Abstract

Time of diagnosis of breast cancer is the single most important factor determining disease outcome. Moreover, breast cancer is a molecularly heterogeneous and the different BC subtypes notably include ER+, HER2- and triple negative (TN) types that are treated as distinct diseases with the two latter being rarer, but more aggressive. A blood-based early diagnostic for cancer is desirable, but will need to address • breast cancer heterogeneity and • measure biomarker proteins at very low concentration to identify tumors as they are still small

We established the antibody colocalization microarray (ACM) that can measure 108 proteins with an LOD as good as 0.6 pg/mL (=35 fm). Here, using the ACM, we profiled the blood of controls and breast patients to identify candidate biomarkers that are differentially expressed either in all subtypes of breast cancers, or specific to one subtype. We hope that this study will lead to a more specific diagnosis of this heterogeneous disease in the future.

Introduction

Breast cancer is a disease that still claims many lives, in part because of the lack of early detection of cancerous lesions in spite of the vast screening programs using mammography. There is a pressing need for a simple, sensitive, non-invasive blood test that can successfully diagnose or help diagnose breast cancers at early stages in women.

Breast cancer is a heterogeneous disease that is currently characterized by the clinical measurement of 3 molecular markers from biopsy samples histology: ER, PR, and HER2 overexpression. Different subtypes of breast cancers are treated differently by oncologists based on their molecular profiles, and therefore it also makes sense to consider the different subtypes as separate, more homogeneous cohorts when comparing to blood samples from healthy women or women who have benign lesions that don’t require surgery.

Because breast cancer is heterogeneous, we evaluated 108 potential biomarkers that are differentially expressed between the different subtypes of breast cancers. By considering the four subtypes as separate, more homogeneous cohorts, the markers found are likely to be more sensitive and more accurate in the diagnosis of breast cancer and its subtype.

Experimental

Two hundred clinical samples were obtained from consenting patients at the Royal Victoria and the Montreal Jewish Hospitals in Montreal, Quebec, Canada. The study was approved by the local ethics committees.

Roughly equal numbers of samples from women who are normal (P), diagnosed with benign lesions (30) or diagnosed with one of four subtypes of breast cancer (ER+/HER2- [34], ER+/HER2+ [34], ER-/HER2- [34], ER-/HER2- also known as triple negative (TN) [31]), were measured in two dilutions using the ACM with 108 protein targets.

Statistical and bioinformatics analyses were performed using Microsoft Excel, GraphPad PRISM and R/Bioconductor.

Results and discussion

Assay Performance

Figure 1. Two hundred patients samples were measured with the ACM and separated in 6 different categories. Normal controls include normal and benign lesions. The other four categories are based on the molecular markers for estrogen receptor (ER) and HER2.

Assay sensitivity is excellent

• Sensitivity is excellent for low-abundance proteins, reaching 0.6 pg/mL for IL-1β (35 femtomolar)
• Reproducibility is acceptable but can be improved by the use of a spotting calibrant

Figure 4. Performance of the ACM platform for this assay in terms of (A) limit of detection (LOD), calculated with the mean+3SD of all blanks, and the (B) coefficient of variation (CV) of multiple replicates of a pooled normal sample.

Table 1. List of protein targets of interest based identified in the different comparison of cohorts

Comparison of different subtypes

7 targets are different when all subtypes are considered together

• These seven low-abundance proteins show p-values < 0.05 and false positive rates < 0.2 which implies a significant difference when compared against controls

Comparison of different sample cohorts

• No difference was found between normal women and women with benign lesions, therefore the two were combined
• Up to five targets with a p-value of < 0.05 and false positive rate of < 0.3 were identified when individual subtypes were compared to normals/benigns
• Comparing different subtypes show that 3 markers are differentially expressed with HER2+ breast cancers

Comparison of different cohorts leads to different markers

• Different markers can be identified when individual cohorts are compared to normal controls or among themselves

Potential markers of one or multiple subtype

• One marker was found to be common between all four subtypes: IL-8
• Five markers appear to distinguish the HER2 marker status specifically
• Nine markers appear to be specific for only one subtype of breast cancer

Conclusion

• Changes in low-abundance proteins can be detected in women who have breast cancer
• The four different breast cancer cohorts based on the ER and HER2 molecular markers show different profiles when compared to healthy women
• This study serves to identify targets of interest that can be further studied and validated in a blood test that could distinguish molecular subtypes in the diagnosis of breast cancer, using more patient samples

References


Acknowledgements

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