Differential expression of ATP binding cassette subfamily A member 10 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 ATP binding cassette subfamily A member 10, ABCA10, when comparing primary tumors of the breast to the tissue of origin, the normal breast. ABCA10 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. ABCA10 mRNA was present at significantly reduced quantity in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of ABCA10 in primary tumors of the breast was correlated with overall survival in patients with stage III cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by cancer progression. ABCA10 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, ABCA10, ATP binding cassette subfamily A member 10, 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. The ATP binding cassette subfamily A member 10 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE109169² and GSE38959³ 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). GSE38959 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=13$ samples of normal mammary gland ductal cells and $n=30$ samples of tumor cells from patients with triple negative breast cancer; analysis was performed using platform GPL4133. 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 ABCA10 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 ABCA10 mRNA expression levels with overall survival (OS) in patients with stage I, stage II, stage III, and stage IV breast cancer ($n=1090$ patients in total).

Results

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

ABCA10 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 ATP binding cassette subfamily A member 10, encoded by the ABCA10 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, ABCA10 ranked 8 out of 19076 total transcripts, equating to 99.96% differential expression (Chart 1). Differential expression of ABCA10 in female breast cancer was statistically significant (Chart 1; $p=2.78E-20$).

Analysis of a second microarray dataset³, here in tumor cells of patients with triple negative breast cancer as compared to normal mammary gland ductal cells, again revealed significant differential expression of ABCA10 in human breast cancer (Chart 2). When sorting each of the genes expressed in the tumor cells of patients with triple negative breast cancer based on significance of difference as compared

to normal mammary gland ductal cells, ABCA10 ranked 4771 out of 45015 total transcripts, equating to 89.4% differential expression (Chart 2). Differential expression of ABCA10 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=3.52E-04$). These data suggested that differential expression of ABCA10 was not an artifact of a single microarray dataset, nor was it associated with one type of breast cancer, rather a general feature of cancers of the breast.

ABCA10 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for ABCA10 from the breast and from breast tumors² to understand the magnitude and direction of ABCA10 expression change. ABCA10 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of ABCA10 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). ABCA10 was expressed at 7.52 ± 0.60 arbitrary units (AU) in normal breast tissue, while it was expressed at 4.60 ± 0.80 AU in tumors of the breast. We calculated a mean fold change of 0.61 in ABCA10 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

ABCA10 expression correlates with survival outcomes in stage III human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate ABCA10 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating interactions between overall survival and cancer progression based on stage of patient disease. We observed a statistically significant correlation between ABCA10 expression and overall survival (OS) in patients with stage III breast cancer (Figure 2; log rank p -value: 0.0075 for overall survival, hazard ratio: 0.44 (0.24-0.82) (Fig. 2)). ABCA10 mRNA levels were a positive prognostic indicator in stage III breast cancer patients. Median OS was 85.03 months for stage III patients with low tumor expression of ABCA10 as compared to stage III patients with high tumor expression of ABCA10, who possessed median OS of 115.37 months (Chart 3). ABCA10 primary tumor expression was not correlated with overall survival in stage I (Figure 2; log rank p -value: 0.74 for OS, hazard ratio: 1.18 (0.43-3.24) (Fig. 2), stage II subtype (Figure 2; log rank p -value: 0.32 for OS, hazard ratio: 1.28 (0.79-2.1) (Fig. 2) or in stage IV breast cancer (Figure 2; log rank p -value: 0.67 for OS, hazard ratio: 0.8 (0.27-2.3) (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 ABCA10 was among the most significant transcriptional features in primary tumors from patients with breast cancer. ABCA10 expression in primary tumors of the breast was correlated with overall survival in patients with stage III breast cancer, with ABCA10 primary tumor mRNA levels a positive prognostic indicator for stage III subtype 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 differential expression of ATP binding cassette subfamily A member 10, encoded by ABCA10, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. ABCA10 was expressed at significantly lower levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with stage III breast cancer, primary tumor expression of ABCA10 was correlated with overall survival. ATP binding cassette subfamily A member 10, 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: 8
Probe ID: 3768880
p-value: 2.78E-20
t: 1.50E+01
B: 35.851395
Gene: ABCA10
Gene name: ATP binding cassette subfamily A member 10

Chart 1: ABCA10 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: 4771
Probe ID: 6337
p-value: 3.52E-04
t: 3.8658312
B: -0.26272
Gene: ABCA10
Gene name: ATP binding cassette subfamily A member 10

Chart 2: ABCA10 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: ABCA10 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of ABCA10 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 ABCA10 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is $p < 0.00001$.

Figure 2: ABCA10 expression correlates with overall survival in patients with stage III cancer, but not in patients with stage I, stage II, or stage IV cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for patients with stage I breast cancer (upper left), patients with stage II breast cancer (upper right), patients with stage III breast cancer (lower left), and patients with stage IV breast cancer (lower right), stratified into two groups, based on low or high expression of ABCA10 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 ABCA10 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 ABCA10 expression: 85.03 months
2 High ABCA10 expression: 115.37 months

3 **Chart 3: Median overall survival is superior in stage III breast cancer patients with high primary**
4 **tumor expression of ABCA10.**

5 The overall survival of stage III breast cancer patients based on stratification into low or high tumor
6 expression of ABCA10 is listed in this chart.
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