Differential expression of melanocortin 2 receptor accessory protein 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 melanocortin 2 receptor accessory protein, MRAP, when comparing primary tumors of the breast to the tissue of origin, the normal breast. MRAP was also differentially expressed in the brain metastases of patients with metastatic breast cancer. MRAP 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 MRAP in primary tumors of the breast was correlated with overall survival in patients with 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. MRAP may be of relevance to initiation, maintenance or progression of cancers of the female breast.

Keywords: breast cancer, MRAP, melanocortin 2 receptor accessory protein, 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. Melanocortin 2 receptor accessory protein emerged as among the most differentially expressed genes in cancer of the female breast.

Methods

We utilized datasets GSE42568² and GSE52604³ 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. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer; analysis was performed using platform GPL6480. 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 MRAP 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 MRAP 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 A 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}.

MRAP 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 melanocortin 2 receptor accessory protein, MRAP, 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, MRAP ranked 27 out of 54675 total transcripts, equating to 99.95% differential expression (Chart 1). Differential expression of MRAP in female breast cancer was statistically significant (Chart 1; $p=9.24E-37$).

Analysis of a second microarray dataset³, here studying global gene expression patterns in the brain metastases of patients with metastatic breast cancer, again revealed significant differential expression of MRAP in tumor tissue of patients with human breast cancer (Chart 2). When sorting each of the genes

expressed in the brain metastases of patients with breast cancer based on significance of difference as compared to normal breast tissue, MRAP ranked 39 out of 41093 total transcripts, equating to 99.9% differential expression (Chart 2). Differential expression of MRAP in the brain metastases of patients with breast cancer was statistically significant (Chart 2; $p=4.49E-15$). These data suggested that differential expression of MRAP was not an artifact of a single microarray dataset, nor was it strictly associated with primary tumors of the breast, rather a reflection of the spectrum of transformation in human cancer.

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

We obtained exact mRNA expression levels for MRAP from the breast and from breast tumors to understand the magnitude and direction of MRAP expression change. MRAP was expressed at lower levels in tumors of the breast as compared to normal breast tissue (Figure 1). Decreased expression of MRAP in primary breast tumors was statistically significant (Figure 1: $p<0.0001$). MRAP was expressed at 5.15 ± 1.55 arbitrary units (AU) in normal breast tissue, while it was expressed at 2.34 ± 0.15 AU in tumors of the breast. We calculated a mean fold change of 0.45 in MRAP mRNA levels when comparing primary tumors of the breast to normal breast tissues.

MRAP expression correlates with survival outcomes in luminal A subtype human breast cancer.

We performed Kaplan-Meier survival analysis to study relationships between tumor MRAP mRNA expression levels and survival outcomes in patients with breast cancer. We observed a correlation between MRAP expression and overall survival (OS) in patients with luminal A subtype breast cancer, in the lower quartile, which trended towards statistical significance (Figure 2; log rank p -value: 0.092 for overall survival, hazard ratio: 0.54 (0.26-1.12) (Fig. 2)). MRAP mRNA levels were a positive prognostic indicator in luminal A subtype breast cancer patients. Median OS was 97.2 months for luminal A patients with low tumor expression of MRAP while median OS was 169.2 months for luminal A patients with high tumor expression of MRAP (Chart 3). MRAP primary tumor expression was not correlated with overall survival in basal-like breast cancer (Figure 2; log rank p -value: 0.25 for OS, hazard ratio: 0.74 (0.45-1.22) (Fig. 2)), luminal B subtype breast cancer (Figure 2; log rank p -value: 0.53 for OS, hazard ratio: 1.25 (0.63-2.5) (Fig. 2)), in HER2+ breast cancer (Figure 2; log rank p -value: 0.28 for OS, hazard ratio: 1.46 (0.73-2.91) (Fig. 2)) or in patients with normal-like breast cancer (Figure 2; log rank p -value: 0.16 for OS, hazard ratio: 3.95 (0.49-31.69) (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 MRAP was among the most significant transcriptional features in primary tumors from patients with breast cancer. MRAP expression in primary tumors of the breast was correlated with overall survival in patients with luminal A subtype disease, with mRNA levels of MRAP a positive 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 melanocortin 2 receptor accessory protein, encoded by MRAP, is a defining transcriptional feature of human breast cancer: in patients diagnosed after age 50, and in brain metastatic tissues from patients with human breast cancer. MRAP was expressed at significantly lower levels in primary tumors from patients with breast cancer as compared to normal breast tissue. Importantly, in patients with luminal A subtype breast cancers,

1 expression of MRAP was correlated with overall survival. Melanocortin 2 receptor accessory protein and
2 the molecular processes to which it pertains to may be relevant to the initiation or progression of human
3 breast cancer.
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Rank: 27
Probe ID: 1555741_at
p-value: 9.24E-37
t: 1.84E+01
B: 73.0433318
Gene: MRAP
Gene name: melanocortin 2 receptor accessory protein

Chart 1: MRAP 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: 39
Probe ID: A_24_P126210
p-value: 4.49E-15
t: 11.3642151
B: 24.051596
Gene: MRAP
Gene name: melanocortin 2 receptor accessory protein

Chart 2: MRAP is differentially expressed in the brain metastases of patients with metastatic 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: MRAP is expressed at significantly lower levels in primary breast tumors as compared to normal breast tissue.

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

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

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=296$ patients with basal-like breast cancer (upper left), $n=198$ patients with HER2+ breast cancer (upper right), $n=222$ patients with luminal A breast cancer (middle left), $n=200$ patients with luminal B breast cancer (middle right), and $n=27$ patients with normal-like breast cancer (below) stratified into two groups, based on low or high expression of MRAP 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 MRAP 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 MRAP expression: 97.2 months

High MRAP expression: 169.2 months

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

The overall survival of $n=200$ luminal A subtype breast cancer patients based on stratification into low or high tumor expression of MRAP is listed in this chart.