Differential expression of pyruvate dehydrogenase kinase 4 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 pyruvate dehydrogenase kinase 4, PDK4, when comparing primary tumors of the breast to the tissue of origin, the normal breast. PDK4 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. PDK4 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 PDK4 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. PDK4 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, PDK4, pyruvate dehydrogenase kinase 4, 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. Pyruvate dehydrogenase kinase 4 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 PDK4 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 PDK4 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}.

PDK4 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 pyruvate dehydrogenase kinase 4, encoded by the PDK4 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, PDK4 ranked 55 out of 19076 total transcripts, equating to 99.7% differential expression (Chart 1). Differential expression of PDK4 in female breast cancer was statistically significant (Chart 1; $p=4.12\text{E-}16$).

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 PDK4 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, PDK4 ranked 250 out of 45015 total transcripts, equating to 99.4% differential expression (Chart 2). Differential expression of PDK4 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=8.66E-11$). These data suggested that differential expression of PDK4 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.

PDK4 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for PDK4 from the breast and from breast tumors² to understand the magnitude and direction of PDK4 expression change. PDK4 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of PDK4 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). PDK4 was expressed at 10.51 ± 0.97 arbitrary units (AU) in normal breast tissue, while it was expressed at 7.53 ± 0.88 AU in tumors of the breast. We calculated a mean fold change of 0.72 in PDK4 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

PDK4 expression correlates with survival outcomes in luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate PDK4 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and overall survival. We observed a correlation between PDK4 expression and overall survival (OS) in patients with luminal A subtype breast cancer which trended towards statistical significance (Figure 2; log rank p -value: 0.071 for overall survival, hazard ratio: 0.75 (0.54-1.03) (Fig. 2)). PDK4 mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median OS was 120 months for luminal A subtype patients with low tumor expression of PDK4 as compared to luminal A subtype patients with high tumor expression of PDK4, who possessed median OS of 169.2 months (Chart 3). PDK4 primary tumor expression was not correlated with overall survival in basal subtype (Figure 2; log rank p -value: 0.74 for OS, hazard ratio: 0.94 (0.64-1.37) (Fig. 2)), HER2+ (Figure 2; log rank p -value: 0.45 for OS, hazard ratio: 1.25 (0.71-2.2) (Fig. 2)), or luminal B subtype breast cancer (Figure 2; log rank p -value: 0.3 for OS, hazard ratio: 1.2 (0.85-1.7) (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 PDK4 was among the most significant transcriptional features in primary tumors from patients with breast cancer. PDK4 expression in primary tumors of the breast was correlated with overall survival in patients with luminal A subtype cancers, with PDK4 primary tumor mRNA levels a positive prognostic indicator for luminal A 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 pyruvate dehydrogenase kinase 4, encoded by PDK4, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. PDK4 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 cancer, primary tumor expression of PDK4 was correlated with overall survival. Pyruvate dehydrogenase

kinase 4 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: 55
Probe ID: 3062082
p-value: 4.12E-16
t: 1.17E+01
B: 26.46532
Gene: PDK4
Gene name: pyruvate dehydrogenase kinase 4

Chart 1: PDK4 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: 250
Probe ID: 42318
p-value: 8.66E-11
t: 8.4063094
B: 14.50764
Gene: PDK4
Gene name: pyruvate dehydrogenase kinase 4

Chart 2: PDK4 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: PDK4 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of PDK4 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 PDK4 expression between normal breast tissue and primary tumors of the breast, a p -value, is $p < 0.0001$.

Figure 2: PDK4 expression correlates with overall survival in patients with luminal A 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 PDK4 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 PDK4 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 PDK4 expression: 120 months

High PDK4 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 PDK4.

The overall survival of $n=794$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of PDK4 is listed in this chart.