SIX1 is differentially expressed in the brain metastases 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. 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 SIX homeobox 1, encoded by SIX1, 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. SIX1 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Up-regulation of SIX1 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, SIX1, 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 SIX1 in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE52604⁵ 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 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 SIX1 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.

SIX1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified SIX homeobox 1, encoded by SIX1, 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, SIX1 195 out of 17418 total transcripts (Table 1), equating to 98.9% differential expression. Differential expression of SIX1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=5.2E-04$).

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 SIX1 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, SIX1 ranked 3870 out of
7 41093 total transcripts (Table 2), equating to 90.6% differential expression. Differential expression of
8 SIX1 in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically
9 significant (Table 2; $p=6.76E-05$).

10 Thus, differential expression of SIX1 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 **SIX1 is expressed at higher levels in the brain metastases of patients with brain metastatic breast 14 cancer.**

15 We obtained exact mRNA expression levels for SIX1, in primary tumors of the breast and in brain
16 metastasis of patients with brain metastatic breast cancer to determine direction and statistical
17 significance of change in SIX1 expression in brain metastatic tissues. SIX1 was expressed at higher
18 levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast,
19 and this difference was statistically significant (Figure 1; $p=0.0002$).

20 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
21 SIX1 as among the genes whose expression was most different, transcriptome-wide, in the brain
22 metastases of patients with breast cancer both when compared to the normal breast and to primary tumors
23 of the breast; SIX1 expression, as compared to primary tumors of the breast, was significantly higher in
24 metastasis to the brain.

25 **Discussion**

26 We provided evidence here that SIX1 is among the genes whose expression is most different in
27 the brain metastases of patients with brain metastatic breast cancer. In an experimental mouse model of
28 breast cancer utilizing 66cl4 breast cancer cells injected into the fourth mammary pad of BALB/c mice,
knockdown of SIX1 resulted in decreased metastatic burden in the lungs⁷. Evaluation of the phenotypic
consequences of genetic depletion of SIX1 in mouse models of metastatic breast cancer on metastasis to
the central nervous system is merited.

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. Salhia, B., Kiefer, J., Ross, J.T., Metapally, R., Martinez, R.A., Johnson, K.N., DiPerna, D.M., Paquette, K.M., Jung, S., Nasser, S. and Wallstrom, G., 2014. Integrated genomic and epigenomic analysis of breast cancer brain metastasis. *PloS one*, 9(1), p.e85448.
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. Wang, C.A., Jedlicka, P., Patrick, A.N., Micalizzi, D.S., Lemmer, K.C., Deitsch, E., Casás-Selves, M., Harrell, J.C. and Ford, H.L., 2012. SIX1 induces lymphangiogenesis and metastasis via upregulation of VEGF-C in mouse models of breast cancer. *The Journal of clinical investigation*, 122(5), pp. 1895-1906.

Rank	ID	p-value	t	B	Gene	Gene name
195	3311	5.2E-04	-4.34	0.011473	SIX1	SIX homeobox 1

Table 1: SIX1 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
3870	A_23_P76914	6.76E-05	4.3731696	1.32897	SIX1	SIX homeobox 1

Table 2: SIX1 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: SIX1 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 SIX1 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 SIX1 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.