Dermatopontin is differentially expressed in brain 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 dermatopontin, encoded by DPT, 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. We observed a significant correlation between DPT expression in primary tumors of the breast and overall survival. Molecular functions of dermatopontin may be relevant to the processes by which tumor cells of the breast metastasize to the brain. Down-regulation of DPT may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, DPT, dermatopontin, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis 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 decreased expression of DPT in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE52604⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. 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 DPT 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). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of DPT mRNA expression levels with distant metastasis-free survival (DMFS) in $n=1746$ breast cancer patients, and overall survival in $n=1402$ breast patients.

Results

We performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain 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.

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

We identified dermatopontin, encoded by DPT, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to the primary tumors of patients with brain metastatic breast cancer, DPT ranked 2 out of 22297 total transcripts (Table 1), equating to 99.991% differential expression. Differential expression of DPT in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=1.25E-07$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus,

we asked whether we could validate differential expression of DPT when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁵ revealed that DPT was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, DPT ranked 122 out of 41093 total transcripts (Table 2), equating to 99.7% differential expression. Differential expression of DPT in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=2.08E-11$).

DPT is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for DPT, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in DPT expression in brain metastatic tissues. DPT was expressed at lower 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; $p<0.0001$). We calculated a mean fold change of 0.53 ± 0.29 in DPT expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

We also obtained exact mRNA expression levels for DPT, in normal breast tissue and in brain metastasis of patients with metastatic breast cancer from a second dataset to determine magnitude and direction of change in DPT expression in brain metastases. DPT was expressed at higher levels in the brain metastases of patients with breast cancer as compared to normal breast tissue, and this difference was statistically significant (Figure 2; $p<0.0001$). We calculated a mean fold change 0.27 ± 0.26 in DPT expression when comparing normal breast tissue to brain metastatic tissues in patients with breast cancer (Table 2).

DPT expression associates with survival outcomes in breast cancer.

We performed Kaplan-Meier survival analysis⁶ to evaluate correlation between DPT mRNA expression levels with survival outcomes in patients with breast cancer. In 1402 breast cancer patients, we found a statistically significant correlation between DPT expression and overall survival (OS) (Figure 3; log rank p -value: 0.0065 for overall survival, hazard ratio: 0.74 (0.6 - 0.92) (Fig. 3)). Lower DPT mRNA levels in primary tumors of the breast, like which we observed in metastases to the brain, were a negative prognostic indicator in patients with breast cancer with respect to overall survival. Median OS was 80.65 months for patients with low tumor expression of DPT while median OS was 123.6 months for patients with high tumor expression of DPT (Table 3).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified DPT as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; DPT was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast. DPT expression in primary tumors of the breast in a large cohort of patients demonstrated a significant correlation with overall survival, with higher expression of DPT correlating with improved survival in humans with breast cancer.

Discussion

In hepatocellular carcinoma, one study demonstrated that dermatopontin could inhibit Wnt signaling through CXXC4, suggesting that down-regulation of dermatopontin that we observed in the brain metastatic tissues in human breast cancer might involve up-regulation of Wnt signal transduction⁷. Dermatopontin can interact with fibronectin⁸, which we recently demonstrated is a differentially expressed gene in human breast cancer, expressed at significantly higher levels in primary tumors of the breast as compared to normal breast tissue⁹. This suggests that dermatopontin-fibronectin interactions might potentially be necessary for anchoring tumor cells to the breast (e.g., tumor stroma or membrane tissues), and that down-regulation of dermatopontin permits tumor cells to detach and exit the breast.

We provided evidence here that dermatopontin is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer and that low expression of dermatopontin in primary tumors of the breast, like that which we observed in brain metastatic tissues, is significantly correlated with worse overall survival in patients with breast cancer. Dermatopontin may be of relevance to any number of steps required for metastasis to the brain; of particular interest its interaction with fibronectin^{8,9}.

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. 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.
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. 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.
7. Liu, S., Qiu, J., He, G., Geng, C., He, W., Liu, C., Cai, D., Pan, H. and Tian, Q., 2020. Dermatopontin inhibits WNT signaling pathway via CXXC finger protein 4 in hepatocellular carcinoma. *Journal of Cancer*, 11(21), p.6288.
8. Kato, A., Okamoto, O., Wu, W., Matsuo, N., Kumai, J., Yamada, Y., Katagiri, F., Nomizu, M. and Fujiwara, S., 2014. Identification of fibronectin binding sites in dermatopontin and their biological function. *Journal of dermatological science*, 76(1), pp.51-59.
9. Mamoor, S., 2020. Differential expression of fibronectin in cancers of the breast.

Rank	ID	p-value	t	B	FC	Gene	Gene name
2	213068_at	1.25E-07	-6.6255619	7.1507	0.53 ± 0.29	DPT	dermatopontin

Table 1: DPT is differentially expressed in brain metastases in 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, fold change (FC) of DPT 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	FC	Gene	Gene name
122	A_23_P200741	2.08E-11	-8.7388695	15.880562	0.27 ± 0.26	DPT	dermatopontin

Table 2: DPT is differentially expressed in brain metastases in metastatic breast cancer 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, fold change (FC) of DPT expression between the brain metastases of patients with breast cancer and normal breast tissue, gene and gene name are listed in this chart.

Figure 1: DPT is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of DPT 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 DPT 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.

Figure 2: DPT is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of DPT in normal breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean DPT levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal breast tissue, a *p*-value, listed above.

Figure 3: DPT expression correlates with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=1402$ total patients stratified into two groups, based on low or high expression of DPT in patient primary tumors. 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 DPT 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.

Low expression cohort (months)	High expression cohort (months)
80.65	123.6

Table 3: Median overall survival is superior in breast cancer patients with high primary tumor expression of DPT.

Overall survival of $n=1402$ breast cancer patients based on stratification into low or high tumor expression of DPT is listed in this chart.