HHATL is differentially expressed in primary tumors and brain metastases in humans with 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 report here the differential expression of the hedgehog acyltransferase-like, encoded by HHATL, in the primary tumors and brain metastases of humans with breast cancer. HHATL mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. These data combined suggest that down-regulation of HHATL is a conserved event, both during transformation of breast tissues and progression to central nervous system metastasis and further point to potential importance of modulation of HHATL during progression of human breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, HHATL, hedgehog acyltransferase-like, 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 trial 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 primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and increased expression of the hedgehog acyltransferase-like, encoded by HHATL, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE109169⁵ 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 performed using platform GPL885. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast and; analysis was performed using platform GPL5175. The tissues from this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 HHATL gene expression was significantly between normal breast tissues 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 tissues from patients with metastatic breast cancer using published microarray data^{4,5} to describe the transcriptional landscape of primary and metastatic tumors in human metastatic breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

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

Through analysis of published microarray data⁴, we identified the hedgehog acyltransferase-like, encoded by HHATL, 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, HHATL ranked 219 out of 22575 total transcripts (Table 1), equating to 99.0% differential expression. Differential expression of HHATL in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=7.45E-04$).

HHATL is differentially expressed in the primary tumors of patients with breast cancer.

Next, we queried a second microarray dataset⁵ generated using primary tumors and normal breast tissues from patients with breast cancer to determine whether differential expression of HHATL was specific to metastatic tissues or could be found in primary tumors as well. Our analysis revealed significant differential expression of HHATL in primary tumors of patients with breast cancer. When sorting each of the genes expressed in primary tumors of the breast based on significance of difference as compared to normal breast tissues in patients with breast cancer, HHATL ranked 5452 out of 19076 total transcripts (Table 2), equating to 71.4% differential expression. Differential expression of HHATL in the primary tumors of patients with breast cancer was statistically significant (Table 2; $p=1.07E-03$). Thus, differential expression of HHATL was conserved in the brain metastases and primary tumors of patients with breast cancer.

HHATL is expressed at higher levels in the brain metastases in humans with breast cancer.

We obtained exact mRNA expression levels for HHATL, in normal breast tissues and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in HHATL expression in brain metastatic tissues. HHATL was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, but this difference was not statistically significant (Figure 1; $p=0.1524$).

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

Discussion

We provided evidence here that hedgehog acyltransferase-like, encoded by HHATL is among the genes whose expression is most different in the primary and metastatic tumors of humans with breast cancer. Evaluation of the effects of genetic depletion of HHATL in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited. Differential expression and up-regulation of HHATL in primary tumors of the breast and metastasis to the brain suggests that its induction may be important for the progression of human breast cancer, but this remains to be experimentally evaluated.

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. Chang, J.W., Kuo, W.H., Lin, C.M., Chen, W.L., Chan, S.H., Chiu, M.F., Chang, I.S., Jiang, S.S., Tsai, F.Y., Chen, C.H. and Huang, P.H., 2018. Wild-type p53 upregulates an early onset breast cancer-associated gene GAS7 to suppress metastasis via GAS7-CYFIP1-mediated signaling pathway. *Oncogene*, 37(30), pp.4137-4150.
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.

Rank	ID	p-value	t	B	Gene	Gene name
219	21169	7.45E-04	4.24	-0.30199	HHATL	hedgehog acyltransferase-like

Table 1: HHATL is differentially expressed in brain metastases in 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
5452	2670874	1.07E-03	-3.47	-1.529618	HHATL	hedgehog acyltransferase-like

Table 2: HHATL is differentially expressed in primary tumors of the breast when compared to normal breast tissue.

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.