ATP-binding cassette transporter sub-family C member 8, ABCC8, is a differentially expressed gene in brain metastatic human breast cancer.

Shahan Mamoor, MS[1]
[1]shahanmamoor@gmail.com
East Islip, NY USA

Metastasis to the brain is a clinical problem in patients with breast cancer[1-3]. We mined published microarray data[4,5] to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that the ATP-binding cassette transporter sub-family C member 8, encoded by ABCC8, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. ABCC8 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of ABCC8 in primary tumors was significantly correlated with patient overall survival. Modulation of ABCC8 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, ATP-binding cassette transporter sub-family C member 8, ABCC8, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in patients treated with trastuzumab for breast cancer[2]. More recently, the NEfERT-T clinical trial[6] which compared administration of either neratinib or trastuzumab in conjunction with paclitaxel demonstrated that in a randomized, controlled setting, in breast cancer patients treated with neratinib, not only was the incidence of central nervous system recurrence significantly lower, the time to central nervous system metastasis was significantly delayed as compared to patients administered trastuzumab[6]. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal[2] and from a randomized, controlled setting[6] illustrating that treatment with trastuzumab may be associated with these events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. We performed a global comparative analysis of primary and metastatic tumors in patients with brain metastatic breast cancer[4,5]. We discovered differential and increased expression of the gene encoding ATP-binding cassette transporter sub-family C member 8, ABCC8, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893[4] and GSE42568[5] for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE10893 was generated using Agilent-011521 Human 1A Microarray G4110A technology with *n*=11 primary breast tumors and *n*=3 brain metastases from patients with breast cancer; analysis was performed using platform GPL885. 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 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 ABCC8 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool[7] for correlation of ABCC8 mRNA expression levels with overall survival in *n*=1402 breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with metastatic breast cancer using published microarray data[4,5] to describe the transcriptional landscape of brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

ABCC8 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data[4], we identified the

ATP-binding cassette transporter sub-family C member 8, encoded by ABCC8, as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, ABCC8 ranked 22 out of 17418 total transcripts (Chart 1), equating to 99.9% differential expression. Differential expression of ABCC8 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; p=2.54E-05).

To validate transcriptome-wide differential expression of ABCC8 in human breast cancer, we queried a second microarray dataset[5], here comparing normal breast tissues and primary tumors of the breast. Again, we identified ABCC8 as a differentially expressed gene in the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to normal breast tissues, ABCC8 ranked 21302 out of 54675 total transcripts (Chart 2), equating to 61.04% differential expression. Differential expression of ABCC8 in the primary tumors of patients with breast cancer approached the level of statistical significance (Chart 2; p=5.20E-02). Thus, differential expression of ABCC8, transcriptome-wide, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

ABCC8 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for ABCC8, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in ABCC8 expression in brain metastatic tissues. ABCC8 was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; p=0.000065).

ABCC8 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis[8] in 1402 breast cancer patients in total, to evaluate whether ABCC8 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of ABCC8 and overall survival in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of ABCC8 possessed median OS of 84 months, while patients whose tumors expressed high levels of ABCC8 possessed median OS of 120 months. This difference in OS based on ABCC8 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p-value: 0.021; hazard ratio: 0.78 (0.63-0.96)).

Thus, by mining published microarray data[4,5] in an unbiased fashion, we identified ATP-binding cassette transporter sub-family C member 8, encoded by ABCC8, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases and primary tumors of patients with breast cancer; we observed significantly increased expression of ABCC8 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between ABCC8 expression and patient survival outcomes, as overall survival was significantly higher in patients whose primary tumors expressed higher levels of ABCC8 as compared to patients whose primary tumors expressed lower levels of ABCC8.

Discussion

We provided evidence here that ATP-binding cassette transporter sub-family C member 8, encoded by ABCC8, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that ABCC8 mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, that ABCC8 is also differentially expressed in primary tumors of patients with breast cancer and that primary tumor ABCC8 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of ABCC8 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. ABCC8 has been identified as an independent prognostic factor in glioma, with patients whose tumors expressed high levels of ABCC8 mRNA possessing improved patient survival[8], consistent with our findings in patients with breast cancer. ABCC8 mRNA primary tumor expression in glioma patients was predictive of sensitivity to temozolomide chemotherapy[8]. Modulation of ABCC8 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

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Rank: 22
Probe ID: 2692
p-value: 2.54E-05
t: -5.86
B: 2.767975
Gene: ABCC8
Gene name: ATP-binding cassette transporter sub-family C member 8

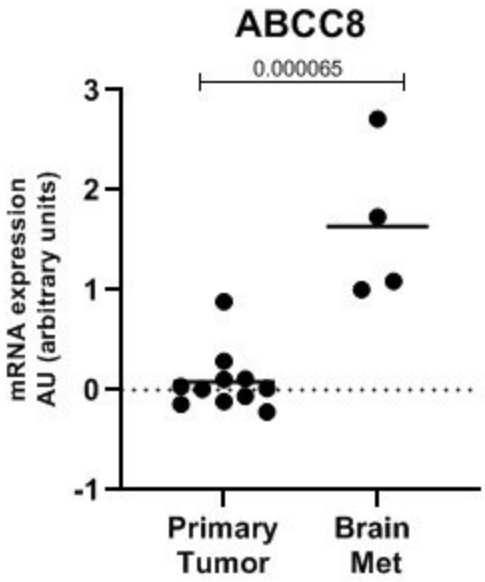
Chart 1: ABCC8 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 21302
probe ID: 210245_at
p-value: 5.20E-02
t: 1,96
B: -5.2984322
Gene: ABCC8
Gene name: ATP-binding cassette transporter sub-family C member 8

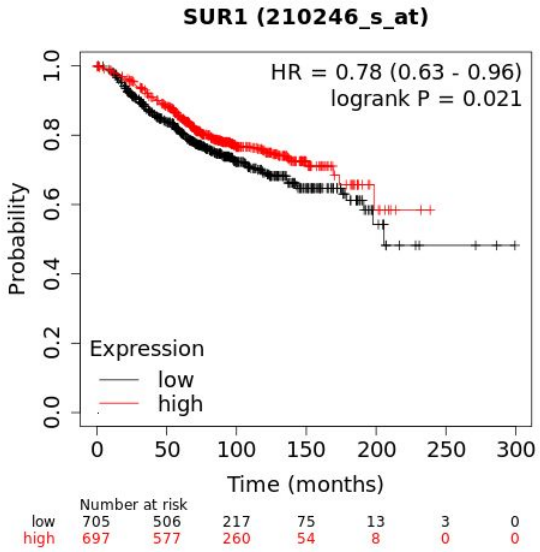
Chart 2: ABCC8 is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.



<u>Figure 1:</u> ABCC8 is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of ABCC8 in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in ABCC8 expression between primary tumors of the breast and brain metastases is p=0.000065.



<u>Figure 2:</u> Significant correlation between ABCC8 primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for *n*=1402 total patients stratified into two groups, based on low or high expression of ABCC8 (SUR1) 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 ABCC8 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.

Low ABCC8 expression: 84 months
High ABCC8 expression: 120 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of ABCC8.

The median OS (overall survival) of patients with low primary tumor expression of ABCC8 and high primary tumor expression of ABCC8 is listed in this chart, for *n*=1402 breast cancer patients, in the upper survival quartile.