Nucleolar complex protein 4, NOC4L, 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 nucleolar complex protein 4, encoded by NOC4L, 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. NOC4L mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of NOC4L in primary tumors was significantly correlated with patient overall survival. Modulation of NOC4L 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, nucleolar complex protein 4, NOC4L, 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 nucleolar complex protein 4, NOC4L, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE42568⁵ 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. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570. 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 NOC4L 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 NOC4L mRNA expression levels with overall survival in $n=1402$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary 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.

NOC4L 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

nucleolar complex protein 4, encoded by NOC4L, 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, NOC4L ranked 67 out of 17418 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of NOC4L in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.26E-04$).

To validate transcriptome-wide differential expression of NOC4L in human breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues and primary tumors of the breast. Again, we identified NOC4L 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 normal breast tissues, NOC4L ranked 20936 out of 54675 total transcripts (Chart 2), equating to 61.7% differential expression. Differential expression of NOC4L in the primary tumors of patients with breast cancer approached the level of statistical significance (Chart 2; $p=4.81E-02$). Thus, differential expression of NOC4L, transcriptome-wide, in the tumor tissues of women with breast cancer was conserved across two independent microarray datasets, both in primary and metastatic tumor tissues.

NOC4L is expressed at higher levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for NOC4L, 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 NOC4L expression in brain metastatic tissues. NOC4L 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; $p=0.000065$).

NOC4L expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁸ in 1402 breast cancer patients in total, to evaluate whether NOC4L tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of NOC4L and overall survival in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of NOC4L possessed median OS of 123.6 months, while patients whose tumors expressed high levels of NOC4L possessed median OS of 80.64 months. This difference in OS based on NOC4L tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.0037; hazard ratio: 1.37 (1.11-1.7)).

1 Thus, by mining published microarray data^{4,5} in an unbiased fashion, we identified
2 nucleolar complex protein 4, encoded by NOC4L, as among the genes whose expression was
3 most different, transcriptome-wide, in the brain metastases and primary tumors of patients with
4 breast cancer; we observed significantly increased expression of NOC4L in brain metastases as
5 compared to primary tumors of the breast. Further, we found a significant correlation between
6 NOC4L expression and patient survival outcomes, as overall survival was significantly lower in
7 patients whose primary tumors expressed higher levels of NOC4L as compared to patients whose
8 primary tumors expressed lower levels of NOC4L.

9 **Discussion**

10 We provided evidence here that nucleolar complex protein 4, encoded by NOC4L, is
11 among the genes whose expression is most different in the brain metastases of patients with brain
12 metastatic breast cancer, that NOC4L mRNA is present at significantly increased quantities in
13 brain metastatic tissues as compared to primary tumors of the breast, that NOC4L is also
14 differentially expressed in primary tumors of patients with breast cancer and that primary tumor
15 NOC4L expression is significantly correlated with patient survival outcomes in human breast
16 cancer. Evaluation of the effects of genetic depletion of NOC4L in mouse models of metastatic
17 breast cancer on metastasis to the central nervous system is merited. Modulation of NOC4L
18 expression may be relevant to the processes by which breast cancer cells exit the breast, enter the
19 vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the
20 blood-brain barrier and colonize the brain.
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Rank: 67
Probe ID: 21412
p-value: 1.26E-04
t: -5.04
B: 1.313923
Gene: NOC4L
Gene name: nucleolar complex protein 4

Chart 1: NOC4L 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: 20936
probe ID: 218860_at
p-value: 4.81E-02
t: -2
B: -5.2335146
Gene: NOC4L
Gene name: nucleolar complex protein 4

Chart 2: NOC4L is differentially expressed in human breast cancer when comparing primary tumors of the breast 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: NOC4L 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 NOC4L 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 NOC4L expression between primary tumors of the breast and brain metastases is $p < 0.00001$.

Figure 2: Significant correlation between NOC4L primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of NOC4L in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall 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 NOC4L 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 NOC4L expression: 123.6 months
High NOC4L expression: 80.64 months

Chart 3: Median overall survival is inferior in patients with high primary tumor expression of NOC4L.

The median OS (overall survival) of patients with low primary tumor expression of NOC4L and high primary tumor expression of NOC4L is listed in this chart, for $n=1402$ breast cancer patients.