Auxilin (DNAJC6) is over-expressed in brain metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

One study reported the incidence of central nervous system metastases in breast cancer patients treated with trastuzumab as 34%¹. We mined published microarray data^{2,3} to discover genes associated with brain metastasis in breast cancer. We identified significant differential expression of auxilin, a molecule with the ability to support assembly and disassembly of the clathrin-coated vesicle lattice^{4,5}, encoded by DNAJC6, in the brain metastases of patients with metastatic breast cancer. DNAJC6 may be relevant to the biology underlying colonization of the brain with metastatic breast cancer clones.

Keyword: breast cancer, metastasis, brain metastasis, central nervous system metastasis, auxilin, DNAJC6,, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study reported a 34% incidence of central nervous system metastases in patients with breast cancer treated with the monoclonal antibody trastuzumab (Herceptin)¹. Treatment options for patients with brain metastatic breast cancer are limited⁶. We mined published microarray datasets^{2,3} to understand in an unbiased and systems-level fashion genes whose expression most significantly distinguished brain metastases from primary tumors of the breast, the cells from which these metastatic clones are presumably derived. We found significant differential expression of DNAJC6 in the brain metastases of patients with brain metastatic breast cancer.

Methods

We utilized microarray datasets GSE125989² and GSE100534³ for this differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=16$ primary tumors from patients with breast cancer and $n=3$ brain metastases from patients with brain metastatic breast cancer. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. 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 DNAJC6 gene expression was significantly between primary breast tumors 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 mined published microarray data^{2,3} to discover genes associated with brain metastasis in breast cancer.

DNAJC6 is differentially expressed in brain metastatic breast cancer.

We identified auxilin, encoded by DNAJC6, as among the genes whose expression changes most significantly when comparing brain metastases to primary tumors of the breast in patients with brain metastatic breast cancer² (Table 1). When sorting each of the genes expressed in brain metastases based on significance of change as compared to primary tumors of the breast, DNAJC6 ranked 248 out of 22277 total transcripts (Table 1), equating to 98.9% differential expression. Differential expression of DNAJC6 in brain metastases in brain metastatic breast cancer was statistically significant (Table 1; $p=2.18E-03$).

We queried a second microarray dataset³ to validate transcriptome-wide differential expression of DNAJC6 in brain metastatic breast cancer. We again found that DNAJC6 was among the genes whose expression changed most significantly when comparing metastases of the brain to primary tumors of the breast in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of change as compared to primary tumors of the breast, DNAJC6 ranked 2210 out of 33297 total transcripts (Table 2), equating to 93.4% differential expression. Differential expression of DNAJC6 in brain metastases in brain metastatic breast cancer was statistically significant (Table 2; $p=1.93E-03$).

DNAJC6 is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in brain metastatic breast cancer.

We obtained exact mRNA expression values for DNAJC6 from brain metastases and primary tumors of the breast to evaluate magnitude and direction of change in DNAJC6 expression in brain metastases in patients with breast cancer. DNAJC6 was expressed at higher levels in brain metastases in patients with breast cancer and this difference was statistically significant (Figure 1; $p=0.0019$). We calculated a mean fold change of 1.72 ± 0.58 in DNAJC6 expression when comparing brain metastatic tissues to primary tumors of the breast (Table 1).

Thus, we found that DNAJC6 was among the genes, globally, whose expression was most different when comparing primary tumors of the breast to brain metastases in patients with breast cancer.

Discussion

Auxilin was originally identified as a coat protein of clathrin-coated vesicles from the bovine brain with the ability to support formation of clathrin cages⁴. On the contrary, in the presence of Hsp70 and ATP, auxilin can interact with both the clathrin triskelion as well as Hsp70, inducing disassembly of the clathrin lattice^{5,7}. Auxilin contains a J-domain at its carboxy-terminus⁵. Thus, auxilin functions both in assembly and disassembly of the clathrin triskelion. In the hippocampal neurons of auxilin-deficient mice, endocytosis of synaptic vesicles induced by clathrin-coated vesicles was disrupted, and empty cages could be found in synapses of neurons from auxilin-deficient mice⁸. However, the cyclin G-dependent kinase GAK could partially compensate for loss of auxilin⁸. Mutation of DNAJC6 has been reported in two patients with juvenile autosomal-recessive Parkinsonism (AJRP)⁹. In cancer, expression of DNAJC was found to be significantly increased in hepatocellular carcinoma tumor tissues, associated with worse patient outcome and could support cell proliferation and invasion in HCC cells *in vitro*. Thus, auxilin, encoded by DNAJC is a critical component of the cellular machinery utilized to assemble and disassemble clathrin-coated vesicles utilized in endocytosis, it can cooperate with Hsc70 in disassembly of the clathrin lattice, mutation of DNAJC has been found in patients with AJRP, and expression of DNAJC is up-regulated in HCC tumor tissues.

We reported here that the gene encoding auxilin, DNAJC6, was among the genes whose expression was most quantitatively different in metastases to the brain when compared to primary tumors of the breast in patients with metastatic breast cancer. DNAJC6 may be of relevance to the biology underlying any number of steps important for metastasis to the brain: exit from the breast, traversing the vasculature and the blood-brain barrier, and colonization of the brain.

References

1. 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.
2. 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.
3. Schulten, H.J., Bangash, M., Karim, S., Dallol, A., Hussein, D., Merdad, A., Al-Thoubaity, F.K., Al-Maghrabi, J., Jamal, A., Al-Ghamdi, F. and Choudhry, H., 2017. Comprehensive molecular biomarker identification in breast cancer brain metastases. *Journal of translational medicine*, 15(1), p.269.
4. Ahle, S. and Ungewickell, E., 1990. Auxilin, a newly identified clathrin-associated protein in coated vesicles from bovine brain. *The Journal of Cell Biology*, 111(1), pp.19-29.
5. Ungewickell, E., Ungewickell, H., Holstein, S.E., Lindner, R., Prasad, K., Barouch, W., Martini, B., Greene, L.E. and Eisenberg, E., 1995. Role of auxilin in uncoating clathrin-coated vesicles. *Nature*, 378(6557), pp.632-635.
6. Robson, M., Im, S.A., Senkus, E., Xu, B., Domchek, S.M., Masuda, N., Delaloge, S., Li, W., Tung, N., Armstrong, A. and Wu, W., 2017. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *New England Journal of Medicine*, 377(6), pp.523-533.
7. Jiang, R.F., Greener, T., Barouch, W., Greene, L. and Eisenberg, E., 1997. Interaction of auxilin with the molecular chaperone, Hsc70. *Journal of Biological Chemistry*, 272(10), pp.6141-6145.
8. Yim, Y.I., Sun, T., Wu, L.G., Raimondi, A., De Camilli, P., Eisenberg, E. and Greene, L.E., 2010. Endocytosis and clathrin-uncoating defects at synapses of auxilin knockout mice. *Proceedings of the National Academy of Sciences*, 107(9), pp.4412-4417.
9. Edvardson, S., Cinnamon, Y., Ta-Shma, A., Shaag, A., Yim, Y.I., Zenvirt, S., Jalas, C., Lesage, S., Brice, A., Taraboulos, A. and Kaestner, K.H., 2012. A deleterious mutation in DNAJC6 encoding the neuronal-specific clathrin-uncoating co-chaperone auxilin, is associated with juvenile parkinsonism. *PloS one*, 7(5), p.e36458.
10. Yang, T., Li, X.N., Li, X.G., Li, M. and Gao, P.Z., 2014. DNAJC6 promotes hepatocellular carcinoma progression through induction of epithelial-mesenchymal transition. *Biochemical and Biophysical Research Communications*, 455(3-4), pp.298-304.

Rank	ID	p-value	t	B	FC	Gene	Gene name
248	204720_s_at	2.18E-03	3.3111925	-1.4187	1.72 ± 0.58	DNAJC6	DnaJ heat shock protein family (Hsp40) member C6

Table 1: DNAJC6 is differentially expressed in brain metastatic breast cancer.

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, fold change (FC) of DNAJC6 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2210	7902043	1.93E-03	3.5650637	-1.460295	DNAJC6	DnaJ heat shock protein family (Hsp40) member C6

Table 2: DNAJC6 is differentially expressed in brain metastatic breast cancer.

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: DNAJC6 is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of DNAJC6 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 DNAJC6 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.