Differential expression of phosphatidic acid phosphatase type 2B 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 phosphatidic acid phosphatase type 2B, PPAP2B, when comparing primary tumors of the breast to the tissue of origin, the normal breast. PPAP2B 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 PPAP2B in primary tumors of the breast was correlated with overall survival in patients with HER2+ and luminal A subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. PPAP2B may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, PPAP2B, phosphatidic acid phosphatase type 2B, 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. phosphatidic acid phosphatase type 2B 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 PPAP2B 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 PPAP2B 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}.

PPAP2B 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 phosphatidic acid phosphatase type 2B, encoded by the PPAP2B 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, PPAP2B ranked 25 out of 19076 total transcripts, equating to 99.9% differential expression (Chart 1). Differential expression of PPAP2B in female breast cancer was statistically significant (Chart 1; $p=3.54E-18$).

To attempt to validate differential transcriptome-wide differential expression of PPAP2B in human breast cancer, we queried a second microarray dataset³, here comparing normal breast tissues and primary tumors of the breast. Again, we identified PPAP2B 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, PPAP2B ranked 5061 out of 41093 total transcripts (Chart 2), equating to 87.7% differential expression. Differential expression of PPAP2B in the primary tumors of patients with breast cancer was statistically significant (Chart 2; $p=2.20E-02$). These data indicated that differential expression of PPAP2B was not an artifact of a single microarray dataset, and perhaps a general feature of cancers of the breast.

PPAP2B is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for PPAP2B from the breast and from breast tumors² to understand the magnitude and direction of PPAP2B expression change. PPAP2B was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of PPAP2B in primary breast tumors was statistically significant (Figure 1; $p<0.0001$). PPAP2B was expressed at 11.13 ± 0.35 arbitrary units (AU) in normal breast tissue, while it was expressed at 9.72 ± 0.40 AU in tumors of the breast. We calculated a mean fold change of 0.87 in PPAP2B mRNA levels when comparing primary tumors of the breast to normal breast tissues.

PPAP2B expression correlates with survival outcomes in HER2+ and luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate PPAP2B 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 PPAP2B expression and overall survival (OS) in patients with HER2+ subtype breast cancer (Figure 2; log rank p -value: 0.033 for overall survival, hazard ratio: 0.53 (0.3-0.96) (Fig. 2)). PPAP2B mRNA levels were a positive prognostic indicator in HER2+ subtype breast cancer patients. Median OS was 33.73 months for HER2+ patients with low tumor expression of PPAP2B as compared to HER2+ patients with high tumor expression of PPAP2B, who possessed median OS of 69.99 months (Chart 3). We also observed a correlation between PPAP2B expression and overall survival (OS) in patients with luminal A subtype breast cancer which approached statistical significance (Figure 2; log rank p -value: 0.056 for overall survival, hazard ratio: 0.73 (0.53-1.01) (Fig. 2)). PPAP2B mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. PPAP2B primary tumor expression was not correlated with overall survival in basal subtype (Figure 2; log rank p -value: 0.44 for OS, hazard ratio: 1.16 (0.79-1.7) (Fig. 2) or in luminal B breast cancer (Figure 2; log rank p -value: 0.61 for OS, hazard ratio: 0.91 (0.64-1.3) (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 PPAP2B was among the most significant transcriptional features in primary tumors from patients with breast cancer. PPAP2B expression in primary tumors of the breast was correlated with overall survival in patients with HER2+ and luminal A subtype cancers, with PPAP2B primary tumor mRNA levels a positive prognostic indicator for both molecular subtypes.

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 phosphatidic acid phosphatase type 2B, encoded by PPAP2B, is a

1 defining transcriptional feature of human breast cancer. PPAP2B was expressed at significantly lower
2 levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with
3 HER2+ and luminal A subtype breast cancers, primary tumor expression of PPAP2B was positively
4 correlated with overall survival. Phosphatidic acid phosphatase type 2B, and the molecular processes to
5 which it pertains to, may be relevant to the initiation or progression of human breast cancer.
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Rank: 25
Probe ID: 2414366
p-value: 3.54E-18
t: 1.33E+01
B: 31.127912
Gene: PPAP2B
Gene name: phosphatidic acid phosphatase type 2B

Chart 1: PPAP2B 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: 5061
Probe ID: A_24_P860797
p-value: 2.20E-02
t: -2.5211132
B: -3.339452
Gene: PAIP2B
Gene name: poly(A) binding protein interacting protein 2B

Chart 2: PPAP2B, also known as PAIP2B, 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: PPAP2B is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of PPAP2B 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 PPAP2B expression between normal breast tissue and primary tumors of the breast, a p -value, is $p < 0.00001$.

Figure 2: PPAP2B expression correlates with overall survival in patients with HER2+ and luminal A breast cancer, but not in patients with basal or luminal B subtype 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 PPAP2B 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 PPAP2B 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.

HER2+ patients

Low PPAP2B expression: 33.73 months

High PPAP2B expression: 69.99 months

Chart 3: Median overall survival is superior in HER2+ subtype breast cancer patients with high primary tumor expression of PPAP2B.

The overall survival of $n=166$ HER2+ subtype breast cancer patients based on stratification into low or high tumor expression of PPAP2B is listed in this chart.