Differential expression of p21 (RAC1) activated kinase 3 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 p21 (RAC1) activated kinase 3, PAK3, when comparing primary tumors of the breast to the tissue of origin, the normal breast. PAK3 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. PAK3 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 PAK3 in primary tumors of the breast was correlated with post-progression survival in patients with luminal B subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by molecular subtype. PAK3 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, PAK3, p21 (RAC1) activated kinase 3, 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. p21 (RAC1) activated kinase 3 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 in conjunction with GEO2R. 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 $n=39$ 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 PAK3 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 PAK3 mRNA expression levels with post-progression survival (PPS) in $n=76$ patients with basal subtype cancer, $n=204$ patients with luminal A subtype cancer, $n=139$ patients with luminal B subtype cancer, and $n=39$ 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}.

PAK3 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 p21 (RAC1) activated kinase 3, PAK3, 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, PAK3 ranked 117 out of 19076 total transcripts, equating to 99.4% differential expression (Chart 1). Differential expression of PAK3 in female breast cancer was statistically significant (Chart 1; $p=2.44E-14$).

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 PAK3 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, PAK3 ranked 3882 out of 45015 total transcripts, equating to 91.4% differential expression (Chart 2). Differential expression of PAK3 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=1.29E-04$). These data suggested that differential expression of PAK3 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.

PAK3 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for PAK3 from the breast and from breast tumors to understand the magnitude and direction of PAK3 expression change. PAK3 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of PAK3 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). PAK3 was expressed at 7.16 ± 0.56 arbitrary units (AU) in normal breast tissue, while it was expressed at 5.73 ± 0.41 AU in tumors of the breast. We calculated a mean fold change of 0.80 in PAK3 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

PAK3 expression correlates with survival outcomes in luminal B subtype human breast cancer.

We performed Kaplan-Meier survival analysis in an attempt to correlate PAK3 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations between molecular subtype and post-progression survival. We observed a statistically significant correlation between PAK3 expression and post-progression survival (PPS) in patients with luminal B subtype breast cancer which trended towards statistical significance (Figure 2; log rank p -value: 0.088 for post-progression survival, hazard ratio: 1.45 (0.94-2.22) (Fig. 2)). PAK3 mRNA levels were a negative prognostic indicator in luminal B subtype breast cancer patients. Median PPS was 39.25 months for luminal B patients with low tumor expression of PAK3 while median PPS was 20.96 months for luminal B patients with high tumor expression of PAK3 (Chart 3). PAK3 primary tumor expression was not correlated with post-progression survival in basal subtype breast cancer (Figure 2; log rank p -value: 0.39 for PPS, hazard ratio: 0.79 (0.45-1.36) (Fig. 2)), luminal A subtype breast cancer (Figure 2; log rank p -value: 0.26 for PPS, hazard ratio: 1.23 (0.86-1.76) (Fig. 2)) or HER2+ breast cancer (Figure 2; log rank p -value: 0.19 for PPS, hazard ratio: 1.64 (0.77-3.46) (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 PAK3 was among the most significant transcriptional features in primary tumors from patients with breast cancer. PAK3 expression in primary tumors of the breast was correlated with post-progression survival in patients with luminal B subtype disease, with mRNA levels of PAK3 a negative prognostic indicator for luminal B 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 p21 (RAC1) activated kinase 3, encoded by PAK3, is a defining transcriptional feature of human breast cancer: in early onset-breast cancer, and in triple negative breast cancer. PAK3 was expressed at significantly lower levels in primary tumors from

1 patients with breast cancer as compared to normal breast tissue. Importantly, in patients with luminal B
2 subtype breast cancer, expression of PAK3 was correlated with post-progression survival. p21 (RAC1)
3 activated kinase 3 and the molecular processes to which it pertains to may be relevant to the initiation or
4 progression of human breast cancer.
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Rank: 117
Probe ID: 3987228
p-value: 2.44E-14
t: 1.05E+01
B: 22.450575
Gene: PAK3
Gene name: p21 (RAC1) activated kinase 3

Chart 1: PAK3 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: 3882
Probe ID: 2767
p-value: 1.29E-04
t: 4.1879769
B: 0.695167
Gene: PAK3
Gene name: p21 (RAC1) activated kinase 3

Chart 2: PAK3 is differentially expressed in the primary 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: PAK3 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of PAK3 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 PAK3 expression between normal breast tissue and primary tumors of the breast, a *p*-value, is $p < 0.0001$.

Figure 2: PAK3 expression correlates with post-progression survival in patients with luminal B subtype breast cancer, but not in patients with basal subtype, luminal A subtype, or HER2+ cancer.

Depicted in this Kaplan-Meier plot is the probability of post-progression survival (PPS) for $n=76$ patients with basal subtype breast cancer (upper left), $n=39$ patients with HER2+ breast cancer (upper right), $n=204$ patients with luminal A breast cancer (lower left), and $n=139$ patients with luminal B breast cancer (lower right), stratified into two groups, based on low or high expression of PAK3, also known as ARHGEF7, in patient primary tumors. The log rank p-value denoting statistical significance of difference in post-progression 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 PAK3 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 B patients

Low PAK3 expression: 39.25 months

High PAK3 expression: 20.96 months

Chart 3: In luminal B breast cancer, median post-progression survival is superior in patients with low primary tumor expression of PAK3.

The post-progression survival of $n=38$ luminal B subtype breast cancer patients based on stratification into low or high tumor expression of PAK3 is listed in this chart.