DHR SX 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 dehydrogenase/reductase X-linked, encoded by DHR SX, 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. DHR SX mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of DHR SX in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of DHR SX 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, dehydrogenase/reductase X-linked, DHR SX, 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 dehydrogenase/reductase X-linked, DHRSX, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ 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 $n=4$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary tumors of the breast and $n=19$ 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 DHRSX 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 DHRSX mRNA expression levels with recurrence-free survival in $n=3951$ 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.

DHRSX 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 dehydrogenase/reductase X-linked, encoded by DHRSX, 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 tumor of the breast in patients with breast cancer, DHRSX ranked 245 out of 17418 total transcripts (Chart 1), equating to 98.6% differential expression. Differential expression of DHRSX in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=7.18E-04$).

To attempt to validate differential transcriptome-wide differential expression of DHRSX in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵; again, we identified DHRSX 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 primary tumors of the breast, DHRSX ranked 4272 out of 61359 total transcripts (Chart 2), equating to 93.04% differential expression. Differential expression of DHRSX in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=0.03010916$). Thus, transcriptome-wide differential expression of DHRSX in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, indicating that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer in humans.

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

We obtained exact mRNA expression levels for DHRSX, 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 DHRSX expression in brain metastatic tissues. We observed significantly higher expression of DHRSX in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: DHRSX was expressed at 0.31 ± 0.52 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 1.66 ± 0.72 AU in brain metastatic tissues. The difference in DHRSX mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.001495$).

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

We performed Kaplan-Meier survival analysis⁸ in 3951 breast cancer patients in total, to evaluate whether DHRSX tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of DHRSX and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of DHRSX possessed median RFS of 37.64 months, while patients whose tumors expressed high levels of DHRSX possessed median RFS of 31 months. This difference in RFS based on DHRSX tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.021; hazard ratio: 1.22 (1.03-1.45)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified dehydrogenase/reductase X-linked, encoded by DHRSX, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast

1 cancer when compared to primary tumors of the breast; we observed significantly increased
2 expression of DHRSX in brain metastases as compared to primary tumors of the breast. Further,
3 we found a significant correlation between DHRSX expression and patient survival outcomes, as
4 recurrence-free survival was significantly greater in patients whose tumors expressed higher
5 levels of DHRSX as compared to patients whose tumors expressed lower levels of DHRSX.

6 **Discussion**

7 We provided evidence here that dehydrogenase/reductase X-linked, encoded by DHRSX,
8 is among the genes whose expression is most different in the brain metastases of patients with
9 brain metastatic breast cancer, that DHRSX mRNA is present at significantly increased quantities
10 in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor
11 DHRSX expression is significantly correlated with patient survival outcomes in human breast
12 cancer. Evaluation of the effects of genetic depletion of DHRSX in mouse models of metastatic
13 breast cancer on metastasis to the central nervous system is merited. Modulation of DHRSX
14 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the
15 vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the
16 blood-brain barrier and colonize the brain.
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Rank: 245
Probe ID: 13538
p-value: 7.18E-04
t: -4.19
B: -0.287611
Gene: DHRSX
Gene name: dehydrogenase/reductase X-linked

Chart 1: DHRSX 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: 4272
probe ID: Hs.11779.0.S1_3p_at
p-value: 0.03010916
t: -2.2431906
B: -3.50789
Gene: DHRSX
Gene name: dehydrogenase/reductase X-linked

Chart 2: DHRSX 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: DHR SX 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 DHR SX 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 DHR SX expression between primary tumors of the breast and brain metastases is $p=0.001495$.

Figure 2: Significant correlation between DHRX 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 DHRX 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 DHRX 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 DHRSX expression: 37.64 months

High DHRSX expression: 31 months

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

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