Apelin is differentially expressed in brain metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Brain metastases are a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data^{4,5} to discover genes associated with brain metastasis in patients with brain metastatic breast cancer. We found that the gene encoding the peptide apelin, APLN, was among those most differentially expressed in the brain metastases of patients with brain metastatic breast cancer. APLN may be of relevance to the biology underlying metastasis to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, brain metastases, central nervous system metastases, APLN, 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 metastases events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets. We performed a global comparative analysis of transcription in the brain metastases of patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and up-regulation of APLN in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE43837⁵ 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. 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 APLN 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 performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain metastatic breast cancer for the discovery of novel therapeutic targets using published microarray data^{4,5}.

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

We identified apelin, a peptide encoded by APLN as among the genes whose expression was most significantly different, transcriptome-wide, 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, APLN ranked 247 out of 22575 total transcripts (Table 1), equating to 98.9% differential expression. Differential expression of APLN in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=9.27E-04$).

We mined a second microarray dataset⁵ to attempt to validate global differential expression of APLN in brain metastatic breast cancer. Again, we found that APLN was among the genes whose expression changed most in the brain metastases of patients with brain metastatic breast cancer when compared to primary tumors of the breast. 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, APLN ranked 3293 out of 61359 total transcripts, equating to 94.6% differential expression (Table 2). Differential expression of APLN in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 2; $p=0.02203619$).

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

We obtained exact mRNA expression levels for APLN, 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 APLN expression in brain metastatic tissues. APLN was expressed at higher 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; $p=0.0354$). We calculated a mean fold change of 2.73 ± 0.71 in APLN expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified APLN as among the genes whose expression was most different in the brain metastases of patients with breast cancer; APLN was expressed at higher levels in brain metastases as compared to primary tumors of the breast.

Discussion

We provided evidence here that the peptide apelin, encoded by APLN, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer when compared to primary tumors of the breast. In retinal epithelial cells, apelin functions as an angiogenic factor by signaling through the apelin receptor, APJ⁶. One study demonstrated that apelin could support tumor neoangiogenesis⁷, and another demonstrated suppression of osteoblast cell death by apelin⁸. Modulation of APLN expression may be of relevance to the biology underlying metastasis to the brain in patients with brain metastatic breast cancer.

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. McMullin, R.P., Wittner, B.S., Yang, C., Denton-Schneider, B.R., Hicks, D., Singavarapu, R., Moulis, S., Lee, J., Akbari, M.R., Narod, S.A. and Aldape, K.D., 2014. A BRCA1 deficient-like signature is enriched in breast cancer brain metastases and predicts DNA damage-induced poly (ADP-ribose) polymerase inhibitor sensitivity. *Breast Cancer Research*, 16(2), pp.1-10.
6. Kasai, A., Shintani, N., Oda, M., Kakuda, M., Hashimoto, H., Matsuda, T., Hinuma, S. and Baba, A., 2004. Apelin is a novel angiogenic factor in retinal endothelial cells. *Biochemical and biophysical research communications*, 325(2), pp.395-400.
7. Sorli, S.C., Le Gonidec, S., Knibiehler, B. and Audigier, Y., 2007. Apelin is a potent activator of tumour neoangiogenesis. *Oncogene*, 26(55), pp.7692-7699.
8. Xie, H., Yuan, L.Q., Luo, X.H., Huang, J., Cui, R.R., Guo, L.J., Zhou, H.D., Wu, X.P. and Liao, E.Y., 2007. Apelin suppresses apoptosis of human osteoblasts. *Apoptosis*, 12(1), pp.247-254.

Rank	ID	p-value	t	B	FC	Gene	Gene name
247	8094	9.27E-04	4.20	-0.47613	2.73 ± 0.71	APLN	apelin

Table 1: APLN is differentially expressed in brain metastases 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 APLN 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
3293	Hs. 248476.0.A1_3p_at	0.02203619	2.376136	-3.26992	APLN	apelin

Table 2: APLN is differentially expressed in brain metastases 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: APLN is expressed at significantly higher levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of APLN 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 APLN 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.