Differential expression of PCNA 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 proliferating cell nuclear antigen, PCNA, when comparing primary tumors of the breast to the tissue of origin, the normal breast. PCNA was also differentially expressed in the tumor cells of patients with triple negative breast cancer. PCNA 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 PCNA 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. PCNA may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, PCNA, proliferating cell nuclear antigen, 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 proliferating cell nuclear antigen 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 PCNA 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 PCNA 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}.

PCNA 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 proliferating cell nuclear antigen, encoded by the PCNA 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, PCNA ranked 192 out of 19076 total transcripts, equating to 99.0% differential expression (Chart 1). Differential expression of PCNA in female breast cancer was statistically significant (Chart 1; $p=3.41E-13$).

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 PCNA 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, PCNA ranked 457 out of 45015 total transcripts, equating to 99.0% differential expression (Chart 2). Differential expression of PCNA in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=2.92E-09$). These data suggested that differential expression of PCNA 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.

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

We obtained exact mRNA expression levels for PCNA from the breast and from breast tumors² to understand the magnitude and direction of PCNA expression change. PCNA was expressed at higher levels in tumors of the breast as compared to normal breast tissue (Figure 1). Increased expression of PCNA in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). PCNA was expressed at 7.52 ± 0.31 arbitrary units (AU) in normal breast tissue, while it was expressed at 8.72 ± 0.55 AU in tumors of the breast. We calculated a mean fold change of 1.16 in PCNA mRNA levels when comparing primary tumors of the breast to normal breast tissues.

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

We performed Kaplan-Meier survival analysis in an attempt to correlate PCNA 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 PCNA expression and overall survival (OS) in patients with luminal A subtype breast cancer (Figure 2; log rank p -value: 0.0051 for overall survival, hazard ratio: 1.57 (1.14-2.16) (Fig. 2)). PCNA mRNA levels were a negative prognostic indicator in luminal A subtype breast cancer patients. Median OS was 175.46 months for luminal A patients with low tumor expression of PCNA while median OS was 105.44 months for luminal A patients with high tumor expression of PCNA (Chart 3). We also observed a correlation between PCNA expression and overall survival (OS) in patients with basal subtype breast cancer which trended towards statistical significance (Figure 2; log rank p -value: 0.094 for overall survival, hazard ratio: 0.72 (0.49-1.06) (Fig. 2)). PCNA mRNA levels were a positive prognostic indicator in basal subtype breast cancer patients. Median OS was 48 months for basal subtype patients with low tumor expression of PCNA while median OS was 85.2 months for basal subtype patients with high tumor expression of PCNA (Chart 3). PCNA primary tumor expression was not correlated with overall survival in luminal B subtype (Figure 2; log rank p -value: 0.49 for OS, hazard ratio: 1.13 (0.8-1.6) (Fig. 2)), or in HER2+ breast cancer (Figure 2; log rank p -value: 0.95 for OS, hazard ratio: 0.98 (0.56-1.73) (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 PCNA was among the most significant transcriptional features in primary tumors from patients with breast cancer. PCNA 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 PCNA associating with superior survival in basal subtype cancer but with low expression of PCNA 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 proliferating cell nuclear antigen, encoded by the PCNA, is a
2 defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative
3 breast cancer. PCNA was expressed at significantly higher levels in primary tumors from patients with
4 breast cancer as compared to normal tissues of the breast. In patients with luminal A subtype breast
5 cancer, low expression of PCNA was associated with superior overall survival, while in patients with basal
6 subtype breast cancer, high expression of PCNA was associated with superior overall survival. PCNA and
7 the molecular processes to which it pertains to may be relevant to the initiation or progression of human
8 breast cancer.
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Rank: 192
Probe ID: 3896200
p-value: 3.41E-13
t: -9.72
B: 19.853061
Gene: PCNA
Gene name: proliferating cell nuclear antigen

Chart 1: PCNA 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: 457
Probe ID: 30021
p-value: 2.92E-09
t: -7.3606038
B: 11.072474
Gene: PCNA
Gene name: proliferating cell nuclear antigen

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

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

Figure 2: PCNA 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 PCNA 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 PCNA 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 PCNA expression: 48 months

High PCNA expression: 85.2 months

Luminal A

Low PCNA expression: 175.46 months

High PCNA expression: 105.44 months

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

The overall survival of $n=404$ basal subtype breast cancer patients and $n=694$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of PCNA is listed in this chart.