SLC16A2 is a differentially expressed gene in brain metastatic human breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. 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 solute carrier family 16 member 2, encoded by SLC16A2, 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. SLC16A2 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of SLC16A2 in primary tumors was significantly correlated with patient distant metastasis-free survival. Modulation of SLC16A2 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, the solute carrier family 16 member 2, SLC16A2, 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 cancer². More recently, the NEfERT-T clinical trial⁶ 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 trastuzumab⁶. The alarmingly high rate of central nervous system metastasis described, as well as data, both anecdotal² and from a randomized, controlled setting⁶ 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 the solute carrier family 16 member 2, SLC16A2, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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. 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 SLC16A2 gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using an unpaired two-tailed t-test with Welch's correction; PRISM 9.0 was utilized for statistical analyses. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of SLC16A2 mRNA expression levels with distant metastasis-free survival in $n=1803$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic 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.

SLC16A2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified the

1 solute carrier family 16 member 2, encoded by SLC16A2, as a differentially expressed gene in
2 the breast metastatic tissues of humans with breast cancer (Table 1). When sorting each of the
3 genes expressed in brain metastases based on significance of difference as compared to primary
4 tumors of the breast in patients with breast cancer, SLC16A2 ranked 156 out of 17418 total
5 transcripts (Chart 1), equating to 99.1% differential expression. Differential expression of
SLC16A2 in the brain metastases of patients with metastatic breast cancer was statistically
significant (Chart 1; $p=3.63E-04$).

6 Differential gene expression analyses that utilize primary and metastatic tumor tissues to
7 discover genes associated with metastasis can be challenging due to the relatively heterogenous
8 cellular composition and constitution of the tumor as compared to normal, untransformed
(benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential
9 expression of SLC16A2 in brain metastases in human metastatic breast cancer, we queried a
10 second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of
11 the breast to brain metastases. Again, we identified SLC16A2 as a differentially expressed gene
12 in the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the
13 genes expressed in brain metastases based on significance of difference as compared to normal
14 breast tissues, SLC16A2 ranked 15554 out of 41093 total transcripts (Chart 2), equating to 62.1%
differential expression. Differential expression of SLC16A2 in the brain metastases of patients
with metastatic breast cancer was statistically significant (Chart 2; $p=3.29E-02$). Thus, differential
expression of SLC16A2, transcriptome-wide, in the brain metastases of women with metastatic
breast cancer was conserved across two independent microarray datasets, both when compared to
benign and transformed breast tissues.

15 **SLC16A2 is expressed at higher levels in the brain metastases of patients with metastatic** 16 **breast cancer.**

17 We obtained exact mRNA expression levels for SLC16A2, in primary tumors of the breast
18 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
19 statistical significance of change in SLC16A2 expression in brain metastatic tissues. SLC16A2
20 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;
 $p=0.000989$).

21 **SLC16A2 expression is significantly correlated with survival outcomes in human breast** 22 **cancer.**

23 We performed Kaplan-Meier survival analysis⁸ in $n=1803$ breast cancer patients in total, to
24 evaluate whether SLC16A2 tumor expression was correlated with survival outcomes in breast
25 cancer. We observed a statistically significant correlation between primary tumor expression of
26 SLC16A2 and distant metastasis-free survival (DMFS) in patients with breast cancer (Figure 2).
27 Patients whose primary tumors expressed low levels of SLC16A2 possessed median DMFS of
130.72 months, while patients whose tumors expressed high levels of SLC16A2 possessed
28 median DMFS of 78.71 months. This difference in DMFS based on SLC16A2 tumor expression
in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value:
0.017; hazard ratio: 1.26 (1.04-1.53)).

1 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
2 identified the solute carrier family 16 member 2, encoded by SLC16A2, as among the genes
3 whose expression was most different, transcriptome-wide, in the brain metastases of patients with
4 breast cancer, both when compared to primary tumors of the breast and to normal breast tissues;
5 we observed significantly increased expression of SLC16A2 in brain metastases as compared to
6 primary tumors of the breast. Further, we found a significant correlation between SLC16A2
expression and patient survival outcomes, as distant metastasis-free survival was significantly
greater in patients whose primary tumors expressed lower levels of SLC16A2 as compared to
patients whose primary tumors expressed higher levels of SLC16A2.

7 **Discussion**

8 We provided evidence here that the solute carrier family 16 member 2, encoded by
9 SLC16A2, is among the genes whose expression is most different in the brain metastases of
10 patients with brain metastatic breast cancer, that SLC16A2 mRNA is present at significantly
11 increased quantities in brain metastatic tissues as compared to primary tumors of the breast, and
12 that primary tumor SLC16A2 expression is significantly correlated with patient survival
13 outcomes in human breast cancer. Evaluation of the effects of genetic depletion of SLC16A2 in
14 mouse models of metastatic breast cancer on metastasis to the central nervous system is merited.
15 Modulation of SLC16A2 expression may be relevant to the processes by which breast cancer
16 cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade
17 immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 156
Probe ID: 13174
p-value: 3.63E-04
t: -4.87
B: 1.007256
Gene: SLC16A2
Gene name: solute carrier family 16 member 2

Chart 1: SLC16A2 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: 15554
probe ID: A_23_P347027
p-value: 3.29E-02
t: -2.1981077
B: -4.412027
Gene: SLC16A2
Gene name: solute carrier family 16 member 2

Chart 2: SLC16A2 is differentially expressed in brain metastatic breast cancer when comparing brain metastases 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.

Figure 1: SLC16A2 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 SLC16A2 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 SLC16A2 expression between primary tumors of the breast and brain metastases, a *p*-value, is $p=0.000989$.

Figure 2: Significant correlation between SLC16A2 primary tumor expression and distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=1803$ total patients stratified into two groups, based on low or high expression of SLC16A2 in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-free 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 SLC16A2 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 SLC16A2 expression: 130.72 months

High SLC16A2 expression: 78.71 months

Chart 3: Median distant metastasis-free survival is inferior in patients with high primary tumor expression of SLC16A2.

The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of SLC16A2 and high primary tumor expression of SLC16A2 is listed in this chart, for $n=1803$ breast cancer patients.