Telethonin 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 telethonin, also known as titin-cap, encoded by TCAP, 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. TCAP mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Up-regulation of TCAP expression may be of relevance to mechanisms by which tumor cells of the breast metastasize to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, telethonin, titin-cap, TCAP, 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 the benign breast, primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of telethonin, encoded by TCAP, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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. 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. 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 TCAP gene expression was significantly between primary tumors of the breast 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.

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

Through analysis of published microarray data⁴, we identified telethonin, also known as titin-cap, encoded by TCAP, 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, TCAP ranked 4 out of 22575 total transcripts (Table 1), equating to 99.98% differential expression. Differential expression of TCAP in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=4.68E-07$).

1 We queried a second microarray dataset³ to validate transcriptome-wide differential expression of
2 TCAP in brain metastatic breast cancer. We again found that TCAP was among the genes whose
3 expression changed most significantly when comparing metastases of the brain to primary tumors of the
4 breast in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain
5 metastases based on significance of change as compared to primary tumors of the breast, TCAP ranked
6 8972 out of 33297 total transcripts (Table 2), equating to 73.1% differential expression. Differential
7 expression of TCAP in brain metastases in brain metastatic breast cancer trended towards statistical
8 significance (Table 2; $p=6.68E-02$).

9 Thus, differential expression of TCAP when compared to primary tumors of the breast in patients
10 with metastatic breast cancer was conserved across two independent microarray datasets^{4,5}.

11 **TCAP is expressed at higher levels in the brain metastases of patients with brain metastatic breast 12 cancer.**

13 We obtained exact mRNA expression levels for TCAP, in primary tumors of the breast and in
14 brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical
15 significance of change in TCAP expression in brain metastatic tissues. TCAP was expressed at higher
16 levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast,
17 and this difference was statistically significant (Figure 1; $p=0.0475$).

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

22 **Discussion**

23 We provided evidence here that telethonin, also known as titin-cap, encoded by TCAP, is among
24 the genes whose expression is most different in the brain metastases of patients with brain metastatic
25 breast cancer. Evaluation of the effects of genetic depletion of TCAP in mouse models of metastatic
26 breast cancer on metastasis to the central nervous system is merited.

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.

Rank	ID	p-value	t	B	Gene	Gene name
4	12356	4.68E-07	8.50	6.28779	TCAP	titin-cap (telethonin)

Table 1: TCAP 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.

Rank	ID	p-value	t	B	Gene	Gene name
8972	8006896	6.68E-02	1.938146	-4.712733	TCAP	titin-cap

Table 2: TCAP is differentially expressed in brain metastases in patients with metastatic breast cancer when compared to primary tumors of the breast, trending towards statistical significance transcriptome-wide.

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: TCAP 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 TCAP 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 TCAP 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.