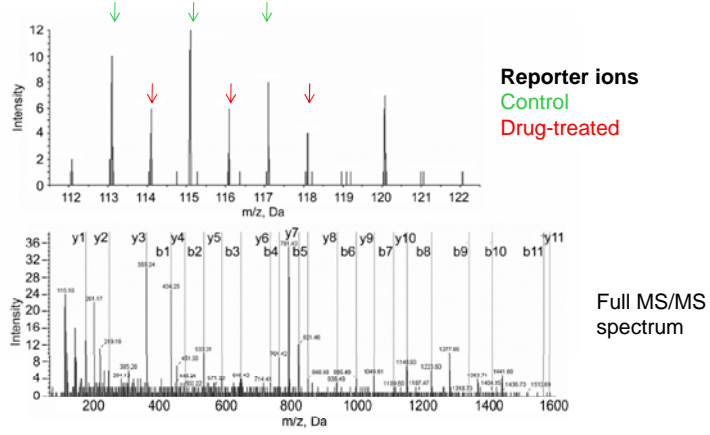
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Sharon Leong

Downregulation of SLC1A5* in breast cancer cells following treatment with doxorubicin and TRAIL



Leong et al, J Proteome Res (in revision)

*Solute carrier family 1 (neutral amino acid transporter), member 5

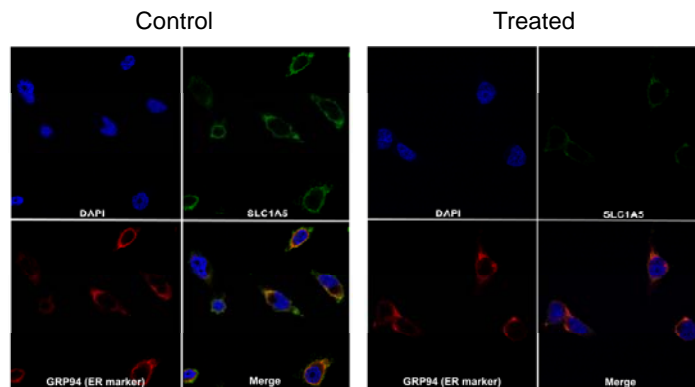
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Mike Lin

Downregulation of SLC1A5 in MDA-MB-231 breast cancer cells following treatment with doxorubicin and TRAIL



Leong et al, J Proteome Res (in revision)

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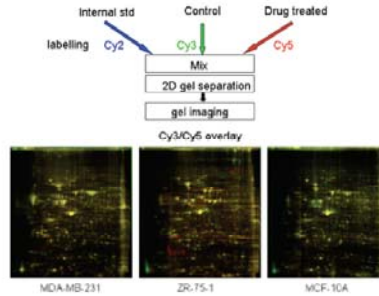
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Sharon Leong

Quantitation of proteomic changes by 2D-DIGE

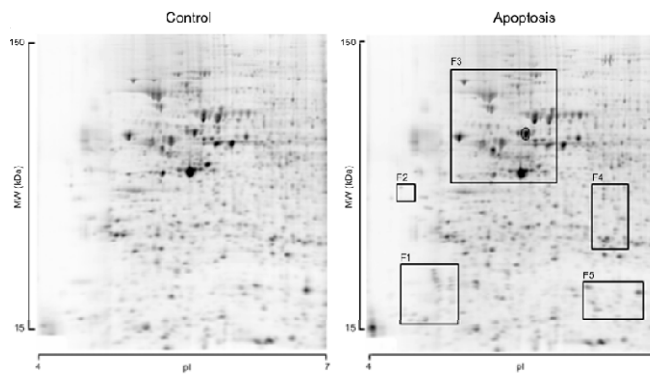
- Proteins extracted into 7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris
- Fluorophore-labelled



- Image analysis and quantitation (DeCyder software)
- Sypro ruby staining, spot cutting, in-gel tryptic digestion
- ID by LC-MS/MS
- Confirmation by selected reaction monitoring (SRM)
(selected tryptic peptides fragmented in triple quadrupole MS to quantifiable product ions)

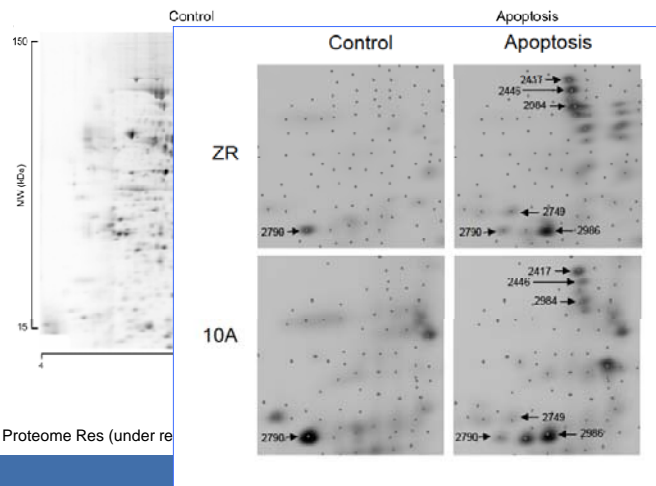
Leong et al, J Proteome Res (under review)

Quantitation of proteomic changes by 2D-DIGE



Leong et al, J Proteome Res (under review)

Quantitation of proteomic changes by 2D-DIGE



Leong et al, J Proteome Res (under review)

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Table 3. Relative quantitation of validated differentially-expressed proteins

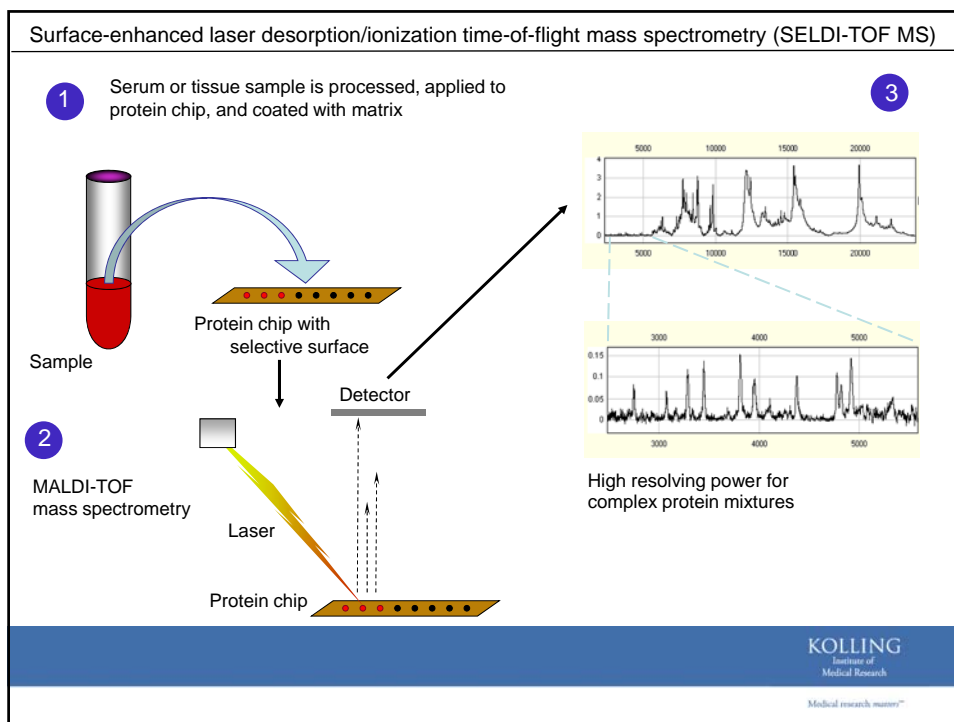
Protein	Dox-TRAIL/DMSO control ratio		
	2D-DIGE ^{a)}	Western blot	SRM
RAD23B	-2.2	-3.3	ND
RPLP0	9.5	ND	26.0
KRT8	5.6	ND	47.5
KRT18	24.0	ND	491.4
HSP105	-2.3	-1.7	-7.2

a) values from ZR-75-1 data; negative values represent decreased protein expression in Dox-TRAIL relative to DMSO control samples; ND, not determined

Leong et al, J Proteome Res (under review)

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Ross Smith Aiqun Xue

Discovery of serum biomarkers for pancreatic adenocarcinoma

- Diagnostic serum biomarkers for pancreatic cancer are unsatisfactory, although many have been investigated
- CA19-9, the 'gold standard', has a 79% (range reported 70–90%) sensitivity and 82% (68–91%) specificity.
- Although grossly elevated CA19-9 predicts unresectable disease and prognosis for chemoradiotherapy, 10–15% of patients cannot produce CA19-9 because of Lewis-negative genotype (Kawai et al, 2008).
- Serum CA19-9 is elevated in other malignancies and benign disorders
- Recently, the necessity for a multivariate serum marker has been proposed

Xue et al, Brit J Cancer (2010) 103, 391 – 400

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Discovery of serum biomarkers for pancreatic adenocarcinoma

Patient Groups

	Training	Validation 1	Validation 2
Pancreatic ductal adenocarcinoma	38	40	33
Disease controls	54	21	28
Healthy volunteers	68	19	18
Total	160	80	79

Xue et al, Brit J Cancer (2010) 103, 391 – 400

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Sample and data analysis

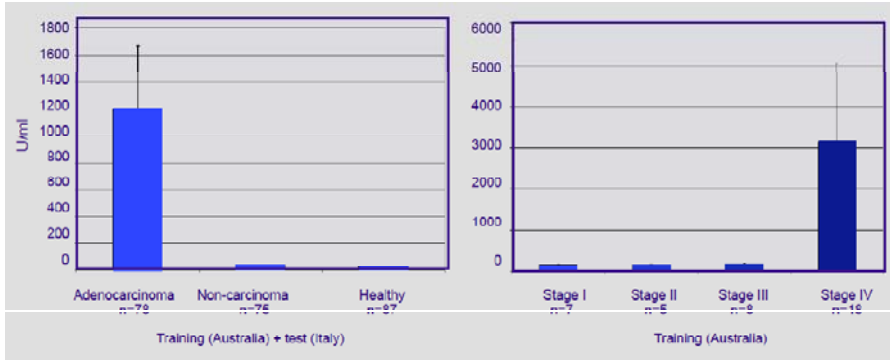
- Samples diluted in 8M urea/1% CHAPS
- Subjected to SELDI-TOF MS in duplicate
- Peaks showing high discriminatory power by univariate analysis were subjected to logistic regression with 10-fold cross-validation to develop a training model
- CA 19-9 also measured by ELISA and added to the model
- Models validated by SELDI-TOF MS on an independent sample set (Validation 1)
- After peak identification, models validated by ELISA on a second independent sample set (Validation 2)

Xue et al, Brit J Cancer (2010) 103, 391 – 400

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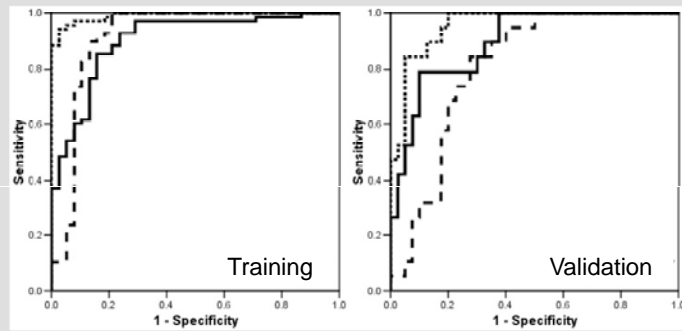
Serum CA19.9 levels by subject group and tumour stage



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Serum Classification Model 1: Cancer vs Healthy



ROC AUC:	Model	ROC AUC:
0.91	CA19.9 (dashed)	0.81
0.90	SELDI Panel (4) (solid)	0.90
0.99	Panel+CA19.9 (dotted)	0.96

SELDI peaks alone: Sensitivity = 71.1%
Specificity = 95.6%

SELDI + CA19.9: Sensitivity = 92.1%
Specificity = 97.1%

Sensitivity = 90.0%
Specificity = 73.3%

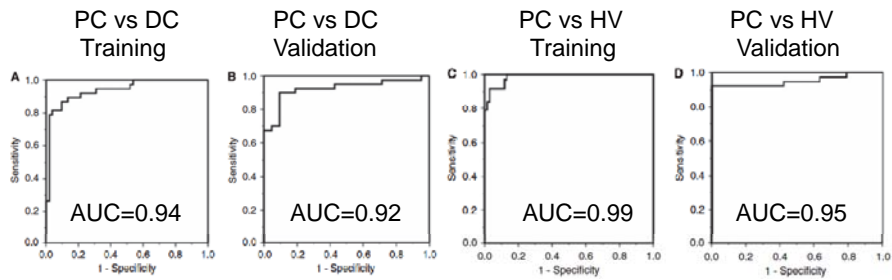
Sensitivity = 92.5%
Specificity = 84.2%

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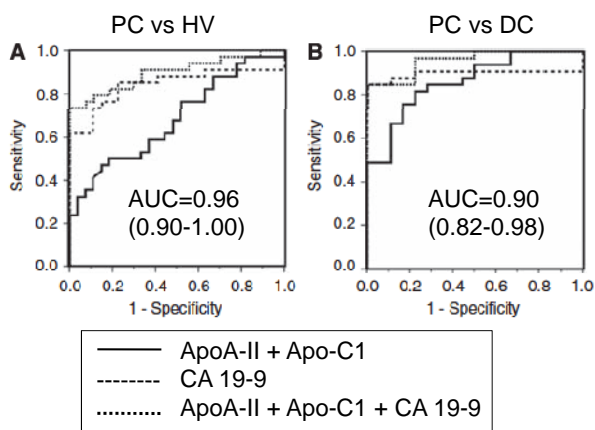
Diagnostic SELDI-based model development and validation

After peak identification by purification and LC-MS/MS and elimination of co-dependent peaks, 4-marker panel was refined to a 2-marker panel + CA 19-9: m/z 6420 = apolipoprotein C-1 and m/z 8614 = apolipoprotein A-II.



Xue et al, Brit J Cancer (2010) 103, 391 – 400

Diagnostic ELISA-based model validation



Xue et al, Brit J Cancer (2010) 103, 391 – 400



Ross Smith

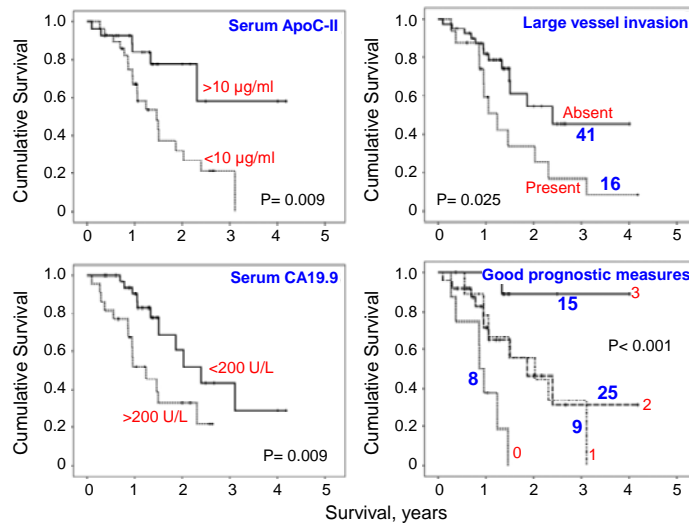


Aiqun Xue

Pancreatic cancer biomarker discovery:

Prognostic marker for long-term survival after pancreatic resection for adenocarcinoma

Relationship of ApoC-II and other biomarkers to patient survival after resection for pancreatic ductal carcinoma





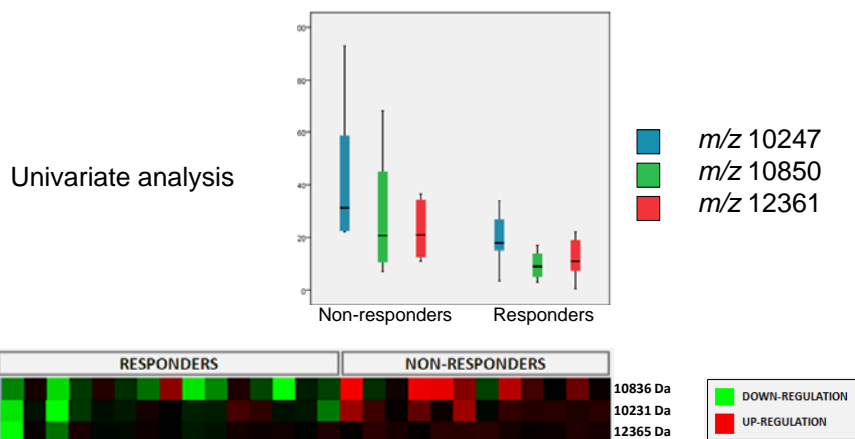
Kerrie McDonald Hatice Sevim

Predicting response to temozolomide combined with radiotherapy in glioblastoma patients

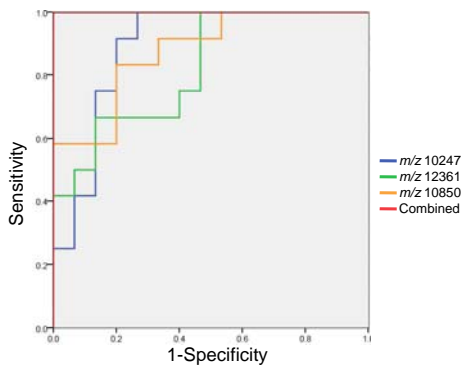
- Median survival time after diagnosis is 14.6 months despite surgery, radiotherapy and chemotherapy
- The efficacy of chemotherapy is often limited because of drug resistance
- Development of objective prognostic, and predictive markers is therefore a priority

3 proteins differentially upregulated in treatment non-responders

Treatment responders (n=12): patients showing >6 months progression-free survival
 Treatment non-responders (n=15): patients showing <6 months progression-free survival



ROC analysis on univariate and multivariate parameters



Area under the curve

Test Variable	Area
m/z10247	0.889
m/z12361	0.822
m/z10850	0.878
Combined	1.000

ID by LC-MS/MS

m/z 10247, identified as **neutrophil defensin 3 (DEF3)**

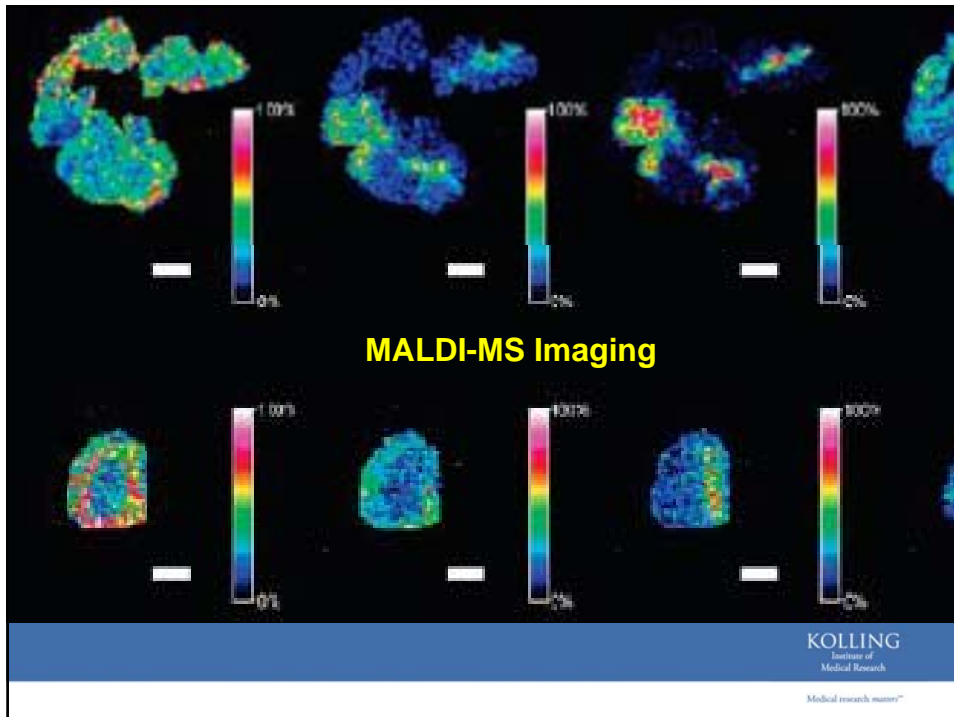
m/z 10850, identified as **calgranulin A (S100-A8)**

m/z 12361, identified as **macrophage migration inhibitory factor (MIF)**

pro-inflammatory proteins expressed by neutrophils, activated macrophages, endothelial cells

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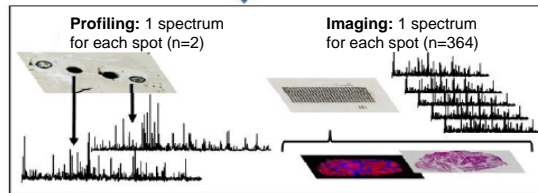
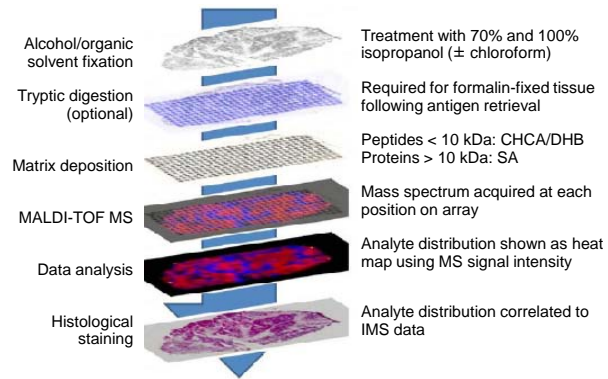


MALDI-MS Imaging

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Imaging MS Workflow

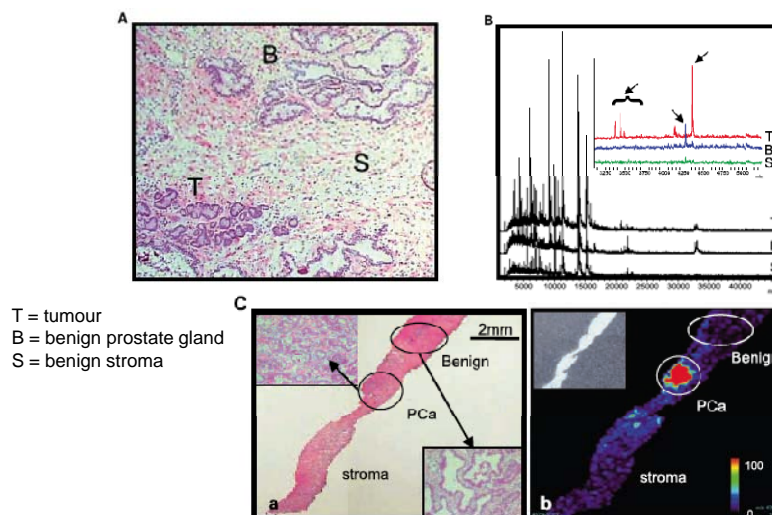


Gustafsson et al, *Int J Mol Sci.* 2011, 12, 773-794.

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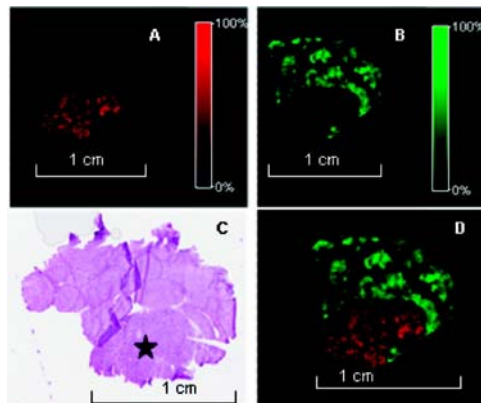
MALDI Imaging of prostate cancer tissue



Cazares et al *Clin Cancer Res* 15:5541, 2009

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Ion intensity maps (m/z 8450 and 3195) in HCC and adjacent liver cirrhosis in a case from the validation cohort. (A) m/z 8450. Ion is overexpressed in HCC. (B) m/z 3195. Ion is overexpressed in cirrhosis. (C) Serial frozen section stained with H&E, with cirrhosis and adjacent carcinoma (star). (D) Merged image of 8450 and 3195 ion intensity maps.

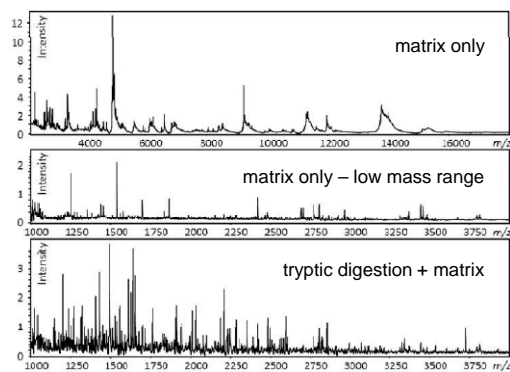
Le Faouder et al, *J. Proteome Res.* 2011, 10, 3755-3765.

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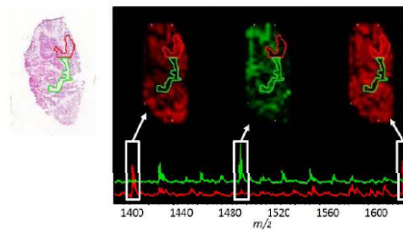
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Imaging MS: Stage IIIC ovarian epithelial carcinoma

Gustafsson et al, *Int J Mol Sci.* 2011, 12, 773-794.



H&E + 3 ion-intensity maps



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Bring on the biomarkers

The dismal patchwork of fragmented research on disease-associated biomarkers should be replaced by a coordinated 'big science' approach, argues **George Poste**.

156 | NATURE | VOL 469 | 13 JANUARY 2011

In a 2009 NIH survey, researchers from 80% of more than 700 responding laboratories said they struggled to obtain standardized specimens for biomarker research. Alarmingly, a similar percentage did not question how specimen quality or handling conditions might affect their results⁵.

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Proteomic Biomarker Discovery

Box 1 | Sources of variability in proteomic analysis of plasma

Listed are some of the sources of variability in the proteomic analysis of plasma. This variability is not associated with disease states and can confound analysis.

- Differences in methodology
- Lack of standardized sample collection and storage, variably affecting comparison groups
- Differences between cases and controls in terms of sex, age and physiological states (for example, fasting, weight gain or loss and hormonal status)
- Differences in genetic make-up
- Changes in inflammation and acute-phase reactants
- Changes in metabolic states
- Other nonspecific changes: for example, cell death and tissue necrosis
- Changes reflecting underlying chronic disease: for example, those caused by smoking and chronic lung disease, in contrast to lung-cancer-specific changes

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Hanesh et al *Nature* 452, 571-579, 2008

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Only 9 single-protein cancer biomarkers in blood are currently approved by the FDA

Biomarker	Clinical Use	Cancer Type	Year Approved
AFP	Staging and monitoring	NGCSTs and HCC	1988
CA-125 (MUC16)	Monitoring	Ovarian cancer	1987
HE4*	Monitoring	Ovarian cancer	2009
Thyroglobulin	Monitoring	Thyroid cancer	1999
PSA	Screening and monitoring	Prostate cancer	1986
CEA	Monitoring	Colon cancer	1980
CA19-9	Monitoring	Colon and pancreatic cancer	2002
CA15-3, CA27-29	Monitoring	Breast cancer	1981
HER2/neu	Monitoring	Breast cancer	2000

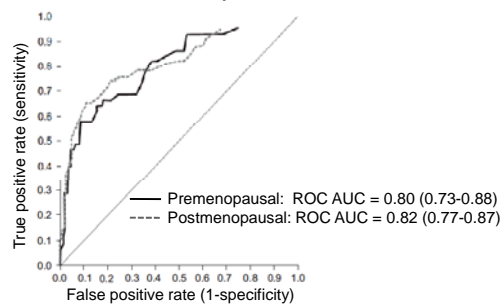
*human epididymis protein 4

OVA1 test (Vermillion Inc.)

5-protein biomarker panel for ovarian cancer, based on proteomic (SELDI-TOF MS) discovery
FDA-approved in September 2009

Components: CA125, transferrin, transthyretin (prealbumin), apolipoprotein AI, and beta2 microglobulin

Evaluated in Ueland FR et al. *Obstet Gynecol* 117:1289, 2011



CANCER BIOMARKERS

SURVIVING THE JOURNEY FROM BENCH TO BEDSIDE

By Jeanne M. Rhee, PhD, and Ross J. Molinaro, MT(ASCP), PhD, D(ABCC), F(ACB)
Medical Laboratory Observer, Mar 2011; 43:10-12.

“None of the nine FDA-approved cancer biomarkers demonstrate the specificity required for diagnosis when used alone.

Thus, the development of panels of proteins such as the FDA-approved OVA1 test may be crucial to achieve the specificity required for early cancer diagnosis, and it is interesting to speculate that members of such panels are likely to have already been identified but not yet implemented”

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Acknowledgements

Markers of apoptosis in breast cancer cells

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Richard Christopherson
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Matthew McKay
Ben Crossett
Mike Lin

Pancreatic cancer diagnosis and survival

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Aiqun Xue
Chris Scarlett
Liping Chung

Glioblastoma response

Hatice Sevim
Kerrie McDonald
Liping Chung

Funding: NHMRC, ARC, Cancer Institute NSW, University of Sydney

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