SYP is a differentially expressed gene in brain metastatic human 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. 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 synaptophysin, encoded by SYP, 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. SYP mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of SYP in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of SYP 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, synaptophysin, SYP, 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 the synaptophysin, SYP, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE125989⁵ 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. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 array technology with $n=16$ primary breast tumors and $n=16$ brain metastases from patients with breast cancer; the samples utilized by investigators for this microarray dataset were paired, matched tumor and metastasis samples; analysis was performed using platform GPL571. 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 SYP 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 tool for correlation of SYP mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients, with auto cut-off selected.

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.

SYP 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 gene

1 encoding synaptophysin, encoded by SYP, as a differentially expressed gene in the breast
2 metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes
3 expressed in brain metastases based on significance of difference as compared to primary tumors
4 of the breast in patients with breast cancer, SYP ranked 72 out of 17418 total transcripts (Chart
5 1), equating to 99.6% differential expression. Differential expression of SYP in the brain
metastases of patients with metastatic breast cancer was statistically significant (Chart 1;
 $p=1.42E-04$).

6 We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide
7 differential expression of SYP in human brain metastatic breast cancer. Again, we identified
8 SYP as a differentially expressed gene in the brain metastatic tissues of patients with breast
9 cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on
10 significance of difference as compared to primary tumors of the breast in patients with breast
11 cancer, in this dataset, SYP ranked 4531 out of 22277 total transcripts (Chart 2), equating to
12 79.7% differential expression. Differential expression of SYP in the brain metastases of patients
with metastatic breast cancer in this dataset was not deemed statistically significant (Chart 2;
 $p=1.61E-01$). Thus, differential expression of SYP, transcriptome-wide, in the brain metastases
of women with metastatic breast cancer was conserved across two independent microarray
datasets.

13 **SYP is expressed at higher levels in the brain metastases of patients with metastatic breast** 14 **cancer.**

15 We obtained exact mRNA expression levels for SYP, in primary tumors of the breast and
16 in brain metastasis of patients with brain metastatic breast cancer to determine direction and
17 statistical significance of change in SYP expression in brain metastatic tissues. SYP was
18 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;
 $p=0.000042$).

19 **SYP expression is significantly correlated with survival outcomes in human breast cancer.**

20 We performed Kaplan-Meier survival analysis⁷ to evaluate correlation in 3951 breast
21 cancer patients in total, to evaluate whether SYP tumor expression was correlated with survival
22 outcomes in breast cancer. We observed a statistically significant correlation between primary
23 tumor expression of SYP and recurrence-free survival (RFS) (Figure 2). Patients whose primary
tumors expressed low levels of SYP possessed median RFS of 36 months, while patients whose
tumors expressed high levels of SYP possessed median RFS of 66 months. This difference in
RFS based on SYP tumor expression in breast cancer was statistically significant (Figure 2, Chart
3; logrank p -value: $3.0e-9$; hazard ratio: 0.72 (0.64-0.8)). The false discovery rate was calculated
at 1%.

26 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
27 identified the synaptophysin, SYP, as among the genes whose expression was most different,
28 transcriptome-wide, in the brain metastases of patients with breast cancer when compared to

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

5 **Discussion**

6 We provided evidence here that the synaptophysin, encoded by SYP, is among the genes
7 whose expression is most different in the brain metastases of patients with brain metastatic breast
8 cancer, and that primary tumor SYP expression is significantly correlated with patient survival
9 outcomes in human breast cancer. Evaluation of the effects of genetic depletion of SYP in mouse
10 models of metastatic breast cancer on metastasis to the central nervous system is merited.
11 Synaptophysin may be of relevance to the processes by which primary tumor cells exit the breast,
12 enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance,
13 breach the blood brain-barrier and colonize the brain.
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., Shabalín, 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. Iwamoto, T., Niikura, N., Ogiya, R., Yasojima, H., Watanabe, K.I., Kanbayashi, C., Tsuneizumi, M., Matsui, A., Fujisawa, T., Iwasa, T. and Shien, T., 2019. Distinct gene expression profiles between primary breast cancers and brain metastases from pair-matched samples. *Scientific reports*, 9(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.
8. Polishchuk, E.V., Concilli, M., Iacobacci, S., Chesi, G., Pastore, N., Piccolo, P., Paladino, S., Baldantoni, D., van IJzendoorn, S.C., Chan, J. and Chang, C.J., 2014. Wilson disease protein SYP utilizes lysosomal exocytosis to maintain copper homeostasis. *Developmental cell*, 29(6), pp.686-700.

Rank: 72
Probe ID: 9539
p-value: 1.42E-04
t: -4.98
B: 1.207376
Gene: SYP
Gene name: synaptophysin

Chart 1: SYP 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: 4531
Probe ID: 213200_at
p-value: 1.61E-01
t: -1.4301665
B: -5.0406
Gene: SYP
Gene name: synaptophysin

Chart 2: SYP 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 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: SYP 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 SYP in primary tumors of the breast (left) and in brain metastases from women diagnosed with breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in SYP expression between primary tumors and brain metastases is $p=0.000042$.

Figure 2: Significant correlation between SYP 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 SYP 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 SYP 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. The false discovery rate is 1%.

Low SYP expression: 36 months

High SYP expression: 66 months

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

The median RFS (recurrence-free survival) of patients with low primary tumor expression of SYP and high primary tumor expression of SYP is listed in this chart.