Proline dehydrogenase (PRODH) is a differentially expressed gene in brain metastatic human 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 for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that proline dehydrogenase, encoded by PRODH, 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. PRODH mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of PRODH in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of PRODH expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, proline dehydrogenase, PRODH, 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 trastuzumab⁶. 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 differential and increased expression of the gene encoding proline dehydrogenase, *PRODH*, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE42568⁵ 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 was performed using platform GPL885. GSE42568 was generated using Affymetrix Human Genome U133 Plus 2.0 array technology with $n=17$ normal breast tissue biopsies and $n=104$ primary breast tumor biopsies from patients with breast cancer; analysis was performed using platform GPL570. 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 *PRODH* gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of *PRODH* mRNA expression levels with recurrence-free survival in $n=3951$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary 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.

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

Through blind, systems-level analysis of published microarray data⁴, we identified proline dehydrogenase, encoded by *PRODH*, 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

1 patients with breast cancer, PRODH ranked 126 out of 17418 total transcripts (Chart 1), equating
2 to 99.3% differential expression. Differential expression of PRODH in the brain metastases of
3 patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.49E-04$).

4 To validate transcriptome-wide differential expression of PRODH in human breast cancer,
5 we queried a second microarray dataset⁵, here comparing normal breast tissues and primary
6 tumors of the breast. Again, we identified PRODH as a differentially expressed gene in the brain
7 metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes
8 expressed in brain metastases based on significance of difference as compared to normal breast
9 tissues, PRODH ranked 16179 out of 54675 total transcripts (Chart 2), equating to 70.4%
10 differential expression. Differential expression of PRODH in the primary tumors of patients with
11 breast cancer approached the level of statistical significance (Chart 2; $p=1.24E-02$). Thus,
12 differential expression of PRODH, transcriptome-wide, in the tumor tissues of women with breast
13 cancer was conserved across two independent microarray datasets, both in primary and metastatic
14 tumor tissues.

15 **PRODH is expressed at higher levels in the brain metastases of patients with metastatic 16 breast cancer.**

17 We obtained exact mRNA expression levels for PRODH, in primary tumors of the breast
18 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
19 statistical significance of change in PRODH expression in brain metastatic tissues. PRODH was
20 expressed at higher levels in the brain metastases of patients with breast cancer as compared to
21 primary tumors of the breast, and this difference was statistically significant (Figure 1;
22 $p=0.000556$).

23 **PRODH expression is significantly correlated with survival outcomes in human breast 24 cancer.**

25 We performed Kaplan-Meier survival analysis⁸ in 3951 breast cancer patients in total, to
26 evaluate whether PRODH tumor expression was correlated with survival outcomes in breast
27 cancer. We observed a statistically significant correlation between primary tumor expression of
28 PRODH and recurrence-free survival (RFS) in patients with breast cancer (Figure 2). Patients
whose primary tumors expressed low levels of PRODH possessed median RFS of 216.66 months,
while patients whose tumors expressed high levels of PRODH possessed median RFS of 228.85
months. This difference in RFS based on PRODH tumor expression in patients with breast
cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.028; hazard ratio: 0.89
(0.79-0.99))

Thus, by mining published microarray data^{4,5} in an unbiased fashion, we identified proline
dehydrogenase encoded by PRODH, as among the genes whose expression was most different,
transcriptome-wide, in the brain metastases and primary tumors of patients with breast cancer; we
observed significantly increased expression of PRODH in brain metastases as compared to
primary tumors of the breast. Further, we found a significant correlation between PRODH
expression and patient survival outcomes, as recurrence-free survival was significantly greater in
patients whose primary tumors expressed higher levels of PRODH as compared to patients whose
primary tumors expressed lower levels of PRODH.

Discussion

We provided evidence here that proline dehydrogenase, encoded by PRODH, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, that PRODH mRNA is present at significantly increased quantities in brain metastatic tissues as compared to primary tumors of the breast, that PRODH is also differentially expressed in primary tumors of patients with breast cancer and that primary tumor PRODH expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of PRODH in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of PRODH expression may be relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

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., Shabalín, 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. Clarke, C., Madden, S.F., Doolan, P., Aherne, S.T., Joyce, H., O'driscoll, L., Gallagher, W.M., Hennessy, B.T., Moriarty, M., Crown, J. and Kennedy, S., 2013. Correlating transcriptional networks to breast cancer survival: a large-scale coexpression analysis. *Carcinogenesis*, 34(10), pp.2300-2308.
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. 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.

Rank: 126
Probe ID: 1756
p-value: 2.49E-04
t: -4.7
B: 0.689597
Gene: PRODH
Gene name: proline dehydrogenase

Chart 1: PRODH is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 16179
probe ID: 214203_s_at
p-value: 1.24E-02
t: -2.54
B: -4.0505164
Gene: PRODH
Gene name: proline dehydrogenase

Chart 2: PRODH is differentially expressed in human breast cancer when comparing primary tumors of the breast to normal breast tissues.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: PRODH is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of PRODH in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in PRODH expression between primary tumors of the breast and brain metastases is $p=0.000556$.

Figure 2: Significant correlation between PRODH primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for $n=3951$ total patients stratified into two groups, based on low or high expression of PRODH in patient primary tumors. The log rank p -value denoting statistical significance of difference in recurrence-free 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 PRODH 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 PRODH expression: 216.66 months
High PRODH expression: 228.85 months

Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of PRODH.

The median RFS (recurrence-free survival) of patients with low primary tumor expression of PRODH and high primary tumor expression of PRODH is listed in this chart, for $n=3951$ breast cancer patients.