PPP2R2D, a regulatory subunit of PP2A, 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 PPP2R2D 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. PPP2R2D mRNA was present at higher quantities in brain metastatic tissues as compared to primary tumors of the breast. PPP2R2D and the substrates of PP2A may be pertinent in understanding post-translational modifications that regulate pathways and processes important for metastasis of primary tumor-derived breast cancer cells to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, PPP2R2D, 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 primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and increased expression of PPP2R2D in the brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ and 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. GSE100534⁵ 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. 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 PPP2R2D 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 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.

PPP2R2D is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through analysis of published microarray data⁴, we identified the protein phosphatase 2, regulatory subunit B, delta isoform, encoded by PPP2R2D as a differentially expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). 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, PPP2R2D ranked 79 out of 22575 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of GRM8 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=1.41E-04$).

1 Next, we queried a second microarray dataset⁵ to determine whether we could validate
2 differential expression of PPP2R2D in brain metastatic tissues in human metastatic breast cancer. We
3 again observed significant differential expression of PPP2R2D in brain metastases of patients with
4 metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on
5 significance of difference as compared to normal breast tissues in patients with breast cancer, PPP2R2D
6 ranked 3811 out of 33297 total transcripts (Table 2), equating to 88.6% differential expression.
7 Differential expression of PPP2R2D in the brain metastases of patients with metastatic breast cancer was
8 statistically significant (Table 2; $p=8.21E-03$). Thus, we observed differential expression of PPP2R2D in
9 the brain metastases of patients with metastatic breast cancer in two independent microarray datasets^{4,5}.

10 **PPP2R2D is expressed at significantly higher levels in the brain metastases of patients with brain**
11 **metastatic breast cancer.**

12 We obtained exact mRNA expression levels for PPP2R2D, in normal breast tissues and in brain
13 metastasis of patients with brain metastatic breast cancer to determine direction and statistical
14 significance of change in PPP2R2D expression in brain metastatic tissues. PPP2R2D was expressed at
15 higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the
16 breast, and this difference was statistically significant (Figure 1; $p=0.0039$).

17 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
18 the interleukin PPP2R2D as among the genes whose expression was most different, transcriptome-wide,
19 in the brain metastases of patients with breast cancer when compared primary tumors of the breast;
20 PPP2R2D expression, as compared to primary breast tumors, was higher in metastasis to the brain.

21 **Discussion**

22 We provided evidence here that PPP2R2D is among the genes whose expression is most different
23 in the brain metastases of patients with brain metastatic breast cancer. One study demonstrated that
24 depletion of PPP2R2D was effective in augmenting cytotoxic function of tumor-infiltrating lymphocytes
25 in an experimental cancer model^{7,8}. Evaluation of the effects of genetic depletion of PPP2R2D in mouse
26 models of metastatic breast cancer on metastasis to the central nervous system is merited; profiling post-
27 translational modifications in sorted tumor cell populations, from primary and metastatic tumor tissues, in
28 conjunction with PP2A inhibition, may be useful in identifying relevant PP2A substrates metastatic breast
cancer and in understanding molecular changes that may be important for breast cancer progression.

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. 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.
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. Ho, W.S., Wang, H., Maggio, D., Kovach, J.S., Zhang, Q., Song, Q., Marincola, F.M., Heiss, J.D., Gilbert, M.R., Lu, R. and Zhuang, Z., 2018. Pharmacologic inhibition of protein phosphatase-2A achieves durable immune-mediated antitumor activity when combined with PD-1 blockade. *Nature communications*, 9(1), pp.1-15.
8. Zhou, P., Shaffer, D.R., Arias, D.A.A., Nakazaki, Y., Pos, W., Torres, A.J., Cremasco, V., Dougan, S.K., Cowley, G.S., Elpek, K. and Brogdon, J., 2014. In vivo discovery of immunotherapy targets in the tumour microenvironment. *Nature*, 506(7486), pp.52-57.

Rank	ID	p-value	t	B	Gene	Gene name
79	8585	1.41E-04	5.09	1.23856	PPP2R2D	protein phosphatase 2, regulatory subunit B, delta isoform

Table 1: PPP2R2D 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
3811	7931405	8.21E-03	2.9324439	-2.822219	PPP2R2D	protein phosphatase 2 regulatory subunit B delta

Table 2: PPP2R2D 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.

Figure 1: PPP2R2D 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 PPP2R2D 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 PPP2R2D 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.