Nebulin is differentially expressed in the brain metastases of patients with metastatic 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 to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that nebulin, encoded by NEB, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to normal breast tissues. NEB mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Up-regulation of NEB expression may contribute to metastasis of tumor cells from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, NEB, 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 trastuzumab. 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 the benign breast, primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and increased expression of NEB in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE26338⁴ and GSE52604⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE26338 was generated using Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer, analysis performed using platform GPL885. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis 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 NEB gene expression was significantly between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

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.

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

Through analysis of published microarray data⁴, we identified nebulin, encoded by NEB, as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer. 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, NEB ranked 27 out of 22575 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of NEB in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=2.94E-05$).

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked

1 how gene expression was most significantly different between brain metastases from patients with breast
2 cancer and normal breast tissue as opposed to primary tumors of the breast, by comparing global gene
3 expression profiles of 10 normal breast tissues to 35 brain metastases⁵. Using this strategy, we validated
4 NEB as a differentially expressed gene in the brain metastases of patients with brain metastatic breast
5 cancer⁴ (Table 2). When sorting each of the genes expressed in brain metastases based on significance of
6 difference in expression between brain metastases and normal breast tissues, NEB ranked 6035 out of
7 41093 total transcripts (Table 2), equating to 85.3% differential expression. Differential expression of
8 NEB in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically
9 significant (Table 2; $p=5.12E-04$).

10 Thus, differential expression of NEB was conserved both when compared to normal tissues of the
11 breast and to primary tumors of the breast in patients with metastatic breast cancer, in two independent
12 microarray datasets^{4,5}

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

14 We obtained exact mRNA expression levels for NEB, in primary tumors of the breast and in brain
15 metastasis of patients with brain metastatic breast cancer to determine direction and statistical
16 significance of change in NEB expression in brain metastatic tissues. NEB was expressed at higher levels
17 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, but this
18 difference was not deemed statistically significant (Figure 1; $p=0.0971$).

19 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
20 nebulin, encoded by NEB, as among the genes whose expression was most different, transcriptome-wide,
21 in the brain metastases of patients with breast cancer both when compared to the normal breast and to
22 primary tumors of the breast; NEB expression, as compared to primary tumors of the breast, was higher in
23 metastasis to the brain.

24 **Discussion**

25 We provided evidence here that nebulin, encoded by NEB, is among the genes whose expression
26 is most different in the brain metastases of patients with brain metastatic breast cancer. Evaluation of the
27 phenotypic consequences of genetic depletion of NEB in mouse models of metastatic breast cancer on
28 metastasis to the central nervous system is merited.

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Rank	ID	p-value	t	B	Gene	Gene name
27	6184	2.94E-05	5.94	2.67141	NEB	nebulin

Table 1: NEB is differentially expressed in brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
6035	A_23_P146783	5.12E-04	3.7319234	-0.597802	NEB	nebulin

Table 2: NEB is differentially expressed in brain metastases in patients with metastatic breast cancer when compared to normal breast tissues.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: NEB is expressed at higher levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

The mRNA expression level of NEB in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean NEB levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.