Differential expression of cyclin dependent kinase 2 (CDK2) 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 CDK2 (cyclin dependent kinase 2) when comparing primary tumors of the breast to the tissue of origin, the normal breast. CDK2 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. CDK2 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 CDK2 in primary tumors of the breast was correlated with recurrence-free survival in patients with luminal A subtype cancer, demonstrating a relationship between correlation of primary tumor expression with patient survival outcomes based on molecular subtype. We have now defined transcriptome-wide differential expression and up-regulation of CDK5⁴, CDK1⁵, and CDK2 in human breast cancer.

Keywords: breast cancer, CDK2, cyclin dependent kinase 2, 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 worldwide⁶. 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^{7,8}. Patients with metastasis to distant sites, like the brain, are provided a 27% 5-year survival rate^{7,8}. 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. Cyclin dependent kinase 2 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 CDK2 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 tool⁹ for correlation of CDK2 mRNA expression levels with recurrence-free survival (RFS) in $n=846$ patients with basal subtype cancer, $n=2277$ patients with luminal A subtype cancer, $n=1491$ patients with luminal B subtype cancer, and $n=315$ 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}.

CDK2 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 cyclin dependent kinase 2 (CDK2) was among those 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, CDK2 ranked 238 out of 19076 total transcripts, equating to 98.8% differential expression (Chart 1). Differential expression of CDK2 in female breast cancer was statistically significant (Chart 1; $p=1.04E-12$).

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 CDK2 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, CDK2 ranked 4152 out of 45015 total transcripts, equating to 90.8% differential expression (Chart 2). Differential expression of CDK2 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=1.77E-04$). These data suggested that differential expression of CDK2 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.

CDK2 is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for CDK2 from the breast and from breast tumors² to understand the magnitude and direction of CDK2 expression change. CDK2 was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of CDK2 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). CDK2 was expressed at 7.76 ± 0.28 arbitrary units (AU) in normal breast tissue, while it was expressed at 8.62 ± 0.37 AU in tumors of the breast. We calculated a mean fold change of 1.11 in CDK2 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

CDK2 expression correlates with survival outcomes in luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate CDK2 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and recurrence-free survival. We observed a statistically significant correlation between CDK2 expression and recurrence-free survival (RFS) in patients with luminal A subtype breast cancer (Figure 2; log rank p -value: 0.035 for recurrence-free survival, hazard ratio: 0.84 (0.71-0.99) (Fig. 2)). CDK2 mRNA levels were a negative prognostic indicator in luminal A subtype breast cancer patients. Median RFS was 228.85 months for luminal A patients with low tumor expression of CDK2 as compared to luminal A patients with high tumor expression of CDK2, who possessed median RFS of 216.66 months (Chart 3). CDK2 primary tumor expression was not correlated with recurrence-free survival in basal subtype (Figure 2; log rank p -value: 0.64 for RFS, hazard ratio: 0.95 (0.76-1.18) (Fig. 2), luminal B subtype (Figure 2; log rank p -value: 1 for RFS, hazard ratio: 1 (0.84-1.19) (Fig. 2) or in HER2+ breast cancer (Figure 2; log rank p -value: 0.63 for RFS, hazard ratio: 0.92 (0.64-1.31) (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 CDK2 was among the most significant transcriptional features in primary tumors from patients with breast cancer. CDK2 expression in primary tumors of the breast was correlated with recurrence-free survival in patients with luminal A subtype cancers, with CDK2 primary tumor mRNA levels a negative 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^{7,8}. 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 cyclin dependent kinase 2, encoded by CDK2, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. CDK2 was expressed at significantly higher levels in primary tumors from patients with breast cancer, as compared to normal breast. In patients with luminal A subtype breast cancer, primary tumor expression of CDK2 was correlated with recurrence-free survival. Though

1 inhibitors of CDK4 and CDK6 are currently FDA-approved for the treatment of metastatic
2 hormone-receptor positive/HER2- breast cancer¹⁰, we have now defined the transcriptional up-regulation
3 and differential expression of CDK5⁴, CDK1⁵, and CDK2 in human breast cancer. For at least CDK1⁵ and
4 CDK2, this phenomena was conserved across hormone-receptor positive and hormone-receptor negative
5 (“triple negative”) breast cancer.
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Rank: 238
Probe ID: 3417146
p-value: 1.04E-12
t: -9.39
B: 18.755348
Gene: CDK2
Gene name: cyclin dependent kinase 2

Chart 1: CDK2 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: 4152
Probe ID: 34292
p-value: 1.77E-04
t: -4.0879334
B: 0.394025
Gene: CDK2
Gene name: cyclin dependent kinase 2

Chart 2: CDK2 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: CDK2 is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of CDK2 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 CDK2 expression between normal breast tissue and primary tumors of the breast, a p -value, is $p < 0.00001$.

Figure 2: CDK2 expression correlates with recurrence-free 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 recurrence-free survival (RFS) for $n=846$ patients with basal subtype breast cancer (upper left), $n=315$ patients with HER2+ breast cancer (upper right), $n=2277$ patients with luminal A breast cancer (lower left), and $n=1491$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of CDK2 (also known as OBFC2B) in patient primary tumors. The log rank p-value denoting statistical significance of difference in recurrence-free 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 CDK2 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 CDK2 expression: 228.85 months

2 High CDK2 expression: 216.66 months

3 **Chart 3: Median recurrence-free survival is superior in luminal A subtype breast cancer patients**
4 **with low primary tumor expression of CDK2.**

5 The recurrence-free survival of $n=2277$ luminal A subtype breast cancer patients based on stratification
6 into low or high tumor expression of CDK2 is listed in this chart.