Differential expression of RNA-binding protein with multiple splicing 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 RNA-binding protein with multiple splicing 2, RBPMS2, when comparing primary tumors of the breast to the tissue of origin, the normal breast. RBPMS2 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 RBPMS2 in primary tumors of the breast was correlated with overall survival in patients with basal-like and luminal B subtype cancer, demonstrating a relationship between primary tumor expression of a differentially expressed gene and patient survival outcomes influenced by PAM50 molecular subtype. RBPMS2 may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, RBPMS2, RNA-binding protein with multiple splicing 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. RNA-binding protein with multiple splicing 2 emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE109169³ 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. 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 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 RBPMS2 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 RBPMS2 mRNA expression levels with overall survival (OS) in $n=296$ patients with basal-like subtype cancer, $n=222$ patients with luminal A subtype cancer, $n=200$ patients with luminal B subtype cancer, $n=198$ patients with HER2+ cancer, and $n=27$ 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}.

RBPMS2 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 RNA-binding protein with multiple splicing 2, RBPMS2, 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, RBPMS2 ranked 143 out of 54675 total transcripts, equating to 99.7% differential expression (Chart 1). Differential expression of RBPMS2 in female breast cancer was statistically significant (Chart 1; $p=4.06E-29$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the tumors of 25 patients with early-onset breast cancer, again revealed significant differential expression of

1 RBPMS2 in human breast cancer (Chart 2). When sorting each of the genes expressed in the tumors of
2 patients with breast cancer based on significance of difference as compared to normal breast tissue,
3 RBPMS2 ranked 3593 out of 19076 total transcripts, equating to 81.2% differential expression (Chart 2).
4 Differential expression of RBPMS2 in the tumors of patients with breast cancer was statistically
5 significant (Chart 2; $p=4.69E-05$). These data suggested that differential expression of RBPMS2 was not
6 an artifact of a single microarray dataset, nor was it strictly associated with early-onset breast cancer,
7 rather a general feature of cancers of the breast.

8 **RBPMS2 is expressed at significantly lower levels in breast tumors as compared to the breast.**

9 We obtained exact mRNA expression levels for RBPMS2 from the breast and from breast tumors
10 to understand the magnitude and direction of RBPMS2 expression change. RBPMS2 was expressed at
11 lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression
12 of RBPMS2 in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). RBPMS2 was
13 expressed at 5.97 ± 1.72 arbitrary units (AU) in normal breast tissue, while it was expressed at 2.86 ± 0.53
14 AU in tumors of the breast. We calculated a mean fold change of 0.48 in RBPMS2 mRNA levels when
15 comparing primary tumors of the breast to normal breast tissues.

16 **RBPMS2 expression correlates with survival outcomes in basal-like and luminal B subtype human breast cancer.**

17 We performed Kaplan-Meier survival analysis to study relationships between tumor RBPMS2
18 mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation
19 between RBPMS2 expression and overall survival (OS) in patients with basal-like subtype breast cancer
20 which approached statistical significance (Figure 2; log rank p -value: 0.056 for overall survival, hazard
21 ratio: 1.58 (0.98-2.54) (Fig. 2)). RBPMS2 mRNA levels were a negative prognostic indicator in basal-like
22 subtype breast cancer patients. Median OS was 89.04 months for basal-like patients with low tumor
23 expression of RBPMS2 while median OS was 54.6 months for basal-like patients with high tumor
24 expression of RBPMS2 (Chart 3).

25 We also observed a correlation between RBPMS2 expression and overall survival (OS) in patients
26 with luminal B subtype breast cancer which trended towards statistical significance (Figure 2; log rank
27 p -value: 0.096 for overall survival, hazard ratio: 1.61 (0.92-2.82) (Fig. 2)). RBPMS2 mRNA levels were a
28 negative prognostic indicator in luminal B subtype breast cancer patients. Median OS was 121.2 months
for luminal B patients with low tumor expression of RBPMS2 while median OS was 63.36 months for
luminal B patients with high tumor expression of RBPMS2 (Chart 3). RBPMS2 primary tumor
expression was not correlated with overall survival in luminal A subtype breast cancer (Figure 2; log rank
 p -value: 0.66 for OS, hazard ratio: 1.17 (0.58-2.38) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank
 p -value: 0.58 for OS, hazard ratio: 1.17 (0.68-2) (Fig. 2)) or in patients with normal-like breast cancer
(Figure 2; log rank p -value: 0.28 for OS, hazard ratio: 2.07 (0.53-8.05) (Fig. 2)).

29 Thus, through comparative transcriptome analysis of primary tumors of the breast and normal
30 breast tissue, we found that differential expression and down-regulation of RBPMS2 was among the most
31 significant transcriptional features in primary tumors from patients with breast cancer. RBPMS2
32 expression in primary tumors of the breast was correlated with overall survival in patients with basal-like
33 and luminal B subtype disease, with mRNA levels of RBPMS2 a negative prognostic indicator for
34 basal-like and luminal B breast cancer patients.

35 **Discussion**

36 Invasive breast cancer is a medical problem with a 27% 5-year survival rate for women whose

1 disease has spread to distant sites^{5,6}. To facilitate understanding of the basic transcriptional differences
2 between primary tumors of the breast and the tissues from which these tumors originate, normal breast
3 tissues, we performed comparative transcriptome analysis using two independently published microarray
4 datasets^{2,3}, providing evidence here that differential expression of RNA-binding protein with multiple
5 splicing 2, encoded by RBPMS2, is a defining transcriptional feature of human breast cancer: in patients
6 diagnosed after age 50, and in early onset-breast cancer. RBPMS2 was expressed at significantly lower
7 levels in primary tumors from patients with breast cancer as compared to normal breast tissue.
8 Importantly, in patients with basal-like and luminal B subtype breast cancers, expression of RBPMS2 was
9 correlated with overall survival. RNA-binding protein with multiple splicing 2 and the molecular
10 processes to which it pertains to may be relevant to the initiation or progression of human breast cancer.
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Rank: 143
Probe ID: 228802_at
p-value: 4.06E-29
t: 1.49E+01
B: 55.6783739
Gene: RBPMS2
Gene name: RNA-binding protein with multiple splicing 2

Chart 1: RBPMS2 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: 3593
Probe ID: 3629243
p-value: 4.69E-05
t: 4.45
B: 1.456416
Gene: RBPMS2
Gene name: RNA-binding protein with multiple splicing 2

Chart 2: RBPMS2 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.

Figure 1: RBPMS2 is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

The mRNA expression level of RBPMS2 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 RBPMS2 expression between normal breast tissue and primary tumors of the breast, a *p*-value, listed above.

Basal-like patients

Low RBPMS2 expression: 89.04 months

High RBPMS2 expression: 54.6 months

Luminal B patients

Low RBPMS2 expression: 121.2 months

High RBPMS2 expression: 63.36 months

Chart 3: In basal-like and luminal B subtype breast cancer, median overall survival is superior in patients with low primary tumor expression of RBPMS2.

The overall survival of $n=296$ basal-like subtype and $n=200$ luminal B subtype breast cancer patients based on stratification into low or high tumor expression of RBPMS2 is listed in this chart.