

Tumor proteomic profiling predicts the resistance of breast cancer to chemotherapy

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USCAP 2009 Abstract #291

Background: Chemotherapy is widely used in breast cancer treatment, but the outcomes vary with some patients responding well and others responding poorly. We hypothesized that the profile of the differentially expressed proteins in tumor tissue may predict individual drug response.

Design: The SELDI-TOF mass spectrometric profiles of tumor tissues obtained from drug resistant and drug sensitive tumors were compared to identify the differences between the two. Fifty-two T2-T4 breast cancer tissues obtained prior to neoadjuvant chemotherapy were analyzed. Of these the first two thirds (35 cases) were allocated to a training set to select m/z peaks characteristic of resistant tumors. The candidate m/z peaks were used to develop a predicting rule to evaluate the remaining 17 specimens in the validation set.

Results: The proteomic peak differences were found most prominent between the drug-resistant breast tumors compared with those with various sensitivity by non-supervised hierarchical clustering. In the supervised classification, the KNN model with K=1 correctly classified 100% of resistant tumors (4/4), and 84.6% of the tumors with favorable response (11/13) with an accuracy rate of 92.3% in the validation set. Furthermore, a single peak at m/z 16,906 correctly separated 88.9% of the tumors with pathologically complete response, and 91.7% of the resistant tumors in the entire group.

Conclusion: The data suggests that breast cancer protein biomarker profiling may be used to pre-select patients for optimal treatment.

Patient information

- Fifty two patients with locally advanced breast cancer (T2-T4) were enrolled. All patients received preoperative chemotherapy as part of an IRB approved phase II clinical trial.
- Tumor specimens were obtained before and after four cycles of Taxotere / Carboplatin / ± Herceptin treatment and protein was extracted from tumor biopsies obtained prior to chemotherapy.
- The cohort was arbitrarily divided into training set (first 2/3=35 patients) and validation set (later 1/3=17 patients).

Experimental methods

- Tissue extraction:** tumor tissues homogenization in liquid nitrogen, repeated freeze and thaw in 1% Triton-X100 and collecting the supernatant by centrifugation.
- Depletion of abundant proteins:** Albumin and IgG were removed by albumin and IgG removal kit (Amersham). Hemoglobin were removed by Ni-NTA magnetic agarose beads (Qiagen).
- Mass spectrometry analysis:** SELDI-TOF with NP20 chips (PBS II, Ciphergen Biosystems, Inc.).
- Statistical analysis:** The original SELDI mass spectrometry data was subjected to baseline subtraction, normalization and peak alignment. A criterion of at least 2-fold expressional difference and statistical significance of $p < 0.05$ by Wilcoxon test was used to select potential markers.
- Bioinformatic analysis:** Unsupervised hierarchical clustering and supervised neural network-based class prediction were performed using a web-based software package, the Gene Expression Profile Analysis Suite (GEPAS v4.0) at <http://gepas.biointo.cipf.es/>.

Results

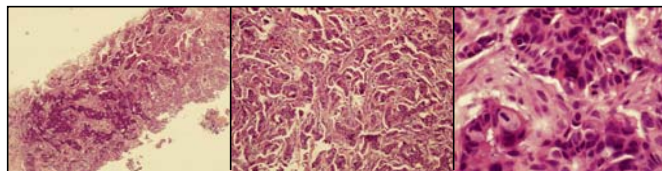


Figure 1. Representative breast cancer tissue biopsy core, H & E staining. (L, x 40; M, x 100; R, x 400)

Table 1. Patient's information

Characteristics	Total patients (%)	Training set (%)	Validation set (%)
Age, years			
≥50	26 (50.0%)	18 (51.4%)	8 (47.1%)
<50	26 (50.0%)	17 (48.6%)	9 (52.9%)
Mean age	49.4	49.3	49.7
Tumor histology			
IDC	42 (80.8%)	27 (77.1%)	15 (88.2%)
ILC	8 (15.4%)	7 (20.0%)	1 (5.9%)
IDC+ILC	1 (1.9%)	0	1 (5.9%)
ITC+IDC	1 (1.9%)	1 (2.9%)	0
Tumor stage			
T2	5 (9.6%)	0	5 (29.4%)
T3	32 (61.5%)	26 (74.3%)	6 (35.3%)
T4	15 (28.8%)	9 (25.7%)	6 (35.3%)
Tumor differentiation			
Poor	13 (25%)	8 (22.9%)	5 (29.4%)
Moderate	7 (13.5%)	6 (17.2%)	1 (5.9%)
Well	6 (11.5%)	3 (8.6%)	3 (17.6%)
Unknown	26 (50%)	18 (51.4%)	8 (47.1%)
Tumor grade			
High	16 (30.8%)	11 (31.4%)	5 (29.4%)
Intermediate	11 (21.2%)	7 (20%)	4 (23.5%)
Low	6 (11.5%)	5 (14.3%)	1 (5.9%)
Unknown	19 (36.5%)	12 (34.3%)	7 (41.2%)
Tumor IHC marker			
ER positive	33 (63.5%)	21 (60.0%)	12 (70.6%)
PR positive	21 (40.4%)	14 (40.0%)	7 (41.2%)
HER2/neu positive	21 (40.4%)	15 (42.9%)	6 (35.3%)
Neoadjuvant chemotherapy			
TC	31 (59.6%)	20 (57.1%)	11 (64.7%)
TCH	10 (19.2%)	8 (22.9%)	2 (11.8%)
TC/H	11 (21.2%)	7 (20.0%)	4 (23.5%)
Ethnicity			
Asian	8 (15.4%)	5 (14.3%)	3 (17.6%)
Black	4 (7.7%)	2 (5.7%)	2 (11.8%)
Hispanic	8 (15.4%)	7 (20.0%)	1 (5.9%)
White	32 (61.5%)	21 (60.0%)	11 (64.7%)
Surgery			
Mastectomy	33 (63.5%)	22 (62.9%)	11 (64.7%)
Breast-conserving treatment	19 (36.5%)	13 (37.1%)	6 (35.3%)

Figure 2. Differential protein expressions in relation to tumor regression rate

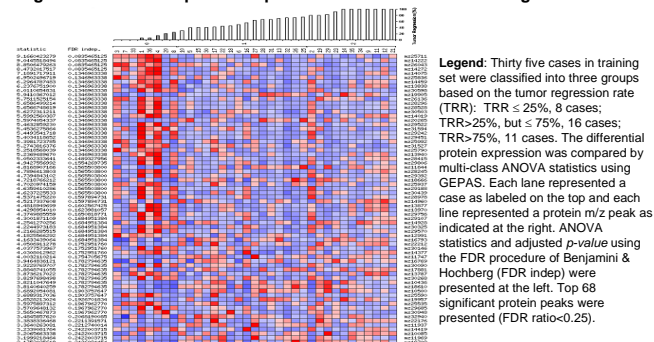


Table 2. Supervised classification of the responses of breast tumors to neoadjuvant chemotherapy in validation set

	Percentage of correctly predicted in NR (%)	Percentage of correctly predicted in ST (%)	Overall accuracy (%)
DLDA	100 (4/4)	0.0 (0/13)	50.0
KNN	100 (4/4)	84.6 (11/13)	92.3
SVM	0 (0/4)	92.3 (12/13)	46.2
PAM	25 (1/4)	84.6 (11/13)	54.8

Legend. The significant protein peaks identified from the training set (n=35) were used to build a prediction rule to classify the chemo-responses in the validation set (n=17). The performances of the four algorithms (DLDA, KNN, SVM, and PAM) were tested and compared.

Summary

- This is the first report of mass spectrometry-based proteomic analysis of breast cancer specimens to predict tumor susceptibility to chemotherapy. The proteomic differences between the groups of drug-sensitive and drug-resistant breast cancers were evaluated by mass spectrometry profiling to predict the tumor response to neoadjuvant chemotherapy.
- Proteomic peak differences were found most prominent between the drug-resistant breast tumors compared with those with various sensitivity by non-supervised hierarchical clustering.
- Fifty-six significant protein m/z peaks were identified from 300 valid protein peaks to develop a predictive model by a supervised neural network-based approach.
- The optimal classification of validation set was achieved by KNN (Nearest Neighbor) model (K=1) which correctly classified 100% of the resistant tumors (4/4) and 84.6% of the responding tumors (11/13), with an accuracy rate of 92.3%.
- Our study suggests that mass spectrometry-based protein profiling of breast cancer tissue samples may play a role in tailored chemotherapy for breast cancer.

Acknowledgement

The study was supported by the funds from NIH (NCI #1R01 CA 093736-01A1), Gonda Foundation, Entertainment Industry Foundation (EIF), and Friends of the Breast Program at UCLA.