NCF1 (p47-phox) is differentially expressed in the lymph nodes of patients with metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. Between the breast and the brain reside the secondary lymphoid organ, the lymph nodes. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with metastasis to the lymph nodes in humans with metastatic breast cancer. We found that the neutrophil cytosolic factor 1, also known as p47-phox, encoded by NCF1, was among the genes whose expression was most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. NCF1 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of NCF1 in primary tumors of the breast was significantly correlated with patient overall survival. Modulation of NCF1 expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph nodes and the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, neutrophil cytosolic factor 1, NCF1, 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 and lymph node metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. The lymph nodes reside between the breast and the brain. We performed a global comparative analysis of lymph node metastatic tumor tissues in patients with metastatic breast cancer, compared to primary tumors and normal breast tissues^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding the neutrophil cytosolic factor 1, NCF1, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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 $n=71$ primary breast tumors and $n=7$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL887. GSE124648 was generated using Affymetrix Human Genome U133A array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL96. 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 NCF1 gene expression was significantly different between primary tumors of the breast and lymph node 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 NCF1 mRNA expression levels with overall survival in $n=241$ breast cancer patients with cancer of the basal subtype.

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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

NCF1 is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified the neutrophil cytosolic factor 1, encoded by NCF1, as a differentially expressed gene in the lymph node metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, NCF1 ranked 15 out of 18034 total transcripts (Chart 1), equating to 99.9% differential expression. Differential expression of NCF1 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.96e-05$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of NCF1 in the lymph node metastases of patients with metastatic breast cancer. We again observed NCF1 to be among the genes expressed most quantitatively differently in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, NCF1 ranked 1983 out of 22283 total transcripts (Chart 2), equating to 91.1% differential expression. Differential expression of NCF1 in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=6.20e-05$).

NCF1 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for NCF1, in primary tumors of the breast and in lymph node metastases of patients with metastatic breast cancer to determine direction and statistical significance of change in NCF1 expression in lymph node metastatic tissues. We observed significantly increased expression of NCF1 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: NCF1 was expressed at 0.62 ± 0.50 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 1.64 ± 1.11 AU in lymph node metastatic tissues (Figure 1). The difference in NCF1 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000024$).

NCF1 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=241$ breast cancer patients in total, to evaluate whether NCF1 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of NCF1 and overall survival (OS) in patients with basal subtype cancer of the breast, in the lower survival tertile (Figure 2). Patients whose primary tumors expressed low levels of NCF1 possessed median OS of 30.31 months, while patients whose tumors expressed high levels of NCF1 possessed median OS of 80.64 months. This difference in OS based on NCF1 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.036; hazard ratio: 0.59 (0.36-0.97)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified NCF1 as among the genes whose expression was most different, transcriptome-wide, in the lymph node metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly increased expression of NCF1 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between NCF1 expression and patient survival outcomes, as overall survival was significantly greater in basal subtype patients whose primary tumors expressed higher levels of NCF1 as compared to patients whose primary tumors expressed lower levels of NCF1.

Discussion

We provided evidence here that the neutrophil cytosolic factor 1, p47-phox, encoded by NCF1, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast and to normal breast tissues, that NCF1 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor NCF1 expression is significantly correlated with patient survival outcomes in human breast cancer of the basal subtype. Evaluation of the effects of genetic depletion of NCF1 in mouse models of metastatic breast cancer on metastasis to the central nervous

1 system is merited. Modulation of NCF1 expression may be relevant to the processes by which breast
2 cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade
3 immune clearance, breach the blood-brain barrier and colonize the brain.
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

References

1. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
2. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.
3. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
4. Weigman, V.J., Chao, H.H., Shabalin, A.A., He, X., Parker, J.S., Nordgard, S.H., Grushko, T., Huo, D., Nwachukwu, C., Nobel, A. and Kristensen, V.N., 2012. Basal-like Breast cancer DNA copy number losses identify genes involved in genomic instability, response to therapy, and patient survival. *Breast cancer research and treatment*, 133(3), pp.865-880.
5. Sinn, B.V., Fu, C., Lau, R., Litton, J., Tsai, T.H., Murthy, R., Tam, A., Andreopoulou, E., Gong, Y., Murthy, R. and Gould, R., 2019. SET ER/PR: a robust 18-gene predictor for sensitivity to endocrine therapy for metastatic breast cancer. *NPJ breast cancer*, 5(1), pp.1-8.
6. Awada, A., Colomer, R., Inoue, K., Bondarenko, I., Badwe, R.A., Demetriou, G., Lee, S.C., Mehta, A.O., Kim, S.B., Bachelot, T. and Goswami, C., 2016. Neratinib plus paclitaxel vs trastuzumab plus paclitaxel in previously untreated metastatic ERBB2-positive breast cancer: the NEfERT-T randomized clinical trial. *JAMA oncology*, 2(12), pp.1557-1564.
7. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*, 123(3), pp.725-731.

Rank: 15
Probe ID: 8993
p-value: 1.96e-05
t: -4.5437009
B: 2.5551
Gene: NCF1
Gene name: neutrophil cytosolic factor 1

Chart 1: NCF1 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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: 1983
Probe ID: 214084_x_at
p-value: 6.20e-05
t: -4.334474
B: 1.184908
Gene: NCF1
Gene name: neutrophil cytosolic factor

Chart 2: NCF1 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node metastases to normal breast tissues.

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.

Figure 1: NCF1 is expressed at significantly higher levels in the lymph node metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of NCF1 in primary tumors of the breast (left) and in lymph node metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in NCF1 expression between primary tumors of the breast and lymph node metastases, a *p*-value, is *p*=0.000024.

Figure 2: Significant correlation between NCF1 primary tumor expression and overall survival in patients with breast cancer of the basal subtype.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=241$ total patients with basal subtype cancer stratified into two groups, based on low or high expression of NCF1 in patient primary tumors, in the lower survival tertile. 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 NCF1 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.

1 Low NCF1 expression: 30.31 months

2 High NCF1 expression: 80.64 months

3
4 **Chart 3: Median overall survival is inferior in basal subtype breast cancer patients with low primary tumor expression of NCF1, for patients in the lower survival tertile.**

5 The median OS (overall survival) of patients with low primary tumor expression of NCF1 and high
6 primary tumor expression of NCF1 is listed in this chart, for $n=241$ breast cancer patients.