Differential expression of PROS1 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 protein S (alpha), PROS1, when comparing primary tumors of the breast to the tissue of origin, the normal breast. PROS1 mRNA was present at significantly lower quantities in tumors of the breast as compared to normal breast tissue. Analysis of human survival data revealed that expression of PROS1 in primary tumors of the breast was correlated with overall survival in patients with luminal A subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. PROS1 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, PROS1, protein S (alpha), 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. Protein S (alpha) 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 PROS1 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 PROS1 mRNA expression levels with overall survival (OS) in $n=404$ patients with basal subtype cancer, $n=794$ patients with luminal A 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}.

PROS1 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 protein S (alpha), encoded by the PROS1 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, PROS1 ranked 84 out of 19076 total transcripts, equating to 99.6% differential expression (Chart 1). Differential expression of PROS1 in female breast cancer was statistically significant (Chart 1; $p=5.04E-15$).

To attempt to validate differential transcriptome-wide differential expression of PROS1 in human breast cancer, we queried a second microarray dataset³, here comparing normal breast tissues and primary tumors of the breast. Again, we identified PROS1 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, PROS1 ranked 1620 out of 41093 total transcripts (Chart 2), equating to 96.1% differential expression. Differential expression of PROS1 in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=5.75E-03$). These data indicated that differential expression of PROS1 was not an artifact of a single microarray dataset, and perhaps a general feature of cancers of the breast.

PROS1 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for PROS1 from the breast and from breast tumors² to understand the magnitude and direction of PROS1 expression change. PROS1 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of PROS1 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). PROS1 was expressed at 9.14 ± 0.34 arbitrary units (AU) in normal breast tissue, while it was expressed at 7.63 ± 0.61 AU in tumors of the breast. We calculated a mean fold change of 0.83 in PROS1 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

PROS1 expression correlates with survival outcomes in luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate PROS1 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 PROS1 expression and overall survival (OS) in patients with luminal A subtype breast cancer (Figure 2; log rank p -value: 0.0082 for overall survival, hazard ratio: 0.65 (0.47-0.9) (Fig. 2)). PROS1 mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median OS was 105.44 months for luminal A patients with low tumor expression of PROS1 while median OS was 169.2 months for luminal A patients with high tumor expression of PROS1 (Chart 3). PROS1 primary tumor expression was not correlated with overall survival in basal subtype breast cancer (Figure 2; log rank p -value: 0.69 for OS, hazard ratio: 1.08 (0.74-1.58) (Fig. 2)), luminal B subtype breast cancer (Figure 2; log rank p -value: 0.95 for OS, hazard ratio: 0.99 (0.7-1.4) (Fig. 2)) or in HER2+ breast cancer (Figure 2; log rank p -value: 0.49 for OS, hazard ratio: 0.82 (0.47-1.44) (Fig. 2)).

Thus, through comparative transcriptome analysis of primary tumors of the breast and normal breast tissue, we found that differential expression and down-regulation of PROS1 was among the most significant transcriptional features in primary tumors from patients with breast cancer. PROS1 expression in primary tumors of the breast was correlated with overall survival in patients with luminal A subtype cancers, with PROS1 primary tumor mRNA levels a positive prognostic indicator for luminal A 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 protein S (alpha), encoded by PROS1, is a defining transcriptional feature of human breast cancer. PROS1 was expressed at significantly lower levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with luminal A subtype breast cancers, primary tumor expression of PROS1 was correlated with overall survival. Protein S (alpha), 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: 84
Probe ID: 2685304
p-value: 5.04E-15
t: 1.10E+01
B: 24.003698
Gene: PROS1
Gene name: protein S (alpha)

Chart 1: PROS1 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: 1620
Probe ID: A_23_P73114
p-value: 5.75E-03
t: 3.160092
B: -2.1551
Gene: PROS1
Gene name: protein S (alpha)

Chart 2: PROS1 is differentially expressed in the primary tumors of patients with 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: PROS1 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of PROS1 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 PROS1 expression between normal breast tissue and primary tumors of the breast, a p -value, is $p<0.0001$.

Figure 2: PROS1 expression correlates with overall survival in patients with luminal A breast cancer, but not in patients with basal, luminal B subtype or HER2+ breast 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 PROS1 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 PROS1 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.

Luminal A patients

Low PROS1 expression: 105.44 months

High PROS1 expression: 169.2 months

Chart 3: In luminal A subtype breast cancer, median overall survival is superior in patients with high primary tumor expression of PROS1.

The overall survival of $n=794$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of PROS1 is listed in this chart.