SIVA1 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 apoptosis regulatory protein, Siva, encoded by SIVA1, 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 breast⁴. SIVA1 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of SIVA1 in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of SIVA1 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 apoptosis regulatory protein, Siva, SIVA1, 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 apoptosis regulatory protein, Siva, SIVA1, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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 metastatic breast cancer; analysis was performed using platform GPL885. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array with $n=16$ primary breast tumors and $n=3$ brain metastases from patients metastatic breast cancer; analysis was performed using platform GPL6244. 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 SIVA1 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 tool⁷ for correlation of SIVA1 mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients, and 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.

SIVA1 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

the apoptosis regulatory protein, Siva, encoded by SIVA1, 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, SIVA1 ranked 65 out of 17418 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of SIVA1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.48e-04$).

To attempt to validate transcriptome-wide differential expression of SIVA1 in brain metastatic tissues of patients with metastatic breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to brain metastases. Again, we identified SIVA1 as a differentially expressed gene in human brain metastatic breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast, SIVA1 ranked 2109 out of 33297 total transcripts (Chart 2), equating to 93.7% differential expression. Differential expression of SIVA1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=1.69e-03$). Thus, differential expression of SIVA1, transcriptome-wide, in brain metastatic tissues of patients with metastatic breast cancer could be observed across two independent microarray datasets.

SIVA1 is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for SIVA1, 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 SIVA1 expression in brain metastatic tissues. We observed significantly higher expression of SIVA1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: SIVA1 was expressed at -0.44 ± 0.25 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -0.03 ± 0.89 AU in brain metastatic tissues (Figure 1). The difference in SIVA1 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000293$).

SIVA1 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in 3951 breast cancer patients in total, to evaluate whether SIVA1 tumor expression was correlated with survival outcomes in human breast cancer. We observed a statistically significant correlation between primary tumor expression of SIVA1 and recurrence-free survival (RFS) in patients with breast cancer, in the upper survival quartile (Figure 2). Patients whose primary tumors expressed low levels of SIVA1 possessed median RFS of 55 months, while patients whose tumors expressed high levels of SIVA1 possessed median RFS of 35 months. This difference in RFS based on SIVA1 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: $1.2e-06$; hazard ratio: 1.34 (1.19-1.51)).

1 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
2 identified the apoptosis regulatory protein, Siva, encoded by SIVA1, as among the genes whose
3 expression was most different, transcriptome-wide, in the brain metastases of patients with breast
4 cancer, when compared to primary tumors of the breasts; we observed significantly increased
5 expression of SIVA1 in brain metastases as compared to primary tumors of the breast. Further,
6 we found a significant correlation between SIVA1 expression and patient survival outcomes, as
recurrence-free survival was significantly greater in patients whose primary tumors expressed
lower levels of SIVA1 as compared to patients whose primary tumors expressed higher levels of
SIVA1.

7 **Discussion**

8 We provided evidence here that the the apoptosis regulatory protein, Siva, encoded by
9 SIVA1, is among the genes whose expression is most different in the brain metastases of patients
10 with brain metastatic breast cancer, that SIVA1 mRNA is present at significantly increased
11 quantities in brain metastatic tissues as compared to primary tumors of the breast, and that
12 primary tumor SIVA1 expression is significantly correlated with patient survival outcomes in
13 human breast cancer. Evaluation of the effects of genetic depletion of SIVA1 in mouse models of
14 metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of
15 SIVA1 expression may be relevant to the processes by which breast cancer cells exit the breast,
16 enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance,
17 breach the blood-brain barrier and colonize the brain.
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Rank: 65
Probe ID: 14656
P-value: 1.48e-04
t: -5.06
B: 1.1843
Gene: SIVA1
Gene name: Siva1 apoptosis-inducing factor

Chart 1: SIVA1 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: 2109
probe ID: 7977288
p-value: 1.69e-03
t: -3.6232755
B: -1.331698
Gene: SIVA1
Gene name: Siva1 apoptosis-inducing factor

Chart 2: SIVA1 is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

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: SIVA1 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 SIVA1 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 SIVA1 expression between primary tumors and brain metastasis, a p -value, is $p=0.000293$.

Figure 2: Significant correlation between SIVA1 primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of SIVA1 in patient primary tumors, in the upper survival quartile. The log rank p -value denoting statistical significance of difference in recurrence-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 SIVA1 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 SIVA1 expression: 55 months

High SIVA1 expression: 35 months

Chart 3: Median recurrence-free survival is inferior in patients with high primary tumor expression of SIVA1.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of SIVA1 and high primary tumor expression of SIVA1 is listed in this chart, for $n=3951$ breast cancer patients, in the upper survival quartile.