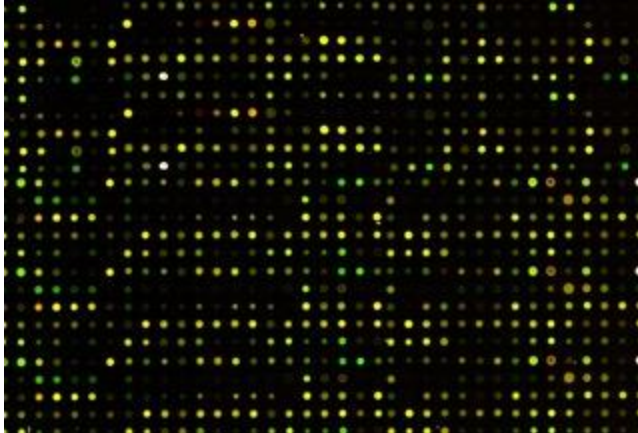# Biomarker candidates for breast cancer found using pre-clinical plasma

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N Zubair

High-throughput antibody array scan.

Image provided by Dr. Jung-hyun Rho

Mammography has become a widely used screening tool for breast cancer. Some studies indicate that routine mammographic screening reduces breast cancer mortality by 30% among women 50-69 years of age; however the magnitude of this mortality benefit remains controversial. Under current guidelines, age and sex remain the sole factors used in determining whether or not a mammogram screening is recommended. This results in millions of healthy women being frequently imaged and biopsied, while other women with cancer falsely identified as cancer-free. More refined methods of breast cancer risk assessment that integrate clinical parameters could tailor screening recommendations; to-date, no clinically useful blood-based biomarkers have been identified for early breast cancer diagnosis. Drs. Matthew Buas, Christopher Li, and colleagues in the Public Health Sciences Division sought to detect candidate biomarkers for estrogen receptor positive/progesterone receptor positive (ER+/PR+) invasive ductal carcinoma, a subtype that accounts for ~45% of invasive breast cancer diagnoses in the U.S. As recently reported in Breast Cancer Research and Treatment, the authors, using pre-clinical plasma specimens with a customized antibody-array, identified a pool of novel candidate protein biomarkers for ER+/PR+ invasive ductal carcinoma; such biomarkers, if validated in independent populations, could be of use in selecting subsets of women for increased mammography screening relative to current guidelines.

To identify novel protein biomarkers for ER+/PR+ invasive ductal carcinoma, the researchers conducted a nested case-control study within the Women's Health Initiative observational study, a

prospective cohort of over 93,000 post-menopausal women in the U.S. For this study, pre-clinical plasma specimens, collected up to 12.5 months before diagnosis from 121 breast cancer cases and 121 matched controls were available; matching covariates included: age, race/ethnicity, body mass index, and hormone replacement therapy use. The researchers divided cases and controls equally into training and testing sets and interrogated their plasma samples using a customized antibody array. Dr. Buas elaborates, "this study made use of an in-house custom-designed antibody array platform developed by Dr. Paul Lampe and colleagues at the Fred Hutch, which enables high-throughput parallel assessment of >2,000 selected plasma proteins. The platform has evolved over the last ~8 years and has been successively refined across multiple generations of array fabrication and development. Several papers have been published describing the platform and its implementation in biomarker discovery studies."

When comparing cases to their matched controls, the researchers found statistically significant mean differences in signal intensity for 37 distinct proteins; these included both known and novel proteins in breast cancer pathogenesis. Four markers remained significant after correcting for multiple testing: CSF2, RYBP, TFRC, ITGB4. The last two of these markers have been the focus of previous breast cancer research. Studies have linked elevated TFRC protein expression in ER+ tumors to higher clinical grade, increased proliferative activity, and worse prognosis. Interestingly, this study observed lower levels of TFRC protein in the pre-clinical plasma of cases relative to controls; it remains uncertain whether reduced levels of circulating TFRC reflect decreased abundance of cellular TFRC, particularly in breast tissue. In contrast to TFRC, the other marker previously reported, ITGB4, was elevated in the plasma of cases relative to controls. This marker has been linked to increased breast tumor size, nuclear grade, and breast cancer progression.

The researchers next determined whether data from The Cancer Genome Atlas (TCGA) might provide further support for any of the markers identified. Of the 37 candidate proteins identified, 14 exhibited significantly altered changes in RNA expression, when comparing breast tumor versus normal tissue.

To assess the utility of combining multiple candidate biomarkers into a composite marker panel, the researchers conducted multivariate modeling. The mean area under the curve (AUC) of the models built using the 39 antibodies was 0.75 with an estimated sensitivity (true positive rate) of ~30% at 95% specificity (true negative rate). According to the authors, a marker panel achieving such a sensitivity and specificity "would have the potential to capture a significant number of additional women who are most likely to benefit from mammography, without flooding the system with those least likely to need imaging."

Breast cancer represents a heterogeneous disease with multiple sub-types, defined by distinct histological and molecular features. Dr. Buas describes, "by analyzing plasma isolated from women who later went on to develop breast cancer (or did not), we could identify proteins that differed in abundance prior to breast cancer diagnosis. These marker candidates require external validation in independent study populations, but may hold potential clinical value down the road, for applications related to early detection or risk stratification."

Additional Fred Hutch investigators contributing to this project were Dr. Jung-hyun Rho, Xiaoyu Chai, Yuzheng Zhang, and Dr. Paul D. Lampe.

Citation:
Buas MF, Rho J-H, Chai X, Zhang Y, Lampe PD, Li CI. 2015. Candidate early detection protein biomarkers for ER+/PR+ invasive ductal breast carcinoma identified using pre-clinical plasma from the WHI observational study. *Breast Cancer Res Treat*. 153(2):445-54.