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Genomic profiling in locally advanced and inflammatory breast cancer and its link to DCE-MRI and overall survival

Sharareh Siamakpour-Reihani¹, Kouros Owzar^{2,3}, Chen Jiang³, Peter M. Scarbrough⁴, Oana I. Craciunescu¹, Janet K. Horton¹, Holly K. Dressman⁵, Kimberly L. Blackwell^{1,6}, and Mark W. Dewhirst¹

¹Department of Radiation Oncology, Duke University Medical Center, Durham, North Carolina

²Department of Biostatistics & Bioinformatics, Duke University Medical Center, Durham, North Carolina

³Bioinformatics Shared Resource, Duke Cancer Institute

⁴Duke Cancer Institute, Duke University Medical Center, Durham, NC

⁵Department of Molecular Genetics and Microbiology and Duke Center for Genomic and Computational Biology, Duke University Medical Center, Durham, NC

⁶Department of Medicine, Division of Medical Oncology, Duke University Medical Center, Durham, NC, USA

Abstract

Purpose—We have previously reported that DCE-MRI perfusion patterns, obtained from LABC patients prior to neoadjuvant therapy, predicted pathologic clinical response. Genomic analyses were also independently conducted on the same patient population. This retrospective study was performed to test two hypotheses: i) gene expression profiles are associated with DCE-MRI perfusion patterns; ii) association between long term overall survival data and gene expression profiles can lead to identification of novel predictive biomarkers.

Methods—We utilized RNA microarray and DCE-MRI data from 47 LABC patients, including 13 IBC patients. Association between gene expression profile and DCE-MRI perfusion patterns (centrifugal and centripetal) was determined by Wilcoxon rank sum test. Association between gene expression level and survival was assessed using a Cox rank score test. Additional genomic analysis of the IBC subset, with up to an 11-year period of follow-up, was conducted. Associations between gene expression and overall survival were further assessed in TCGA database.

Results—Differences in gene expression profiles were seen between centrifugal and centripetal perfusion patterns in the: sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1 and 2 (SULT1A1, SULT1A2), poly (ADP-ribose) polymerase, member 6 (PARP6), and metastasis tumor antigen1 (MTA1). In the IBC subset, our analyses demonstrated that differential expression of 45 genes was associated with long term survival.

Conclusions—Here we have demonstrated an association between DCE-MRI perfusion patterns and gene expression profiles. In addition we have reported on candidate prognostic biomarkers in IBC patients, with some of the genes being significantly associated with survival in IBC and LABC.

Keywords

Breast Cancer; Inflammatory Breast Cancer; Locally Advanced Breast Cancer; Survival; DCE-MRI; Genomic; Imaging; Perfusion Patterns

Introduction

Locally advanced breast cancer (LABC) and inflammatory breast cancer (IBC) have poor 5-year survival rates (55% and 33%, respectively) when compared to early-stage breast cancer (80%). (1–3) The purpose of neoadjuvant chemotherapy is to improve the treatment outcome of the breast cancer patients by down staging the tumor. Effective downstaging of the tumor before surgery will provide for more conservative surgery and potentially converting inoperable tumors to an operable state. (4, 5) In breast cancer patients who receive neoadjuvant chemotherapy, 20–30% will achieve pathologic clinical response (pCR). It has been shown that patients who achieve a pCR have a better prognosis. However, not all patients respond to neoadjuvant treatment, and thus there is a clear need for biomarkers that accurately predict response to treatment. (5)

Between 2000 and 2004, 47 LABC patients were enrolled in an IRB-approved phase I/II clinical trial at Duke University Medical Center (Durham, NC). (6) The patients enrolled in this clinical trial received a neoadjuvant combination of breast hyperthermia, liposomal doxorubicin (Evacet™), and paclitaxel. Hyperthermia was administered within one hour of completing chemotherapy infusion. The hyperthermia prescription was to achieve 41–41.5 °C in greater than 90% of measured points for 60–70 minutes. The neoadjuvant treatment was followed by surgery, radiation therapy and adjuvant chemotherapy. Safety, tolerability and pathological response of this combination treatment has been reported previously. (4, 6)

RNA from pre-treatment ultrasound guided core biopsies were assayed using Affymetrix GeneChip® Human Genome U133 Plus 2.0 arrays. Of the 47 patients enrolled in the clinical trial, a 20-patient subgroup in the phase II portion of the trial, received the same paclitaxel and liposomal drug dose. Pre-treatment DCE-MRI (dynamic contrast-enhanced magnetic resonance imaging) studies were performed on this subset. The DCE-MRI enhancement features were analyzed for association with treatment response. (7) DCE-MRI patterns can vary between tumors, based on factors such as the size of the extra cellular space and vascularity of the tumor. Thus, the results can be indicative of angiogenesis, perfusion and/or hypoxic conditions in the tissue, all of which play vital roles in tumor development, progression and sensitivity to treatment. (7–11) In this patient dataset, we observed two distinct patterns of image enhancement: i) centripetal (**CP**), an inhomogeneous ring-type enhancement and ii) centrifugal (**CF**), characterized by more homogenous signal enhancement from center to periphery (Figure 1). (7, 9, 10)

In this current study, we tested two hypotheses: **i)** gene expression profiles are associated with the DCE-MRI perfusion patterns; **ii)** association between long term overall survival (OS) data and gene expression profiles can lead to identification of novel predictive biomarkers.

In order to test our hypotheses, we analyzed potential linkages between gene expression profiles and DCE-MRI parameters in LABC patients. In addition, we analyzed associations between long term OS and gene expression profiles in the IBC subgroup. Our OS analysis results were further validated using “The Cancer Genome Atlas” (TCGA) database.

Materials and Methods

Study population

Institutional Review Board (IRB) approval was obtained for this retrospective study (Protocol # Pro00028021). The Duke Health System Institutional Review Board for Clinical Investigations (DUHS IRB) has determined the specific components above to be in compliance with all applicable Health Insurance Portability and Accountability Act (“HIPAA”) regulations. In addition, DUHS IRB has approved and provided us with a “*Waiver of Consent and HIPAA Authorization*”. We were not required to register this study on clinicaltrials.gov, because retrospective studies do not qualify as ACT (Applicable Clinical trial) studies.

The original selection of the study population has been described in detail elsewhere including the patients’ treatment, clinical characteristics, and demographic data. (4, 6) Briefly, between 2000 and 2004, 47 LABC patients (Stage IIB and III) were enrolled on an IRB-approved phase I/II clinical trial at Duke University Medical Center (Durham, NC, Protocol # Pro1884-99-10). Patients were treated with neoadjuvant liposomal doxorubicin and paclitaxel in combination with breast hyperthermia, every three weeks for four cycles. The median patients’ age was 50 years (range 27–75 years). From the total of 47 patients enrolled in the clinical trial, pretreatment biopsies, collected under ultrasound guidance, were available from 37 patients, including 13 with a diagnosis of IBC. All 37 pretreatment biopsies were confirmed to have at least 60% invasive disease throughout the core sample before RNA harvesting. RNA was prepared, probe generated, and hybridized to the Affymetrix GeneChip® Human Genome U133 Plus 2.0 array. (4, 6) For the current analysis, our study population had long term surveillance of 11 years (median=8.4 years; range 0.3 – 11 years). All genomic data and samples were linked to clinical data.

DCE-MRI

The image analysis protocol has previously been described in detail for characterization of the CP and CF perfusion patterns. (7, 12) A 20-patient subgroup in the phase II portion of the trial was chosen for the DCE_MRI analysis from the 47 patients. These 20 patients were chosen because their breast DCE-MRI data was attained using the same protocol, and because all received the same dosage of neoadjuvant chemotherapy and hyperthermia. The DCE-MRI was done over 30 minutes following bolus injection of gadopentetate-based

contrast agent (gadopentetate dimeglumine (Gd-DTPA). The contrast agent was administered through an indwelling IV catheter with a $2\text{cm}^3/\text{sec}$ rate. (7)

Data Analysis & Statistical Considerations

The genomic data discussed in this paper has been deposited in the NCBI's Gene Expression Omnibus (GEO, <http://www.ncbi.nlm.nih.gov/geo/>). (13) The deposited data are accessible through the GEO Series accession number GSE52322; "Novel linkages between DCE-MRI and genomic profiling in locally advanced and inflammatory breast cancer". (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE52322>)

A) Analysis of association between gene expression profiles and DCE-MRI perfusion patterns—LABC samples were assayed using the Affymetrix GeneChip® Human Genome U133 Plus 2.0 array, which interrogates mRNA expression, based on 54,675 probe sets. All statistical analyses were conducted using the R statistical environment. (14, 15) Pre-processing was conducted on the basis of all available samples from the LABC study deemed to be of good quality, regardless of the availability of the imaging phenotype. (16) The arrays were pre-processed using the RMA algorithm. (17) Ordination methods (e.g., multi-dimensional scaling) were used to identify experimental artifacts, such as batch effects. (18) From the 20 patients subgroup who had the DCE-MRI, 15 had both imaging and genomic data available. Association between the gene expression profile and the DCE-MRI CP versus CF morphology was determined by the Wilcoxon rank sum test to prioritize the features (probesets) according to statistical significance. (16, 19)

B) Analysis of gene expression profiles and patient survival—Association between feature expression level and survival was assessed using a Cox rank score test. (16) Corresponding effect size was quantitatively assessed using a hazard ratio assuming proportional hazards. Conditional inference trees were used to find optimal cutpoints. (20) As the feature selection process was considered to be hypothesis generating, the P-values reported have not been adjusted for multiple testing. The associations between expression level and OS for the implicated genes were further assessed in a published cohort from TCGA (<https://tcga-data.nci.nih.gov/tcga/>) using the Breast Invasive Carcinoma project dataset. (21) The TCGA mRNA data were retrieved from the Cancer Genomic Data Server (CGDS) through the Computational Biology Center Portal (cBio): <http://www.cbioportal.org/>. The `cdgsr` extension package (cran.rproject.org/web/packages/cdgsr/) was used to execute the retrieval.

Results

A) Association between gene expression profiles and DCE-MRI perfusion patterns—Of the fifteen patients with both genomic and imaging information, ten patients had the CP perfusion pattern while five had the CF pattern. (Figure 1) Following data analysis on RNA microarray results we identified genes that were differentially expressed in the CP vs CF. (Figure 2) The CP pattern was found to have higher expression of genes involved in a component of sulfur metabolism pathway. In particular, genes included those

in the sulfotransferase family: i) sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1 (SULT1A1), and ii) sulfotransferase family, cytosolic, 1A, phenol-preferring, member 2 (SULT1A2). (Figure 3-A and 3-B) In addition, CP had similarly higher RNA expression levels of poly (ADP-ribose) polymerase, member 6 (PARP6). (Figure 3-C) The metastasis tumor antigen 1 (MTA1) was also differentially expressed in the CP vs. CF morphology. However in contrast to the previous three genes, MTA1 was overexpressed in CF when compared to CP (Figure 3-D). Even though MTA functions as an estrogen receptor 1 (ESR1) co-repressor, we did not find a positive association between the CP and CF enhancement patterns and levels of ESR1 expression in our dataset. Only one of the nine probe sets representing ESR1 demonstrated a positive association. (ESR1:205225_at; p-value=0.028; with lower ESR1 expression in the CF cases compared to CP)

B) Association between long term overall survival (OS) data and gene expression profiles—Our follow up time for the patients in this trial was 11 years and the median follow up time was 8.4 years. We analyzed the association between the genomic expression data and OS in the subgroup of 13 IBC patients. *We limited the sample analysis to the IBC cases because they provided the largest subgroup out of the total 47 that had a uniform tumor type.* In our survival analysis overexpression of 45 genes were found to be associated with OS in IBC patients (p-value <0.001). The genes identified were involved in the sulfur metabolism pathway and the focal adhesion kinase (FAK) pathway. (Table 1 and supplemental data) Overexpression of two genes involved in the sulfur metabolism pathway was associated with shorter OS, these included carbohydrate (chondroitin 4) sulfotransferase 11 (CHST11, p-value= 7.88E-05, HR= 2.4) and carbohydrate (chondroitin 6) sulfotransferase 3 (CHST3, p-value= 5.84E-04, HR= 1.5). In addition, over expression of four members of the FAK pathway were significantly associated with shorter OS in IBC patients. These included: collagen, type IV, alpha 4 (COL4A4, p-value= 7.82 E-04, HR= 1.6), protein kinase C, alpha (PRKCA, p-value= 6.56 E-04, HR= 1.6), talin 2 (TLN2, p-value= 5.25E-04, HR= 1.6) and tenascin C (TNC p-value= 9.01E-04, HR= 1.7). In our OS analysis we also noticed that our list included two of the genes associated tumor invasiveness and treatment resistance: i) hypoxia up-regulated protein 1 (HYOU1, p-value= 2.00E-04, HR=1.7), and ii) apoptosis caspase activation inhibitor (AVEN, p-value= 1.03E-03, HR=1.5).

We used the publically available TCGA database for external confirmation of our survival analysis results. Currently TCGA does not provide access to imaging data to be used for CP vs. CF classification; therefore we were not able to use the TCGA database for confirmation of linking DCE-MRI and gene expression profiles. We analyzed the survival association between the expression level of the 45 genes of interest in the TCGA's LABC group with a sample size of N=462 (we did not have the data available from TCGA to specifically select the IBC cases from the main LABC group). In TCGA's LABC cohort, differential expression of four genes from our gene list exhibited the same significant concordance and association with overall survival as in IBC patients. The genes included **i)** actin related protein 2/3 complex, subunit 5-like (ARPC5L, Duke p-value = 4.42E-04, HR=1.8, TCGA p-value = 1.20E-03, HR =2.3); **ii)** regulator of G protein signaling 16 (RGS16, Duke p-value = 6.26E-04, HR= 1.6 TCGA p-value= 1.58E-03, HR =2.8); **iii)** cyclin-dependent kinase-like 2

(CDKL2, Duke p-value = 1.50E-03, HR=1.5, TCGA p-value= 9.66E-03, HR =2.0); iv) Cysteine protease cathepsin B (CTSB) (, Duke p-value = 4.63E-04, HR=1.6, TCGA p-value= 1.95E-02 HR =2.1). (Figure 4 and 5; Table 1)

Discussion

A) Association between gene expression profiles and DCE-MRI perfusion patterns—Our group previously reported that DCE-MRI parameters are linked to treatment outcome in canine and human cancers. (7, 22) We reported that DCE-MRI parameters were predictive of overall and metastasis-free survival in 37 dogs with soft tissue sarcomas that were treated with thermoradiotherapy. This was the first study to report a linkage between DCE-MRI parameters in sarcomas and long-term outcome. (22) In humans, we previously reported that DCE-MRI perfusion patterns, obtained from LABC patients prior to neoadjuvant and hyperthermia therapy were predictive of pCR. (7)

Here we have analyzed links in gene expression profiles and DCE-MRI parameters in LABC patients. Our results indicate that the two DCE-MRI enhancement patterns of CF and CP have distinct gene expression profiles. Out of the top 200 probesets associated with 144 genes, which were differentially expressed in CF vs. CP (p-value < 0.008), 80% of the probes had higher expression levels in the CP enhancement pattern and only 20% had higher expression levels in CF.

CP was found to have higher expression in genes involved in a component of sulfur metabolism pathway. Cytosolic sulfotransferase (SULTs) function as catalysts for biotransformation of steroid hormones, drugs and environmental toxins. Table 2 provides some background information and references for SULTs. Through sulfoconjugation, SULTs play a key role in the metabolism and inactivation of estrogen. It is therefore theorized that overexpression of the SULTs genes in CP morphologies could decrease the levels of unconjugated estrogens (the active form of estrogen) in breast cancer tissue and consequently block the proliferative effect of the hormone. In addition estrogen directly affects tumor microenvironment. Estrogen has direct effect on endothelial cells resulting in endothelial cell migration, proliferation and modulating angiogenesis. (23) This suggests that patients with the CP enhancement pattern with higher expressions of *SULTs* could have lower levels of active estrogen. This observation potentially draws an important connection between genomics, imaging, and therapy, and thus merits further investigation.

Here we have reported that the CP morphology is also associated with higher expression levels of *PARP6*. (Table 2) Recently PARP inhibitors have been used in clinical trials and suggested as a favorable target in breast cancer therapy. (24–27) Our findings suggest that an enhancement pattern of CP might benefit more from PARP inhibitor treatment. Recent studies in animal models of endometrioid carcinoma, the major subtype of human endometrial cancers, have demonstrated that high estrogen levels interfered with the PARP inhibitor AZD2281 (olaparib). In lower serum estrogen levels, the PARP inhibitors resulted in more reduction in tumor size. (28) In addition, as mentioned previously the higher levels of *SULTs* in the CP tumors could result in inactivation of estrogen. The inactivation of

estrogen potentially could also make tumors with CP enhancement a better target for PARP inhibitor treatment, and affect the patients' pCR and prognosis.

Our imaging and gene expression analysis also demonstrated that *MTA1* was overexpressed in CF tumors compare to CP. The metastasis tumor antigen (MTA) family of proteins (MTA1, MTA2, and MTA3) are transcriptional co-repressors. MTA1 is overexpressed in a wide range of human cancers, such as gastrointestinal, ovarian, prostate, and breast cancers (29–33). MTA1 is considered a potential predictor for breast tumor aggressiveness (Table 2). We noted that MTA1 was overexpressed in CF tumors, which were characterized to have a more homogenized vascular network, and thus a better potential for drug delivery. The improved drug delivery characteristics could potentially be counteracted by increased aggressiveness in tumors with CF phenotypes which at the end could affect pCR rate.

B) Association between overall survival data and gene expression profiles

—In the second part of our study we focused on the association between gene expression profiles and long term survival of breast cancer patients. The long follow up time of 11 years and availability of clinical data provided the unique opportunity to analyze the association between gene expression profiles and long term OS. Limiting our analysis to the IBC cases provided the largest subgroup out of the total 47 patients with a more uniform tumor type. IBC is a rare subtype of LABC, and the most aggressive form of breast cancer. IBC survival rates are relatively poor, averaging 35% to 40%. IBC has no known etiology and has no proven effective therapy. In spite of aggressive multimodality therapies over the past three decades, there has been relatively little improvement in the overall survival rate of IBC. (2, 3, 34)

Here we were able identify candidate gene expression profiles that defined signaling pathways associated with survival in IBC patients. For the Duke IBC patient subgroup, overexpression of 45 genes was associated with poor survival; this included genes involved in the sulfur metabolism pathway and the FAK pathway. The expression patterns reflect themes common to the aggressive biology of breast cancer (e.g. metastasis, tumor invasiveness, cell migration). (Table 2)

Our results demonstrated that overexpression of *CHST11* and *CHST3*, genes involved in the sulfur metabolism pathway, was associated with shorter OS in IBC patients. Chondroitin sulfates are involved in breast tumor aggressiveness and metastasis and *CHST11* and *CHST3* overexpression have been reported to be associated with more aggressive breast tumors. (35) This is in concordance with our findings since the patients with higher expression levels of *CHST11* and *CHST3*, had shorter OS (HR of 2.4 and 1.5 respectively).

Activation of FAK signaling pathway in tumor cells, including breast tumors, can affect cellular processes such as metastasis, cell spreading, adhesion, proliferation, angiogenesis, progression and tumor survival. The FAK pathway can be a potential cancer therapeutic target to inhibit tumor invasion and progression. (36, 37) Our results demonstrated that in IBC patients, over expression of four FAK pathway genes: i) *COL4A4*, ii) *PRKCA*, iii) *TLN2*, and iv) *TNC* were significantly associated with shorter OS.

When we analyzed the association between survival and the expression level of the 45 genes of interest in the TCGA's LABC cases (N=462), 4 genes had common levels of significance and concordant direction for LABC patients. Those 4 common genes between IBC and LABC are: **i) ARPC5L, ii) RGS16, iii) CDKL2, and iv) CTSB.**

The TCGA analysis provides an external confirmation for the importance of these 4 genes in LABC. *These 4 genes have been implicated in other studies for their roles in:* tumor invasion and metastasis (ARPC5L and TNC); (38, 39) Infiltrative tumor border pattern and histologic high grade breast carcinomas (CDKL2); (40) potential prognosis biomarker associated with poor survival and increased number of positive metastatic lymph nodes in IBC patients (*CTSB*); (41) and potential therapeutic targets in cancer therapy (*RGS16*). (42, 43)

Conclusion

In conclusion, although these findings are preliminary we feel these results are especially important and merit further investigation and validation in independent datasets with a larger sample size and more homogenous tumor types. The major limitation of our study is the small sample size and lack of access to tumor samples from the patients enrolled in the original phase I/II clinical trial to perform additional validation experiments such as qPCR and western blot analysis. However, our preliminary results have shown unique links to changes in DCE-MRI and gene expression profiles. Here we reported on the differential genes expression profile between the CP and CF enhancement patterns. In addition we have reported on candidate prognostic biomarkers in IBC patients, with some of the genes being significantly associated with OS in IBC and LABC. While not all these genes have previously been reported to be associated with prognosis in IBC or LABC, they are consistent with other studies which associate these genes and gene products with aggressiveness and poor outcomes in other cancers.

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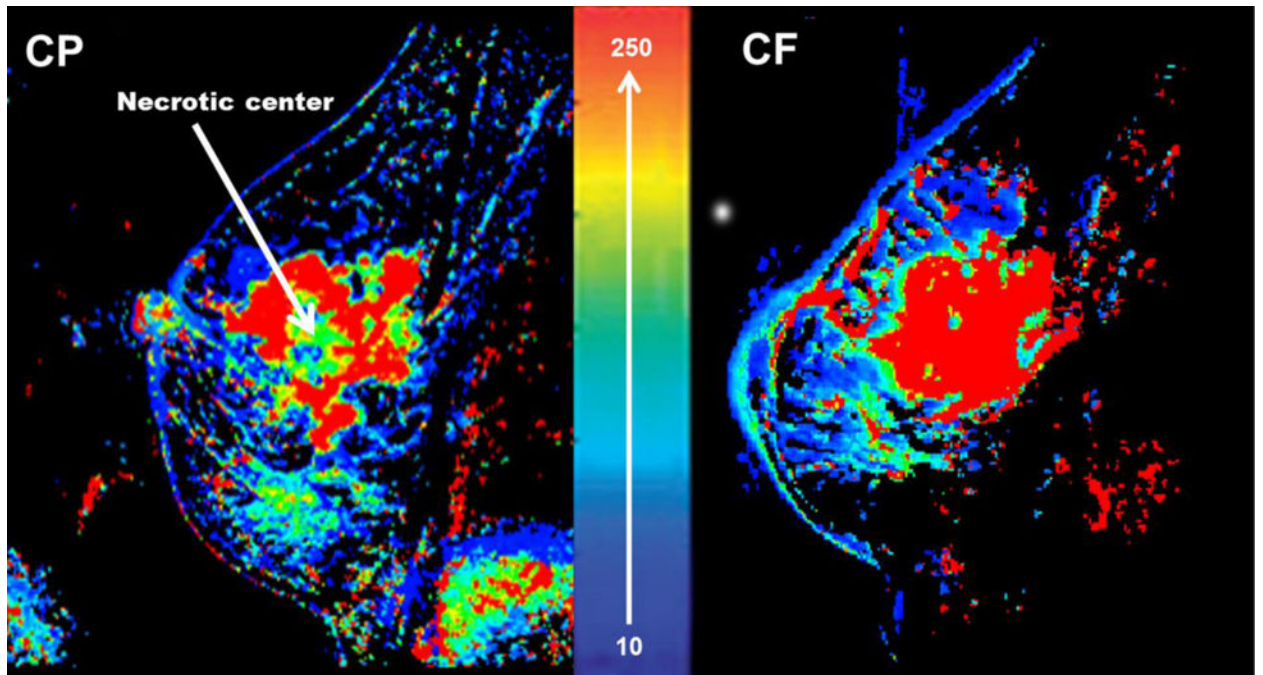


Figure 1. Wash in Parameter maps generated from DCE-MRI in LABC patients

Wash in Parameter (WiP) maps are surrogates for perfusion/permeability. (7) The right side image shows the CF enhancement pattern which is a uniform enhancement from center to periphery. The left side image is the CP enhancement pattern which is an inhomogeneous ring-type enhancement. The 10–250 scale represents the range of the WiP as previously described in detail. (7)

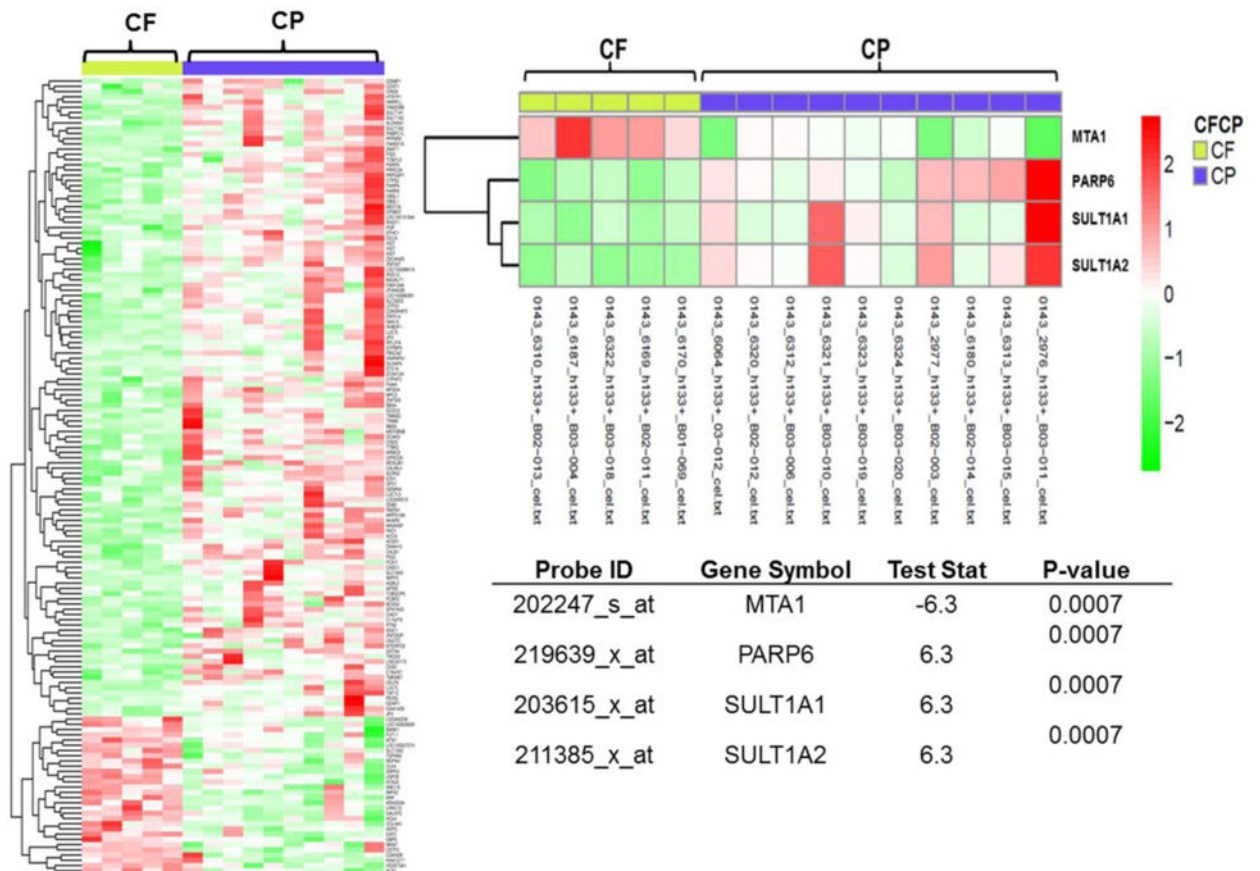


Figure 2. Heatmap cluster plot of Affymetrix microarray data in LABC samples

This heatmap plot records post-normalization between-probes distances measured by their absolute median difference. The left diagram represents all the top probesets associated with 144 genes, which were differentially expressed in CF vs. CP (p -value < 0.008). 80% of the probes had higher expression levels in the CP enhancement pattern and only 20% had higher expression levels in CF. The right diagram is the enlarged heatmap for *MTA1*, *PARP6*, *SULT1A1* and *SULT1A2*. In the table test statistic (teststat) is the smaller of two sums of ranks of given sign. The negative test statistics represent higher expression level of a gene in the CF pattern, and a positive test statistics represents higher expression level in the CP perfusion pattern.

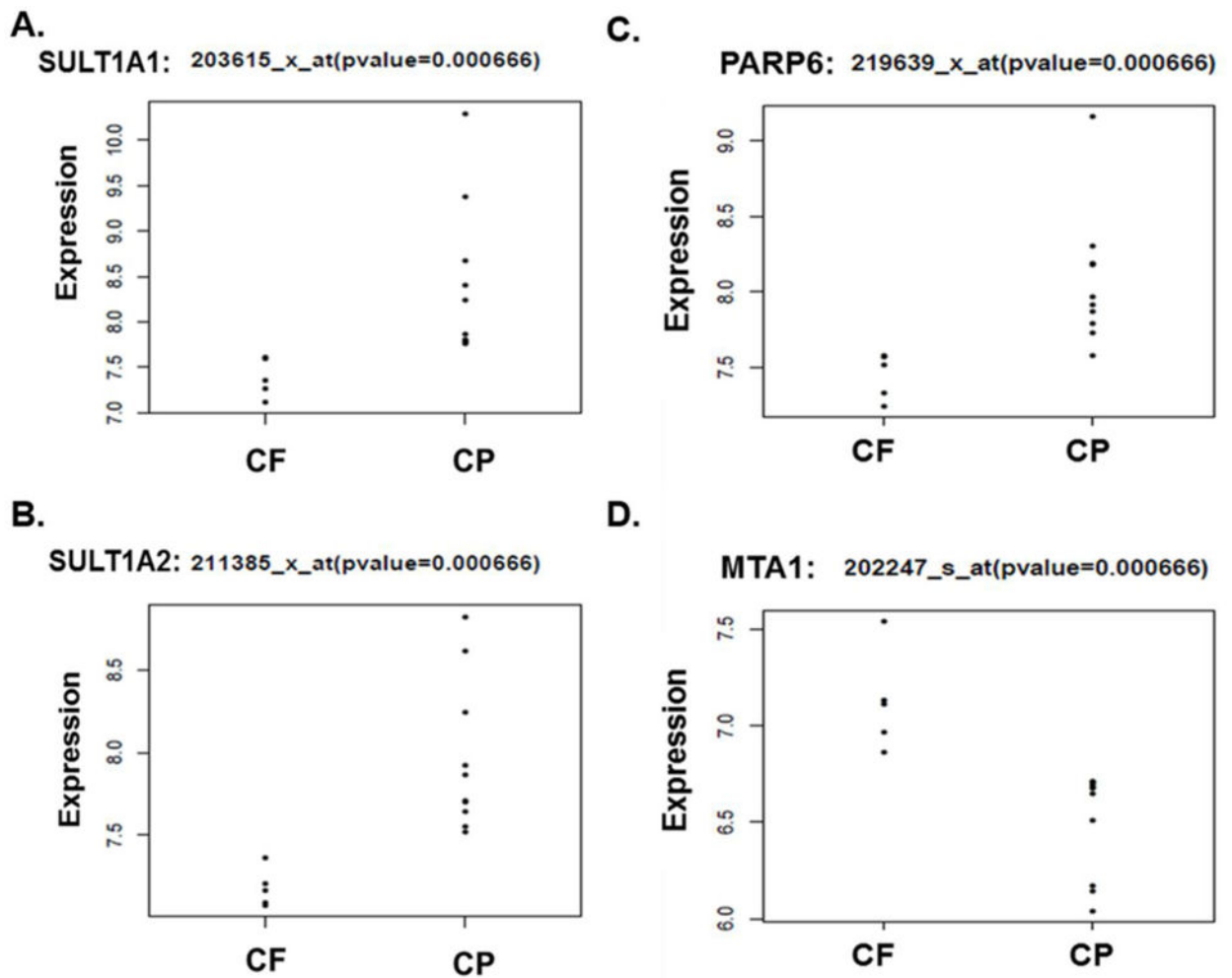


Figure 3. Differential expression levels in the CF enhancement pattern compare to the CP
 A–B) Our analysis demonstrates an over expression of SULTs (SULT1A1 and SULT1A2) in the CP enhancement compare to CF. C) The CP enhancement pattern is associated with higher expression levels of PARP6. D) MTA1 shows higher expression levels in the CF enhancement pattern compared to the CP.

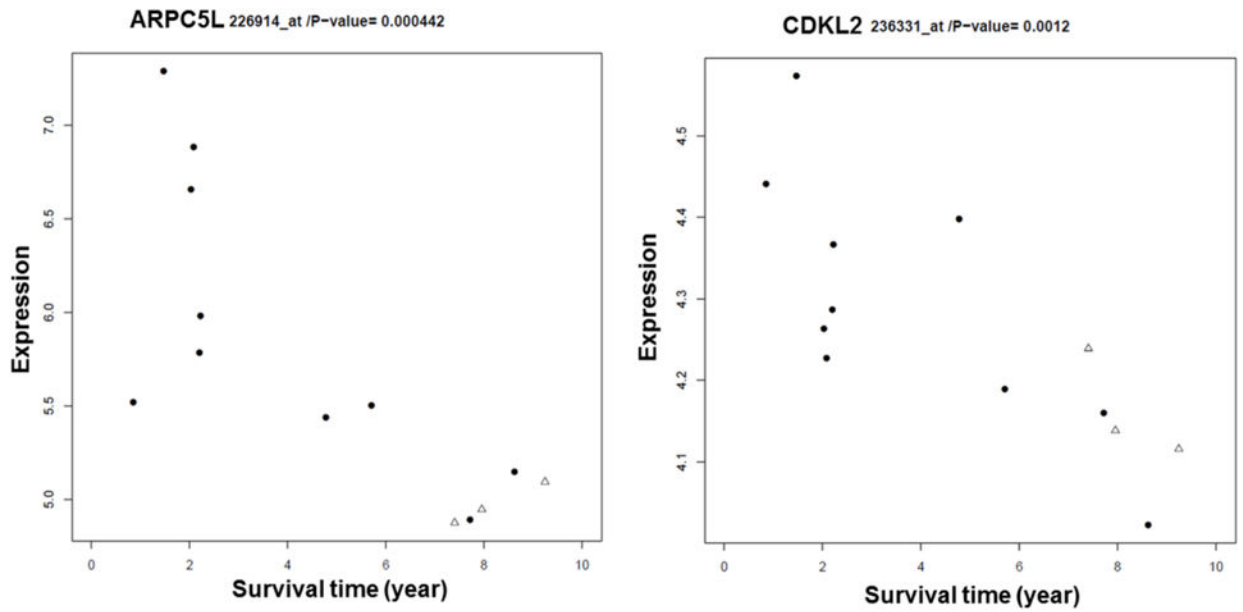
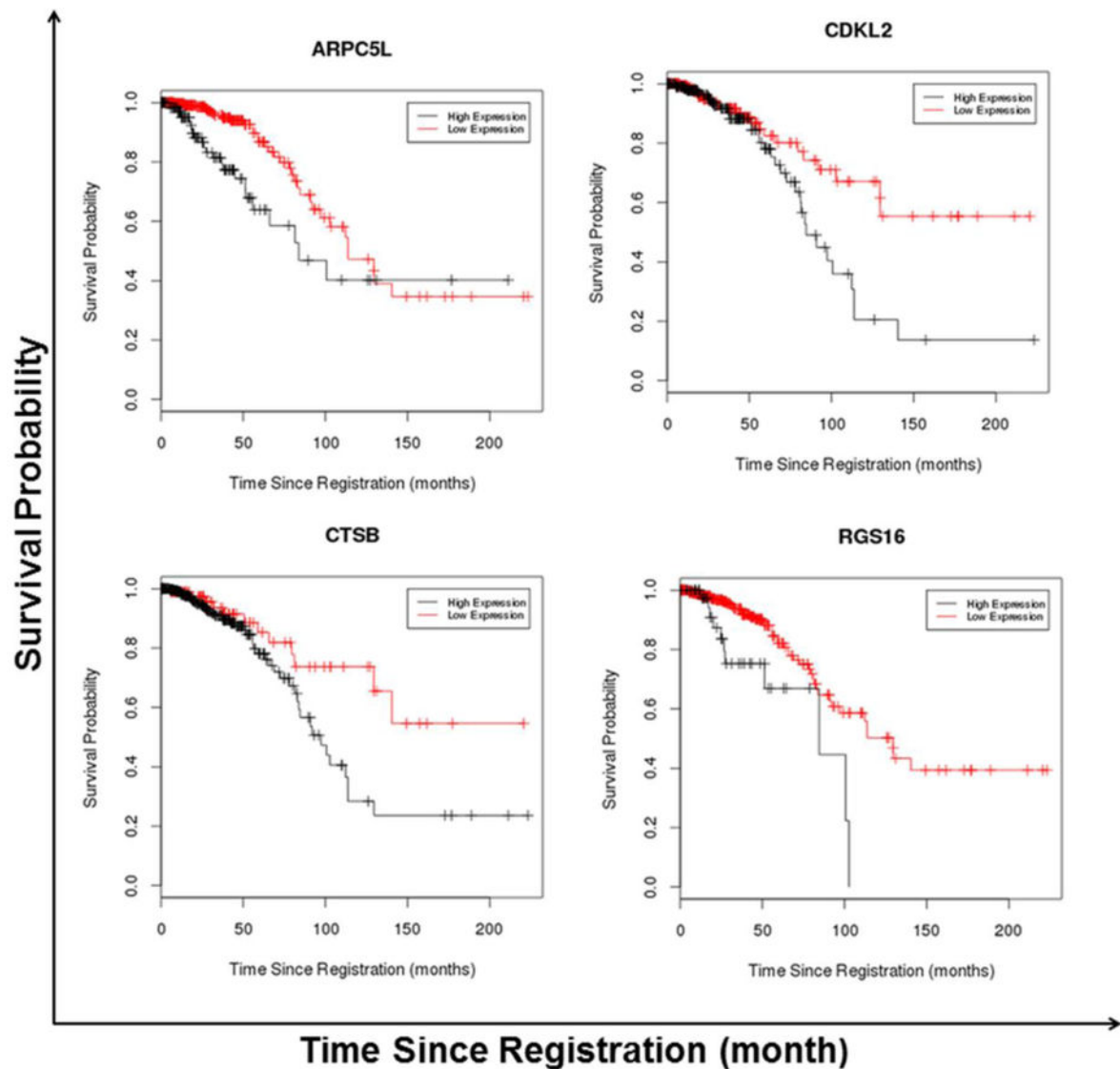


Figure 4. Gene expression patterns and overall survival in IBC patients
ARPC5L and *CDKL2* expression levels are significantly associated with long term OS in IBC patients. The “Survival Time” represents years. Three of the patients from the Duke clinical trial who are alive are represented with a “triangles” symbol in the graph.



Probe ID	Gene Symbol	sctest p-value	HR
226914_at	ARPC5L	1.20E-03	2.3
209325_s_at	RGS16	1.58E-03	2.8
236331_at	CDKL2	9.66E-03	2.0
213274_s_at	CTSB	1.95E-02	2.1

Figure 5. External confirmation for gene expression patterns and overall survival in LABC using TCGA database

In TCGA LABC cohort differential expression of four genes exhibited the same significant concordance and association with overall survival as in the IBC patients. The black lines in the graph represent high expression levels and the red lines represent low expression levels of the genes. The “sctest p-value” and “HR” (which is a cox HR) columns are based on the log rank test.

Table 1

Association between gene expression patterns and overall survival in IBC patients.

Probe	Symbol	p-value	HR	Scetest p-value	Cox HR
226914_at	ARPC5L	4.00E-04	1.8	4.42E-04	1.8
219366_at	AVEN	1.90E-03	1.5	1.03E-03	1.5
236331_at	CDKL2	1.50E-03	1.5	1.20E-03	1.5
226372_at	CHST11	0.00E+00	2.4	7.88E-05	2.4
32094_at	CHST3	7.00E-04	1.5	5.84E-04	1.5
229779_at	COL4A4	9.00E-04	1.6	7.82E-04	1.6
213274_s_at	CTSB	6.00E-04	1.6	4.63E-04	1.6
200825_s_at	HYOU1	1.00E-04	1.7	1.99E-04	1.7
213093_at	PRKCA	1.00E-03	1.6	6.56E-04	1.6
209325_s_at	RGS16	1.00E-03	1.6	6.26E-04	1.6
212701_at	TLN2	5.00E-04	1.6	5.25E-04	1.6
201645_at	TNC	1.50E-03	1.7	9.01E-04	1.7

* The complete list of the 45 genes associated with OS is presented in the supplemental data.

** The HR and p-value is computed based on the permutation resampling inference test for survival data. The "scetest p-value" and "cox HR" columns are based on the log rank test.

Table 2

Overview of some of the biological processes associated with differential expression of our genes of interest.

Gene Symbol	Gene Function
A. Genes differentially expressed in the DCE-MRI enhancement patterns (CP vs. CF)	
<i>MTA1</i>	MTA1 is a potential predictor for breast tumor aggressiveness; Is involved in angiogenesis and is a predictor of early disease relapse; (44, 45); MTA1 can repress the ESR1 trans-activation function; (46, 47) MTA1 can inactivate the tumor suppressor TP53; (48, 49), and deacetylate and stabilize the hypoxia-inducible factor-1alpha (HIF-1α) which is a key regulator of angiogenic factors. (50–52) MTA proteins, including MTA1, have been reported as possible set of “master co-regulatory molecules” involved in the carcinogenesis and progression of various malignancies. (47)
<i>PARP6</i>	PARP superfamily is involved in the regulation of angiogenesis, differentiation, proliferation, cell death, tumor transformation and DNA damage recovery. (53, 54); PARP inhibitors can decrease angiogenesis since inhibition of PARP will affect VEGF-induced proliferation and migration. (55, 56)
<i>SULT1A1 & SULT1A2</i>	Alterations in the expression of SULT enzymes and their genetic polymorphisms have been reported in lung, prostate, and breast cancer; (57, 58) Several members SULT family are responsible for the sulfation of estrogens, therapeutic estrogenic compounds, and hormone replacement therapy (HRT) agents; SULTs play a key role in the metabolism and inactivation of estrogen. (59)
B. Genes differentially expressed in the survival analysis for the IBC patients**	
<i>ARPC5L</i>	ARPC5L belongs to the ARPC5 family, and has been implicated in invasion and metastasis in human head and neck squamous cell carcinoma (HNSCC). (38)
<i>AVEN</i>	Aven is considered a regulator of apoptosis. Resistance to apoptosis is associated with carcinogenesis, tumor progression, and treatment resistance In various tumors such as childhood acute lymphoblastic leukemia and acute myeloid leukemia overexpression of AVEN has been associated with poor prognosis. (60–62)
<i>CDKL2</i>	CDKL2 is a member of a large family of CDC2-related serine/threonine protein kinase. CDC2 overexpression in breast tumors has been associated with infiltrative tumor border pattern and histologic high grade breast carcinomas. (40)
<i>CHST11 & CHST3</i>	CHST11 and CHST3, are involved in the sulfur metabolism pathway. CHST11 mediates 4-O sulfation of chondroitin, while CHST3 mediates 4-O sulfation of chondroitin. (63) Chondroitin sulfates are involved in breast tumor aggressiveness and metastasis and CHST11 and CHST3 overexpression have been reported to be associated with more aggressive breast tumors. (35)
<i>COL4A4</i>	COL4A4 is a protein that belongs to the type IV collagen family. Overexpression of the type IV collagen family members s have been implicated to be associated with tumor size, higher grade, metastasis and invasion in malignancies such as breast, ovarian, cervical, colorectal and gastric cancers. (64–66)
<i>CTSB</i>	Over expression of CTSB, has reported to be associated with tumor invasiveness and poor patient prognosis in different tumor types including in melanoma, prostate and breast cancers. (67) In inflammatory breast cancer, CTSB over expression has been suggested as a prognosis biomarker associated with poor survival and increased number of positive metastatic lymph nodes. (41)
<i>HYOU1</i>	HYOU1 has been associated with tumor invasiveness and resistance to therapy. HYOU1 suppression also results in increased apoptosis. (68–70)
<i>PRKCA</i>	PRKCA is a member of the Protein kinase C (PKC) family which can play an important role in cell signaling pathways by phosphorylating protein targets. High expression levels of PRKCA has been reported as a potential marker for poor prognosis and associated with breast cancer progression. (71)
<i>RGS16</i>	RGS16 is a member of the RGS super family of proteins which are a class of intracellular signaling regulators. The RGS family has been considered as therapeutic targets, including in breast cancer. (42, 43)
<i>TLN2</i>	In the FAK pathway, FAK and TLN are two key players in matrix-cell adhesion assembly, which is required for cell migration which in turn can result in tumor metastasis and progression. (72, 73)
<i>TNC</i>	TNC is frequently up-regulated in breast cancer and is promoting tumor invasion. TNC has been associated with promoting cell migration, angiogenesis and can acts as a cell survival factor. (39)

** The complete list of the 45 genes associated with OS is presented in the supplemental data.