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Association of Clinicopathologic Features with Plasma Protein Expression in Invasive Breast Cancer Patients

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BACKGROUND

- Liquid biopsies using blood provide a less invasive method for detecting cancer and assessing treatment responses compared to tissue biopsies.
- The plasma proteome involves secreted proteins from various organs and tissues, making it a prime candidate for extensive biomarker discovery.
- In this study, we used a highly sensitive aptamer-based assay to explore plasma proteins from primary breast cancer (BC) patients.

METHODS

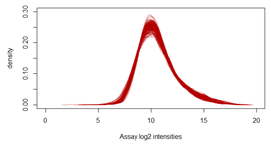
Cohort Selection

- **239** heparinized plasma samples were selected from breast cancer patients at primary diagnosis prior to neoadjuvant chemotherapy and excisional surgery.
- Patients with stage IV, prior DCIS, synchronous breast cancer, or relapse within 6 months were excluded.

SomaScan Assay* (7K)

- 239 samples x 7289 human protein aptamers.
- 2 Batches: Batch correction done using Combat package.
- Median normalization and $\log_2$ transformation.

*Gold, L., et al., Aptamer-Based Multiplexed Proteomic Technology for Biomarker Discovery. PLOS ONE, 2010. 5(12): p. e15004.



Matrix after QC: **239 x 6888**

Association Study

- Generalized linear models (GLM) using each clinical feature (Table 1) and plasma protein expression to estimate effect size.
- Significance: FDR < 0.05 (Fig.1a, 1b), FDR < 0.1 (Fig.1c).

Differential Expression Analysis

- Limma analysis for features significant in association study using matched subsets
  - Sample groups were matched based on age, race, menopausal status, HR status, BMI etc.
- Ingenuity Pathway Analysis (IPA) tool for pathway analysis.
  - Significance: p val ≤ 0.05, |z score| ≥ 2

RESULTS & DISCUSSION

Table 1. Distribution of clinical features

Sample Characteristics [n = 239]		N
Age Group	Young (< 40 years old)	41
	Middle Age (40 – 60)	110
	Old (≥ 60)	88
Race	Caucasian	171
	African American	63
	Other	5
Menopausal Status	Pre-menopausal	96
	Post-menopausal	111
	Surgically menopausal	30
	Unknown	2
Stage	Stage I	76
	Stage II	107
	Stage III	56
IHC Subtype	LA	34
	LB1	63
	LB2	15
	LA/LB1	29
	LA/LB	7
	HER2+	28
	TN	55
	Not Available	8
HR* Status	Positive	157
	Negative	82
HER2 Status	Positive	45
	Negative	175
	Indeterminate	17
	Not Available	2

*Hormone Receptor Status: ER/PR ≥ 1%

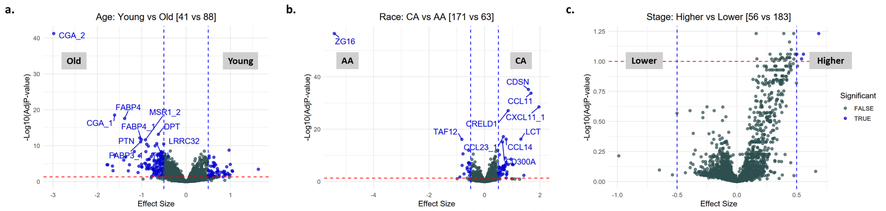


Fig.1 Association analysis between plasma protein expression and (a) Age, (b) Race*, (c) Stage#

*CA: Caucasians, AA: African Americans; #Higher: Stage III, Lower: Stage I/II

Differential protein expression analysis using matched subsets

Age (41 Young vs 41 Old, Adjusted covariate: BMI)

- 424 significant (|$\log_2$ FC| > 0.27, FDR < 0.05) differentially expressed proteins (DEPs).
- Distinct clusters for age observed (Fig. 2a).
- Pathways related to immune response, age (e.g. arthritis), and cancer were upregulated in old (Table 2).

Race (62 African Americans vs 62 Caucasians)

- 175 significant DEPs (|$\log_2$ FC| > 0.27, FDR < 0.05).
- Distinct clusters for race observed (Fig.2b).
- 8 pathways upregulated in Caucasians (Table 3).
- 4 pathways overlapped with age result (given in blue).

Stage (53 Higher vs 53 Lower)

- Higher group included Stage III, lower group included Stage I patients.
- 16 significant DEPs (|FC| > 1.2, p val < 0.05).
- No distinct clusters (Fig.2c).
- No significant pathways identified.
- Further analysis of samples that had relapse (n = 32) vs no relapse (n = 21) within the higher stage group identified 37 possible markers of relapse (|FC| > 1.2, p val < 0.05).

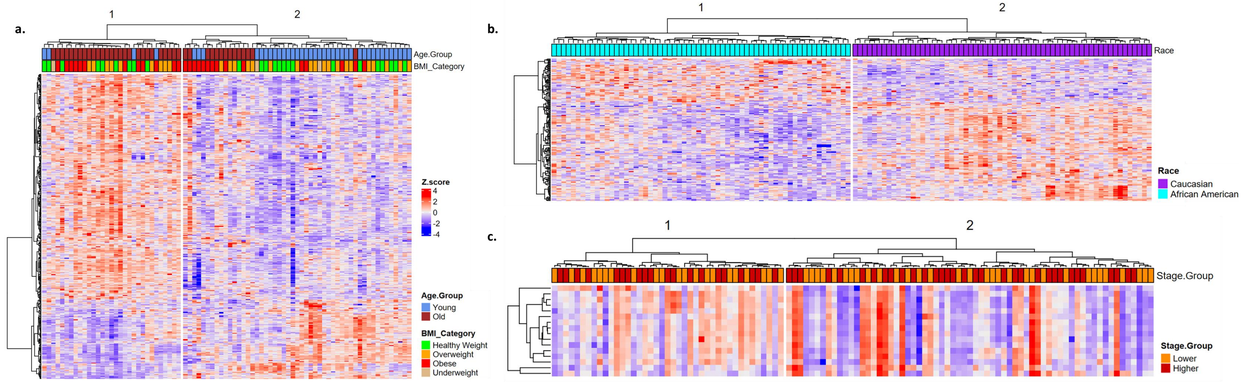


Fig.2 Unsupervised clustering using significant aptamers from limma results for (a) Age Group, (b) Race and (c) Stage.

Table 2. Significant pathways upregulated in Old.

#	Pathway Name	p-value	z-score$
1	Signaling by PDGF	0.013	-2.00
2	Inhibition of ARE-Mediated mRNA Degradation	0.014	-2.00
3	S100 Family Signaling	0.023	-2.07
4	Irritable Bowel Syndrome Signaling	0.006	-2.11
5	Liver Disease Related*	0.026	-2.12
6	Ovarian Cancer Signaling	0.001	-2.24
7	MSP-RON Signaling in Cancer Cells	0.025	-2.24
8	p38 MAPK Signaling	0.046	-2.24
9	Integrin Signaling	0.000	-2.31
10	Post-Translational Modifications (PTMs)	0.000	-2.31
11	Pulmonary Fibrosis Idiopathic Signaling	0.000	-2.32
12	Wnt Signaling	0.001	-2.33
13	Colorectal Cancer Metastasis Signaling	0.011	-2.33
14	SNARE Signaling	0.022	-2.45
15	Cachexia Signaling	0.006	-2.50
16	Collagen Biosynthesis/Degradation	0.000	-2.65
17	Pulmonary Healing Signaling	0.000	-2.71
18	Rheumatoid Arthritis	0.004	-2.71
19	Osteoarthritis	0.000	-2.84
20	GPCR Signaling	0.021	-3.00
21	Immune Response**	0.000	-3.46
22	Synapse Related#	0.004	-3.46

*Included hepatic fibrosis, cholestasis, and NAFLD signaling.

**Included NK, dendritic, lymphoid cell interactions, neutrophil degranulation, wound healing, platelet activation, GP6, interleukin signaling etc.

#Included synaptogenesis, neurexins and neuroligins, and EPH-Ephrin signaling.

$Old as reference.

Table 3. Significant pathways upregulated in Caucasians (CA).

#	Pathway Name	p val	z-score#
1	Immune Response*	0.000	3.30
2	Cardiac Hypertrophy Signaling (Enhanced)	0.025	2.65
3	Human Embryonic Stem Cell Pluripotency	0.004	2.24
4	Pulmonary Fibrosis Idiopathic Signaling	0.001	2.12
5	S100 Family Signaling	0.032	2.12
6	Post-Translational Modifications (PTMs)**	0.003	2.00
7	Glioblastoma Multiforme Signaling	0.026	2.00
8	Regulation of the Epithelial Mesenchymal Transition	0.038	2.00

*Included neutrophil degranulation, lymphoid cell interactions, defensins, inflammation, and complement cascade.

**Synthesis of GPI-anchored proteins

#AA as reference

CONCLUSIONS

- We identified plasma proteins associated with age group, race and tumor stage in breast cancer patients.
- Several cancer related pathways (MSP-RON, ovarian cancer etc.) were upregulated in old compared to young.
- We identified 37 potential relapse markers for higher stage patients, which require validation in a larger cohort.
- These findings suggest that BC related plasma protein expression varies by age and race, a factor to consider in personalized treatment.

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