Differential expression of G protein-coupled receptor associated sorting protein 2 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 G protein-coupled receptor associated sorting protein 2, GPRASP2, when comparing primary tumors of the breast to the tissue of origin, the normal breast. GPRASP2 was also differentially expressed in the tumor cells of patients with triple negative breast cancer. GPRASP2 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 GPRASP2 in primary tumors of the breast was correlated with overall survival in black patients with high mutational burden, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by race and mutational burden. GPRASP2 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, GPRASP2, G protein-coupled receptor associated sorting protein 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. G protein-coupled receptor associated sorting protein 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 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 $n=30$ 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 GPRASP2 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 GPRASP2 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}.

GPRASP2 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 G protein-coupled receptor associated sorting protein 2, GPRASP2, 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, GPRASP2 ranked 135 out of 19076 total transcripts, equating to 99.3% differential expression (Chart 1). Differential expression of GPRASP2 in female breast cancer was statistically significant (Chart 1; $p=6.22E-14$).

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 GPRASP2 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, GPRASP2 ranked 2976 out of 45015 total transcripts, equating to 93.4% differential expression (Chart 2). Differential expression of GPRASP2 in the tumor cells of patients with triple negative breast cancer was statistically significant (Chart 2; $p=3.41E-05$). These data suggested that differential expression of GPRASP2 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.

GPRASP2 is expressed at significantly lower levels in breast tumors as compared to the breast.

We obtained exact mRNA expression levels for GPRASP2 from the breast and from breast tumors to understand the magnitude and direction of GPRASP2 expression change. GPRASP2 was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of GPRASP2 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). GPRASP2 was expressed at 6.29 ± 0.31 arbitrary units (AU) in normal breast tissue, while it was expressed at 5.47 ± 0.26 AU in tumors of the breast. We calculated a mean fold change of 0.87 in GPRASP2 mRNA levels when comparing primary tumors of the breast to normal breast tissues.

In human breast cancer, GPRASP2 expression correlates with survival outcomes in black patients with high mutational burden.

We performed Kaplan-Meier survival analysis in an attempt to correlate GPRASP2 mRNA expression levels with survival outcomes in patients with breast cancer, evaluating correlations with race and mutational burden. We observed a statistically significant correlation between GPRASP2 expression and overall survival (RFS) in patients with black patients with high mutational burden (Figure 2; log rank p -value: 0.015 for overall survival, hazard ratio: 4.55 (1.2-17.26) (Fig. 2)). GPRASP2 mRNA levels were a positive prognostic indicator in black patients with high mutational burden. Median RFS was 131.97 months for black patients with high mutational burden that possessed low tumor expression of GPRASP2 while median RFS was 78.7 months for black patients with low mutational burden that possessed high tumor expression of GPRASP2 (Chart 3). GPRASP2 primary tumor expression was not correlated with overall survival in white patients with high mutational burden (Figure 2; log rank p -value: 0.22 for RFS, hazard ratio: 1.43 (0.81-2.51) (Fig. 2)), white patients with low mutational burden (Figure 2; log rank p -value: 0.81 for RFS, hazard ratio: 1.07 (0.61-1.86) (Fig. 2)), nor in black patients with low mutational burden (Figure 2; log rank p -value: 0.32 for RFS, hazard ratio: 0.55 (0.16-1.83) (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 GPRASP2 was among the most significant transcriptional features in primary tumors from patients with breast cancer. In human breast cancer, GPRASP2 expression in primary tumors of the breast was correlated with overall survival in black patients with high mutational burden, with mRNA levels of GPRASP2 a negative prognostic indicator for 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 G protein-coupled receptor associated sorting protein 2, encoded by GPRASP2, is a defining transcriptional feature of human breast cancer: in

early onset-breast cancer, and in triple negative breast cancer. GPRASP2 was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly, in black patients with high mutational burden, expression of GPRASP2 was correlated with overall survival. G protein-coupled receptor associated sorting protein 2 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: 135
Probe ID: 3985305
p-value: 6.22E-14
t: 1.02E+01
B: 21.529952
Gene: GPRASP2
Gene name: G protein-coupled receptor associated sorting protein 2

Chart 1: GPRASP2 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: 2976
Probe ID: 21797
p-value: 3.41E-05
t: 4.6009578
B: 1.969874
Gene: GPRASP2-AS1
Gene name: GPRASP2 antisense RNA 1

Chart 2: An antisense RNA encoded by the GPRASP2 locus 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: GPRASP2 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of GPRASP2 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 GPRASP2 expression between normal breast tissue and primary tumors of the breast, a p -value, is $p < 0.0001$.

Figure 2: GPRASP2 expression correlates with overall survival in black patients with high mutational burden, but not in black patients with low mutational burden, nor in white patients with high or low mutational burden.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=1090$ patients, segregated based on mutational burden and race (white or black), in black women with low mutational burden (upper left), in black women with high mutational burden (upper right), in white women with low mutational burden (lower left), and in white women with high mutational burden (lower right), further stratified into two groups, based on low or high expression of GPRASP2 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 GPRASP2 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.

Black females; high mutational burden

Low GPRASP2 expression: 131.97 months

High GPRASP2 expression: 78.7 months

Chart 3: In black females with high mutational burden, median overall survival is superior in patients with primary tumor expression of GPRASP2.

The overall survival of black female cancer patients with high mutational burden, based on stratification into low or high tumor expression of GPRASP2 is listed in this chart.