Differential expression of calcium and integrin binding 1 in cancers of the breast.

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Breast cancer affects women at relatively high frequency¹. We mined published microarray datasets^{2,3} to determine in an unbiased fashion and at the systems level genes most differentially expressed in the primary tumors of patients with breast cancer. We report here significant differential expression of the gene encoding calcium and integrin binding 1, CIB1, when comparing primary tumors of the breast to the tissue of origin, the normal breast. CIB1 mRNA was present at significantly higher quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of CIB1 in primary tumors of the breast was correlated with overall survival in patients with HER2+ subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. CIB1 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, CIB1, calcium and integrin binding 1, systems biology of breast cancer, targeted therapeutics in breast cancer.

Invasive breast cancer is diagnosed in over a quarter of a million women in the United States each year¹ and in 2018, breast cancer was the leading cause of cancer death in women worldwide⁴. While patients with localized breast cancer are provided a 99% 5-year survival rate, patients with regional breast cancer, cancer that has spread to lymph nodes or nearby structures, are provided an 86% 5-year survival rate^{5,6}. Patients with metastasis to distant sites, like the brain, are provided a 27% 5-year survival rate^{5,6}. Understanding how primary tumors are most transcriptionally different from the tissue from which they originate, the breast, can facilitate development of novel diagnostic and therapeutics to promote early detection and enhanced treatment, and contribute to efforts to prevent progression to metastatic stages. We mined published microarray data^{2,3} to understand at the transcriptome level and in an unbiased fashion genes most differentially expressed in primary tumors of the breast as compared to normal breast tissue. calcium and integrin binding 1 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE72653³ for this differential gene expression analysis of female breast cancer. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast; analysis was performed using platform GPL5175. The tissues from this first dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). GSE72653 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=8$ normal epithelium and $n=8$ tumors of the breast from patients with breast cancer; the tissues from this second dataset are paired tissues (8 tumors matching 8 normal breast epithelial tissues from 8 patients); analysis was performed using platform GPL6480. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether CIB1 expression was significantly different between primary breast tumors and breast tissue using a two-tailed t -test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of CIB1 mRNA expression levels with overall survival (OS) in $n=404$ patients with basal subtype cancer, $n=794$ patients with HER2+ subtype cancer, $n=515$ patients with luminal B subtype cancer, and $n=166$ patients with HER2+ cancer.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets^{2,3}.

CIB1 is differentially expressed in primary tumors of the breast.

Comparison of 25 normal breast tissues to 25 tumors of the breast² from patients with early-onset breast cancer revealed that the gene encoding calcium and integrin binding 1, encoded by the CIB1 gene, was among the genes most differentially expressed in tumors of the breast in human breast cancer (Chart 1). When sorting each of the genes expressed in tumors of the breast based on significance of difference as compared to normal breast tissue, CIB1 ranked 250 out of 19076 total transcripts, equating to 98.7% differential expression (Chart 1). Differential expression of CIB1 in female breast cancer was statistically significant (Chart 1; $p=1.49\text{E-}12$).

To attempt to validate differential transcriptome-wide differential expression of CIB1 in human breast cancer, we queried a second microarray dataset³, here comparing normal breast tissues and primary tumors of the breast. Again, we identified CIB1 as a differentially expressed gene in the tumor tissues of

patients with breast cancer (Chart 2). When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal breast tissues, CIB1 ranked 4574 out of 41093 total transcripts (Chart 2), equating to 88.9% differential expression. Differential expression of CIB1 in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=1.92E-02$). These data indicated that differential expression of CIB1 was not an artifact of a single microarray dataset, and perhaps a general feature of cancers of the breast.

CIB1 is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for CIB1 from the breast and from breast tumors² to understand the magnitude and direction of CIB1 expression change. CIB1 was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of CIB1 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). CIB1 was expressed at 7.65 ± 0.34 arbitrary units (AU) in normal breast tissue, while it was expressed at 8.52 ± 0.33 AU in tumors of the breast. We calculated a mean fold change of 1.11 in CIB1 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

CIB1 expression correlates with survival outcomes in HER2+ human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate CIB1 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and overall survival. We observed a statistically significant correlation between CIB1 expression and overall survival (OS) in patients with HER2+ subtype breast cancer (Figure 2; log rank p -value: 0.04 for overall survival, hazard ratio: 1.83 (1.02-3.28) (Fig. 2)). CIB1 mRNA levels were a negative prognostic indicator in HER2+ subtype breast cancer patients. Median OS was 72.2 months for HER2+ patients with low tumor expression of CIB1 as compared to HER2+ patients with high tumor expression of CIB1, who possessed median OS of 35.7 months (Chart 3). CIB1 primary tumor expression was not correlated with overall survival in basal subtype (Figure 2; log rank p -value: 0.23 for OS, hazard ratio: 1.26 (0.86-1.84) (Fig. 2), luminal A subtype (Figure 2; log rank p -value: 0.72 for OS, hazard ratio: 0.94 (0.69-1.29) (Fig. 2) or in luminal B breast cancer (Figure 2; log rank p -value: 0.19 for OS, hazard ratio: 0.79 (0.56-1.12) (Fig. 2)).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and up-regulation of CIB1 was among the most significant transcriptional features in primary tumors from patients with breast cancer. CIB1 expression in primary tumors of the breast was correlated with overall survival in patients with HER2+ subtype cancers, with CIB1 primary tumor mRNA levels a negative prognostic indicator for HER2+ patients.

Discussion

Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose disease has spread to distant sites^{5,6}. To facilitate understanding of the basic transcriptional differences between primary tumors of the breast and the tissues from which these tumors originate, normal breast tissues, we performed comparative transcriptome analysis using two independently published microarray datasets^{2,3}, providing evidence here that calcium and integrin binding 1, encoded by CIB1, is a defining transcriptional feature of human breast cancer. CIB1 was expressed at significantly higher levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with HER2+ subtype breast cancer, primary tumor expression of CIB1 was negatively correlated with overall survival. Calcium and integrin binding 1, and the molecular processes to which it pertains to, may be relevant to the initiation or progression of human breast cancer.

References

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Rank: 250
Probe ID: 3638819
p-value: 1.49E-12
t: -9.29
B: 18.40001
Gene: CIB1
Gene name: calcium and integrin binding 1

Chart 1: CIB1 is differentially expressed in the primary tumors of patients with early-onset human breast cancer.

Rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank: 4574
Probe ID: A_23_P257638
p-value: 1.92E-02
t: -2.5872047
B: -3.221104
Gene: CIB1
Gene name: calcium and integrin binding 1

Chart 2: CIB1 is differentially expressed in the tumor cells of patients with triple-negative breast cancer.

Rank of differential expression, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: CIB1 is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of CIB1 in normal breast tissue (left) and in primary tumors of the breast (right) is graphically depicted with the result of a statistical test evaluating significance of difference in CIB1 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is $p < 0.00001$.

Figure 2: CIB1 expression correlates with overall survival in patients with HER2+ breast cancer, but not in patients with basal, luminal B or HER2+ cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=404$ patients with basal subtype breast cancer (upper left), $n=166$ patients with HER2+ breast cancer (upper right), $n=794$ patients with luminal A breast cancer (lower left), and $n=515$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of CIB1 in patient primary tumors. The log rank p-value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on CIB1 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

1 Low CIB1 expression: 72.2 months

2 High CIB1 expression: 35.7 months

3 **Chart 3: Median overall survival is superior in HER2+ subtype breast cancer patients with low**
4 **primary tumor expression of CIB1.**

5 The overall survival of $n=166$ HER2+ subtype breast cancer patients based on stratification into low or
6 high tumor expression of CIB1 is listed in this chart.