Differential expression of cyclin dependent kinase inhibitor 2C 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 cyclin dependent kinase inhibitor 2C, CDKN2C, when comparing primary tumors of the breast to the tissue of origin, the normal breast. CDKN2C was also differentially expressed in the tumor cells of patients with triple negative breast cancer. CDKN2C 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 CDKN2C in primary tumors of the breast was correlated with overall survival in patients with basal-like and luminal A subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by PAM50 molecular subtype. CDKN2C may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, CDKN2C, cyclin dependent kinase inhibitor 2C, 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. Cyclin dependent kinase inhibitor 2C emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE38959³ for this global differential gene expression analysis of female breast cancer. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570; the majority of patients whose tumors were analyzed were age 50 or older. 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 tissues whose expression was profiled in this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 CDKN2C expression was significantly different between primary breast tumors and normal breast tissue using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of CDKN2C mRNA expression levels with overall survival (OS) in $n=431$ patients with basal-like subtype cancer, $n=596$ patients with luminal A subtype cancer, $n=439$ patients with luminal B subtype cancer, $n=362$ patients with HER2+ cancer, and $n=51$ patients with normal-like subtype cancer.

Results

We performed discovery of genes associated with breast cancer in females by mining two independently published microarray datasets^{2,3}.

CDKN2C is differentially expressed in primary tumors of the breast.

Studying the global gene expression profiles of 104 breast cancers from patients aged 31 to 89 revealed that the gene encoding cyclin dependent kinase inhibitor 2C, CDKN2C, 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, CDKN2C ranked 132 out of 54675 total transcripts, equating to 99.8% differential expression (Chart 1). Differential expression of CDKN2C in female breast cancer was statistically significant (Chart 1; $p=2.64E-29$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the

tumor cells of patients with triple negative breast cancer (TNBC), again revealed significant differential expression of CDKN2C in human breast cancer (Chart 2). When sorting each of the genes expressed in the tumors of patients with breast cancer (TNBC) based on significance of difference as compared to normal breast tissue, CDKN2C ranked 681 out of 45015 total transcripts, equating to 98.5% differential expression (Chart 2). Differential expression of CDKN2C in the tumor cells of patients with TNBC was statistically significant (Chart 2; $p=2.14e-08$). These data suggested that differential expression of CDKN2C was not an artifact of a single microarray dataset, nor was it strictly associated with one type of breast cancer, rather a general feature of cancers of the breast.

CDKN2C is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for CDKN2C from the breast and from breast tumors to understand the magnitude and direction of CDKN2C expression change. CDKN2C was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of CDKN2C in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). CDKN2C was expressed at 7.68 ± 1.69 arbitrary units (AU) in normal breast tissue, while it was expressed at 4.52 ± 0.56 AU in tumors of the breast. We calculated a mean fold change of 0.59 in CDKN2C mRNA levels when comparing primary tumors of the breast to normal breast tissues.

CDKN2C expression correlates with survival outcomes in basal-like and luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis to study relationships between tumor CDKN2C mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation between CDKN2C expression and overall survival (OS) in patients with basal-like subtype breast cancer which was statistically significant (Figure 2; log rank p -value: 0.034 for overall survival, hazard ratio: 0.66 (0.44-0.97) (Fig. 2)). CDKN2C mRNA levels were a positive prognostic indicator in basal-like subtype breast cancer patients. OS was, on average, markedly greater for basal-like patients with low tumor expression of CDKN2C as compared to for basal-like patients with high tumor expression of CDKN2C (Chart 3).

We also observed a correlation between CDKN2C expression and overall survival (OS) in patients with luminal A subtype breast cancer which was statistically significant (Figure 2; log rank p -value: 0.0074 for overall survival, hazard ratio: 0.55 (0.36-0.86) (Fig. 2)). CDKN2C mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median OS was 136.8 months for luminal A patients with low tumor expression of CDKN2C while median OS was 191.21 months for luminal A patients with high tumor expression of CDKN2C (Chart 3).

CDKN2C primary tumor expression was not correlated with overall survival in luminal B subtype breast cancer (Figure 2; log rank p -value: 0.96 for OS, hazard ratio: 0.99 (0.7-1.41) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank p -value: 0.3 for OS, hazard ratio: 0.78 (0.53-1.14) (Fig. 2)) or in patients with normal-like breast cancer (Figure 2; log rank p -value: 0.28 for OS, hazard ratio: 0.57 (0.21-1.58) (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 CDKN2C was among the most significant transcriptional features in primary tumors from patients with breast cancer. CDKN2C expression in primary tumors of the breast was correlated with overall survival in patients with basal-like and luminal A subtype disease, with mRNA levels of CDKN2C a positive prognostic indicator for basal-like and luminal A breast cancer 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 cyclin dependent kinase inhibitor 2C, encoded by CDKN2C, is a defining transcriptional feature of human breast cancer: in patients diagnosed after age 50, and in early onset-breast cancer. CDKN2C was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly, in patients with basal-like and luminal A subtype breast cancers, expression of CDKN2C was correlated with overall survival. Cyclin dependent kinase inhibitor 2C 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: 132
Probe ID: 211792_s_at
p-value: 2.64E-29
t: 1.50E+01
B: 56.1023019
Gene: CDKN2C
Gene name: cyclin dependent kinase inhibitor 2C

Chart 1: CDKN2C is differentially expressed in the primary tumors of patients with 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: 681
Probe ID: 2001
p-value: 2.14e-08
t: -6.7777495
B: 9.128411
Gene: CDKN2C
Gene name: cyclin dependent kinase inhibitor 2C

Chart 2: CDKN2C 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: CDKN2C is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of CDKN2C 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 CDKN2C expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Figure 2: CDKN2C expression correlates with overall survival in patients with basal-like and luminal A subtype breast cancer, but not in patients with luminal B, HER2+, or normal-like subtype cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=431$ patients with basal-like breast cancer (upper left), $n=362$ patients with HER2+ breast cancer (upper right), $n=596$ patients with luminal A breast cancer (middle left), $n=439$ patients with luminal B breast cancer (middle right), and $n=51$ patients with normal-like breast cancer (below) stratified into two groups,

based on low or high expression of CDKN2C 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 CDKN2C 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 CDKN2C expression: 136.8 months

High CDKN2C expression: 191.21 months

Chart 3: In luminal A subtype breast cancer, median overall survival is superior in patients with high primary tumor expression of CDKN2C.

The overall survival of $n=596$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of CDKN2C is listed in this chart.