Differential expression of CKS2 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 CDC28 protein kinase regulatory subunit 2, CKS2, when comparing primary tumors of the breast to the tissue of origin, the normal breast. CKS2 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. CKS2 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 CKS2 in primary tumors of the breast was correlated with overall survival in patients with basal and luminal A subtype cancer, but in a contrary manner. CKS2 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, CKS2, CDC28 protein kinase regulatory subunit 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 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 CDC28 protein kinase regulatory subunit 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 CKS2 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 CKS2 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}.

CKS2 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 CDC28 protein kinase regulatory subunit 2, encoded by the CKS2 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, CKS2 ranked 191 out of 19076 total transcripts, equating to 99.0% differential expression (Chart 1). Differential expression of CKS2 in female breast cancer was statistically significant (Chart 1; $p=3.35E-13$).

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 CKS2 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, CKS2 ranked 47 out of 45015 total transcripts, equating to 99.9% differential expression (Chart 2). Differential expression of CKS2 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=5.61E-14$). These data suggested that differential expression of CKS2 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.

CKS2 is expressed at significantly higher levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for CKS2 from the breast and from breast tumors² to understand the magnitude and direction of CKS2 expression change. CKS2 was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of CKS2 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). CKS2 was expressed at 6.08 ± 0.77 arbitrary units (AU) in normal breast tissue, while it was expressed at 8.68 ± 1.14 AU in tumors of the breast. We calculated a mean fold change of 1.43 in CKS2 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

CKS2 expression correlates with survival outcomes in basal and luminal A human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate CKS2 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 CKS2 expression and overall survival (OS) in patients with luminal A subtype breast cancer (Figure 2; log rank p -value: 0.0018 for overall survival, hazard ratio: 1.66 (1.2-2.29) (Fig. 2)). CKS2 mRNA levels were a negative prognostic indicator in luminal A subtype breast cancer patients. Median OS was 169.2 months for luminal A patients with low tumor expression of CKS2 while median OS was 99 months for luminal A patients with high tumor expression of CKS2 (Chart 3). We also observed a correlation between CKS2 expression and overall survival (OS) in patients with basal subtype breast cancer which approached statistical significance (Figure 2; log rank p -value: 0.06 for overall survival, hazard ratio: 0.69 (0.47-1.02) (Fig. 2)). CKS2 mRNA levels were a positive prognostic indicator in basal subtype breast cancer patients. Median OS was 52.92 months for basal subtype patients with low tumor expression of CKS2 while median OS was 80.64 months for basal subtype patients with high tumor expression of CKS2 (Chart 3). CKS2 primary tumor expression was not correlated with overall survival in luminal B subtype (Figure 2; log rank p -value: 1 for OS, hazard ratio: 1 (0.71-1.42) (Fig. 2)), or in HER2+ breast cancer (Figure 2; log rank p -value: 0.22 for OS, hazard ratio: 1.43 (0.81-2.53) (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 CKS2 was among the most significant transcriptional features in primary tumors from patients with breast cancer. CKS2 expression in primary tumors of the breast was correlated with overall survival in patients with basal and luminal A subtype cancers, with high expression of CKS2 associating with superior survival in basal subtype cancer but with low expression of CKS2 associating with superior survival in luminal A subtype cancer.

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

1 datasets^{2,3}, providing evidence here that CDC28 protein kinase regulatory subunit 2, encoded by the
2 CKS2, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in
3 triple negative breast cancer. CKS2 was expressed at significantly higher levels in primary tumors from
4 patients with breast cancer as compared to normal tissues of the breast. In patients with luminal A subtype
5 breast cancer, low expression of CKS2 was associated with superior overall survival, while in patients
6 with basal subtype breast cancer, high expression of CKS2 was associated with superior overall survival.
7 CKS2 and the molecular processes to which it pertains to may be relevant to the initiation or progression
8 of human breast cancer.
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Rank: 191
Probe ID: 3178583
p-value: 3.35E-13
t: -9.72
B: 19.86992
Gene: CKS2
Gene name: CDC28 protein kinase regulatory subunit 2

Chart 1: CKS2 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: 47
Probe ID: 4913
p-value: 5.61E-14
t: -10.7064004
B: 21.645774
Gene: CKS2
Gene name: CDC28 protein kinase regulatory subunit 2

Chart 2: CKS2 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: CKS2 is expressed at significantly higher levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of CKS2 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 CKS2 expression between normal breast tissue and primary tumors of the breast, a p -value, is $p<0.0001$.

Figure 2: CKS2 expression correlates with overall survival in patients with basal subtype or luminal A subtype breast cancer, but not in patients with 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 CKS2 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 CKS2 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.

Basal

Low CKS2 expression: 52.92 months

High CKS2 expression: 80.64 months

Luminal A

Low CKS2 expression: 169.2 months

High CKS2 expression: 99 months

Chart 3: In basal subtype breast cancer, median overall survival is superior in patients with high primary tumor expression of CKS2, while in luminal A subtype breast cancer, median overall survival is superior in patients with low primary tumor expression of CKS2.

The overall survival of $n=404$ basal subtype breast cancer patients and $n=794$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of CKS2 is listed in this chart.