NDUFB1 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 NADH:ubiquinone oxidoreductase subunit beta 1, encoded by NDUFB1, 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. NDUFB1 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of NDUFB1 in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of NDUFB1 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, NADH:ubiquinone oxidoreductase subunit beta 1, NDUFB1, 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 NADH:ubiquinone oxidoreductase subunit beta 1, *NDUFB1*, 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 *NDUFB1* 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 *NDUFB1* 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.

NDUFB1 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 NADH:ubiquinone oxidoreductase subunit beta 1, encoded by *NDUFB1*, as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When

1 sorting each of the genes expressed in brain metastases based on significance of difference as
2 compared to primary tumor of the breast in patients with breast cancer, NDUF B1 ranked 237 out
3 of 17418 total transcripts (Chart 1), equating to 98.6% differential expression. Differential
4 expression of NDUF B1 in the brain metastases of patients with metastatic breast cancer was
statistically significant (Chart 1; $p=7.03E-04$).

5 To attempt to validate differential transcriptome-wide differential expression of NDUF B1
6 in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵;
7 again, we identified NDUF B1 as a differentially expressed gene in the brain metastatic tissues of
8 patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain
9 metastases based on significance of difference as compared to primary tumors of the breast,
10 NDUF B1 ranked 5592 out of 61359 total transcripts (Chart 2), equating to 90.9% differential
11 expression. Differential expression of NDUF B1 in the brain metastases of patients with
12 metastatic breast cancer was statistically significant (Chart 2; $p=0.04188868$). Thus,
13 transcriptome-wide differential expression of NDUF B1 in the brain metastases of women with
14 metastatic breast cancer was conserved across two independent microarray datasets, indicating
15 that this may likely be a *bona fide* biological phenomena associated with metastatic breast cancer
16 in humans.

17 **NDUF B1 is expressed at higher levels in the brain metastases of patients with metastatic 18 breast cancer.**

19 We obtained exact mRNA expression levels for NDUF B1, in primary tumors of the breast
20 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
21 statistical significance of change in NDUF B1 expression in brain metastatic tissues. NDUF B1
22 was expressed at higher levels in the brain metastases of patients with breast cancer as compared
23 to primary tumors of the breast, and this difference was statistically significant (Figure 1;
24 $p=0.001159$).

25 **NDUF B1 expression is significantly correlated with survival outcomes in human breast 26 cancer.**

27 We performed Kaplan-Meier survival analysis⁸ in 3951 breast cancer patients in total, to
28 evaluate whether NDUF B1 tumor expression was correlated with survival outcomes in breast
cancer. We observed a statistically significant correlation between primary tumor expression of
NDUF B1 and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients
whose primary tumors expressed low levels of NDUF B1 possessed median RFS of 216.66
months, while patients whose tumors expressed high levels of NDUF B1 possessed median RFS
of 228.85 months. This difference in RFS based on NDUF B1 tumor expression in patients with
breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.051; hazard ratio:
1.11 (1-1.24)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
identified NADH:ubiquinone oxidoreductase subunit beta 1, encoded by NDUF B1, as among the
genes whose expression was most different, transcriptome-wide, in the brain metastases of

1 patients with breast cancer when compared to primary tumors of the breast; we observed
2 significantly increased expression of NDUFB1 in brain metastases as compared to primary
3 tumors of the breast. Further, we found a significant correlation between NDUFB1 expression
4 and patient survival outcomes, as recurrence-free survival was significantly greater in patients
5 whose tumors expressed higher levels of NDUFB1 as compared to patients whose tumors
6 expressed lower levels of NDUFB1.

6 **Discussion**

7 We provided evidence here that NADH:ubiquinone oxidoreductase subunit beta 1,
8 encoded by NDUFB1, is among the genes whose expression is most different in the brain
9 metastases of patients with brain metastatic breast cancer, that NDUFB1 mRNA is present at
10 significantly increased quantities in brain metastatic tissues as compared to primary tumors of the
11 breast, and that primary tumor NDUFB1 expression is significantly correlated with patient
12 survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of
13 NDUFB1 in mouse models of metastatic breast cancer on metastasis to the central nervous
14 system is merited. Modulation of NDUFB1 expression may be relevant to the processes by
15 which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the
16 lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.
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Rank: 237
Probe ID: 3329
p-value: 7.03E-04
t: -4.2
B: -0.267612
Gene: NDUFB1
Gene name: NADH:ubiquinone oxidoreductase subunit beta 1

Chart 1: NDUFB1 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: 5592
probe ID: g4758775_3p_a_at
p-value: 0.04188868
t: 2.0974641
B: -3.75669
Gene: NDUFB1
Gene name: NADH:ubiquinone oxidoreductase subunit beta 1

Chart 2: NDUFB1 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: NDUFB1 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 NDUFB1 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 NDUFB1 expression between primary tumors of the breast and brain metastases is $p=0.001159$.

Figure 2: Significant correlation between NDUFB1 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 NDUFB1 in patient primary tumors. 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 NDUFB1 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 NDUFB1 expression: 216.66 months
High NDUFB1 expression: 228.85 months

Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of NDUFB1.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of NDUFB1 and high primary tumor expression of NDUFB1 is listed in this chart, for $n=3951$ breast cancer patients.