Isolation and detection of breast cancer cells in blood samples using antibody cocktails selected by large-scale genome-wide screening

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BACKGROUND

Metastatic breast cancer is the second leading cause of cancer death for women. The development of a new blood test for breast cancer screening could offer significant improvements with the potential to be more accurate, less costly, less intimidating, not subject to age differences, and able to discriminate between cancers subtypes.

OBJECTIVE

Develop an antibody cocktaill/genetic signature with specificity and sensitivity that outperforms the current EpCAM enrichment method and provides a proof of feasibility for screening of breast cancer (BC).

METHODS

Discovery: To identify the targets we first used gene expression microarrays to select genes whose mRNA was specifically expressed in breast tumor cells but not blood cells. Affymetrix HG-U133 plus 2.0 microarrays were used to compare breast tumor tissue biopsies and breast cancer cell lines with blood samples from patients with conditions other than cancer. A total of 924 samples were used in this comparison, including 417 breast tumor tissue biopsies (Hess 2006, Foekens, 2006), 41 breast cancer cell lines (Elstrodt 2006, Fournier 2006), and 401 blood samples (GSE19314) (**Figure 1**).

External Validation: We tested the expression these genes selected in 8 tumor types (breast, colon, lung, ovarian, prostate, pancreatic, gastric cancers, and leukemia) (**Table 1**), comparing blood from healthy individuals (N=162), totaling 3,725 samples in 17 datasets.

RESULTS

Expression in independent samples: Expression of the BIOARRAY 80 were studied in an independent set of breast tumor samples not used for the original selection procedure and normalized to an independent set of controls (GSE19314). 76 of 80 genes (95%) were expressed at least 20 fold greater in at least one breast tumor biopsy (**Figure 2**).

Expression in paired tumor and blood from breast cancer patients: Expression of BIOARRAY 80 was studied in paired tumor and blood samples from 3 patients with breast cancer. Expression was normalized to blood samples from 48 individuals with conditions other than breast cancer (**Figure 3**).

Hierarchical clustering using the BIOARRAY 80 accurately classified all 3 breast cancer patient blood samples into a separate cluster from 48 control blood samples (**Figure 3).**

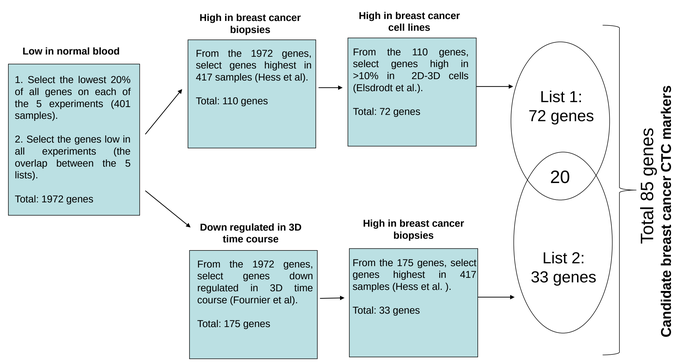

Figure 1. Scheme for the discovery of 85 candidate genes for detecting circulating tumor cells

Table 1. Database used to validate the expression of 80 genes

Tissue	Accession	N	Tissue	Accession	N
Breast (BR1)	GSE25055	310	Lung (LU1)	GSE19188	156
Breast (BR2)	E-TABM-157	51	Lung (LU2)	GSE30219	307
Colon (CO1)	GSE39582	585	Ovarian (OV1)	GSE26712	192
Colon (CO2)	GSE68468	366	Ovarian (OV2)	GSE9891	285
Gastric (GA1)	GSE13911	69	Pancreatic (PA1)	GSE15471	78
Gastric (GA2)	GSE54129	132	Pancreatic (PA2)	GSE16515	52
Leukemia (LK1)	GSE13159	568	Prostate (PR1)	GSE17951	154
Leukemia (LK2)	GSE14471	110	Prostate (PR2)	GSE8218	148

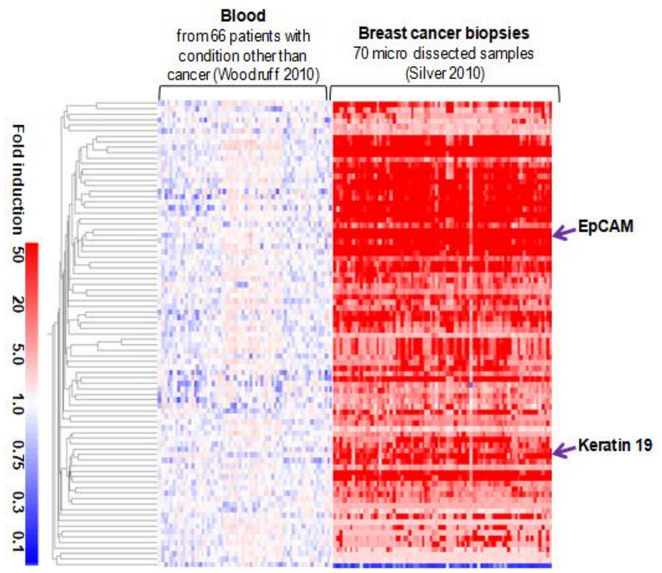

Figure 2. Confirmation of tumor specificity of Bioarray 80 genes in samples not used for gene selection. Expression levels of micro dissected tumor biopsies were normalized to the median of the 66 blood samples.

Prioritization of 20 antibody-target candidates: Of the BIOARRAY 80, 20 genes have a membrane localized product. The expression in micro dissected biopsies was markedly higher than non-breast cancer blood for most (**Figure 4**). Also, most were expressed at lower levels than EpCAM in normal breast tissue and had low expression profiles in isolated normal blood cells.

17 datasets: The studies were batch-corrected by making the average of 22K genes equal in each data set (**Figure 5**). After comparing blood expression to the 8 cancer types, 63 of the 80 genes showed their lowest expression in blood (**Figure 6**). Some genes showed high expression in specific tissues (**Table 2**).

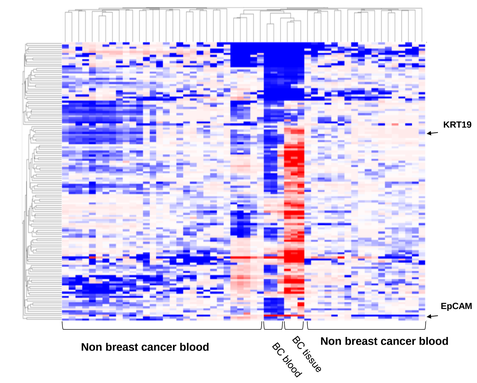

Figure 3. Expression of BIOARRAY 80 in 3 breast cancer patients. Expression levels are shown in paired blood and tumor samples obtained from 3 breast cancer patients normalized to the median of 48 blood samples obtained from individuals with conditions other than cancer (Fury 2009). Hierarchical cluster analysis in two dimensions was used to organize samples. Some probe set duplicates are included. Same expression key as Figure 2.

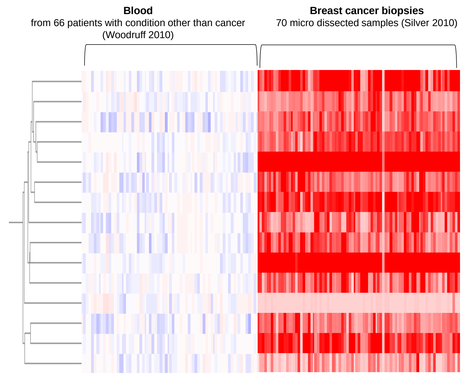

Figure 4. mRNA expression of 20 antibody-target candidates in non-breast cancer blood compared with breast tumor tissue. Color scale same as Figure 2.

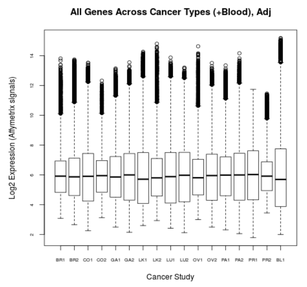

Figure 5. The studies were batch-corrected by making the average of 22K genes equal in each data set

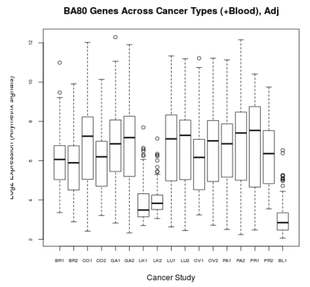

Figure 6. Expression profile of 80 genes. Blood and leukemia are the low boxplots.

Table 2. Genes that showed high expression in specific tissue

Tissue	Genes	High
Breast	4	S100A7, SCGB2A2, PRLR, TFAP2B
Pancreatic	2	SRPX and CTGF
Lung	1	GJA1
Ovarian	2	ESR1 and OBSL1
Gastric	2	TOM1L1, AGR2
Prostate	7	ALDH1A3, FZD7, GHR, GREB1, AZGP1, CYP4F8, TBX3

CONCLUSIONS AND FUTURE DIRECTIONS

We identified 20 potential markers for isolation of circulating breast tumor cells. Expression in micro dissected biopsies was markedly higher than non-breast cancer blood for most. Also, most were expressed at lower levels than EpCAM in normal breast tissue and had background level expression in isolated normal blood cells. The validation of antibodies in breast cancer cells is being performed. 10-20 antibodies specific for BC surface molecules are being assessed individually and in a cocktail. Cocktails will be ranked for their ability to detect a range of different breast cancer cells.

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