The peptidase inhibitor 16, PI16, is differentially expressed in the brain metastases of patients with metastatic breast cancer.

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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 the peptidase inhibitor PI16, encoded by PI16, was among the genes whose expression was most different in the brain metastatic tumor tissues of patients with metastatic breast cancer. PI16 messenger RNA was present in significantly decreased quantities in metastasis to the brain when compared to normal breast tissues. PI16 was also differentially expressed in primary tumors of the breast when compared to normal breast tissues. Down-regulation of PI16 may be important for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, PI16, peptidase inhibitor 16, 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 metastatic breast cancer^{4,5}. We discovered transcriptome-wide differential expression of PI16 in the brain metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE22384⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. 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 was performed using platform GPL6480. GSE22384 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with $n=9$ normal breast tissues and $n=12$ primary tumors of the breast; analysis was performed using platform GPL8264. 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 PI16 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 using published microarray data^{4,5} to describe the transcriptional landscape of metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

PI16 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the peptidase inhibitor 16, encoded by PI16 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, PI16 ranked 32 out of 22277 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of PI16 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=3.76E-16$).

We asked whether differential expression of PI16 was specific to brain metastasis or whether PI16 differential expression could also be found in primary tumors of the breast. When sorting each of the genes expressed in primary tumors of the breast based on significance of difference in expression as compared to primary tumors of the breast, PI16 ranked 3447 out of 243504 total transcripts (Table 2), equating to 98.6% differential expression. Differential expression of PI16 in the primary tumors of patients with breast cancer was statistically significant (Table 2; $p=2.83E-05$).

1 **PI16 is expressed at significantly lower levels in the brain metastases of patients with brain**
2 **metastatic breast cancer.**

3 We obtained exact mRNA expression levels for PI16, in primary tumors of the breast and in brain
4 metastasis of patients with brain metastatic breast cancer to determine direction and statistical
5 significance of change in PI16 expression in brain metastatic tissues relative to the untransformed breast.
6 PI16 mRNA was present at lower levels in the brain metastases of patients with breast cancer as
7 compared to primary tumors of the breast, and this difference was statistically significant (Figure 1;
8 $p<0.0001$).

9 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
10 PI16 as a differentially expressed gene in the brain metastases of patients with metastatic breast cancer
11 when compared to tissues of the breast, transformed and benign. PI16 was expressed at significantly
12 lower levels in brain metastases as compared to primary tumors of the breast.

13 **Discussion**

14 We provided evidence here that PI16 is among the genes whose expression is most different in
15 the brain metastases of patients with metastatic breast cancer. PI16 has been described as an inhibitor of
16 matrix metalloproteinase 2, as well as a regulator of neuropathic brain by influencing permeability of the
17 blood nerve barrier when expressed by fibroblasts. PI16 may be of relevance to any number of steps
18 required for metastasis to the brain, and down-regulation of PI16 in metastatic tissues of the brain in
19 conjunction with differential expression of PI16 in primary tumors of the breast relative to normal breast
20 tissue suggests that changes in PI16 expression at some point during progression of primary tumor to
21 brain metastases - including exit from the breast, entry into the vasculature or lymphatics, or breach of the
22 blood-brain barrier - may be important for colonization of the brain with primary tumor-derived clones.
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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. 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.
5. 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.
6. Elloumi, F., Hu, Z., Li, Y., Parker, J.S., Gulley, M.L., Amos, K.D. and Troester, M.A., 2011. Systematic bias in genomic classification due to contaminating non-neoplastic tissue in breast tumor samples. *BMC medical genomics*, 4(1), p.54.
7. Hazell, G.G., Peachey, A.M., Teasdale, J.E., Sala-Newby, G.B., Angelini, G.D., Newby, A.C. and White, S.J., 2016. PI16 is a shear stress and inflammation-regulated inhibitor of MMP2. *Scientific reports*, 6, p.39553.
8. Singhmar, P., Ma, J., Huo, X., Peng, B., Heijnen, C.J. and Kavelaars, A., 2020. The fibroblast-derived protein PI16 controls neuropathic pain. *Proceedings of the National Academy of Sciences*, 117(10), pp. 5463-5471.

Rank	ID	p-value	t	B	Gene	Gene name
32	A_23_P161197	3.76E-16	12.1916877	26.433234	SEC61A2	Sec61 translocon alpha 2 subunit

Table 1: PI16 is differentially expressed in brain metastases in metastatic breast cancer when compared to normal breast tissues.

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 PI16 expression between the brain metastases of patients with breast cancer and normal breast tissues, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
3447	96557	2.83E-05	-5.27	2.5676456	PI16	peptidase inhibitor 16

Table 2: PI16 is differentially expressed in primary tumors of the breast in patients with 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, gene and gene name are listed in this chart.

Figure 1: PI16 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of PI16 in normal breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean PI16 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal (benign) tissues of the breast, a *p*-value, listed above.