Differential expression of PDZ domain containing 11 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 the PDZ domain containing 11, PDZD11, when comparing primary tumors of the breast to the tissue of origin, the normal breast. PDZD11 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. PDZD11 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 PDZD11 in primary tumors of the breast was correlated with overall survival in patients with HER2+ cancer. PDZD11 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, PDZD11, PDZ domain containing 11, 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 PDZ domain containing 11 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 PDZD11 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 PDZD11 mRNA expression levels with overall survival (OS) in $n=278$ patients with basal subtype cancer, $n=377$ patients with luminal A subtype cancer, $n=177$ patients with luminal B subtype cancer, and $n=111$ 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}.

PDZD11 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 the PDZ domain containing 11, encoded by the PDZD11 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, PDZD11 ranked 163 out of 19076 total transcripts, equating to 99.1% differential expression (Chart 1). Differential expression of PDZD11 in female breast cancer was statistically significant (Chart 1; $p=1.46e-13$).

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 PDZD11 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, PDZD11 ranked 1042 out of 45015 total transcripts, equating to 97.7% differential expression (Chart 2). Differential expression of PDZD11 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=1.72\text{e-}07$). These data suggested that differential expression of PDZD11 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.

PDZD11 is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for PDZD11 from the breast and from breast tumors² to understand the magnitude and direction of PDZD11 expression change. PDZD11 was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of PDZD11 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). PDZD11 was expressed at 5.84 ± 0.23 arbitrary units (AU) in normal breast tissue, while it was expressed at 6.60 ± 0.31 AU in tumors of the breast. We calculated a mean fold change of 1.13 in PDZD11 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

PDZD11 expression correlates with survival outcomes in HER2+ human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate PDZD11 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 PDZD11 expression and overall survival (OS) in patients with HER2+ breast cancer (Figure 2; log rank p -value: 0.0096 for overall survival, hazard ratio: 0.39 (0.18-0.82) (Fig. 2)). PDZD11 mRNA levels were a positive prognostic indicator in HER2+ subtype breast cancer patients. Median OS was 31.08 months for HER2+ patients with low tumor expression of PDZD11 as compared to HER2+ patients with high tumor expression of PDZD11, who possessed median OS of 68.04 months (Chart 3). PDZD11 primary tumor expression was not correlated with overall survival in basal subtype (Figure 2; log rank p -value: 0.9 for OS, hazard ratio: 1.03 (0.65-1.64) (Fig. 2), luminal A subtype (Figure 2; log rank p -value: 0.8 for OS, hazard ratio: 1.06 (0.66-1.7) (Fig. 2) or in luminal B breast cancer (Figure 2; log rank p -value: 0.9 for OS, hazard ratio: 0.96 (0.52-1.77) (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 PDZD11 was among the most significant transcriptional features in primary tumors from patients with breast cancer. PDZD11 expression in primary tumors of the breast was correlated with overall survival in patients HER2+ subtype cancers, with PDZD11 primary tumor mRNA levels a positive 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 PDZ domain containing 11, encoded by PDZD11, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. PDZD11 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 PDZD11 was correlated with overall survival. The inositol-pentakisphosphate 2-kinase, 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: 163
Probe ID: 4011637
p-value: 1.46e-13
t: -9.96
B: 20.690931
Gene: PDZD11
Gene name: PDZ domain containing 11

Chart 1: PDZD11 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: 1042
Probe ID: 22121
p-value: 1.72e-07
t: -6.1701299
B: 7.096085
Gene: PDZD11
Gene name: PDZ domain containing 11

Chart 2: PDZD11 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: PDZD11 is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of PDZD11 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 PDZD11 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is *p*<0.00001.

Figure 2: PDZD11 expression correlates with overall survival in patients with HER2+ breast cancer, but not in patients with basal, luminal A or luminal B cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=278$ patients with basal subtype breast cancer (upper left), $n=111$ patients with HER2+ breast cancer (upper right), $n=278$ patients with luminal A breast cancer (lower left), and $n=377$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of PDZD11 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 PDZD11 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 PDZD11 expression: 31.08 months

2 High PDZD11 expression: 68.4 months

3 **Chart 3: Median overall survival is superior in HER2+ breast cancer patients with high primary**
4 **tumor expression of PDZD11.**

5 The overall survival of $n=111$ HER2+ breast cancer patients based on stratification into low or high tumor
6 expression of PDZD11 is listed in this chart.