Vascular endothelial growth factor D, VEGF-D, encoded by *FIGF* is differentially expressed in metastatic breast cancer, both in metastases to the brain and to the lymph nodes.

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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 c-fos induced growth factor, FIGF was among the genes whose expression was most different in the metastatic tumor tissues of patients with metastatic breast cancer, both in metastases to brain and to the lymph nodes when compared to primary tumors of the breast or normal breast tissue, respectively. We observed significant down-regulation of FIGF in metastasis to the brain. Molecular functions and down-regulation of FIGF may be important for metastasis of primary tumor-derived cancer cells to the lymph nodes and to the brain in humans with metastatic breast cancer and suggests some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, FIGF, c-fos induced growth factor, vascular endothelial growth factor D, VEGF-D, 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 FIGF in brain and lymph node metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE124648⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE124648, a microarray dataset, was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissues and $n=44$ lymph node metastases from patients with breast cancer; platform GPL96 was used for this analysis. 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 FIGF 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 and lymph node 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.

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

We identified C-fos induced growth factor, encoded by FIGF, 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, FIGF ranked 7 out of 22277 total transcripts (Table 1), equating to 99.97% differential expression. Differential expression of FIGF in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=3.26E-23$).

We asked whether we could validate differential expression of FIGF was specific to brain metastasis or if we could observe differential expression of FIGF in lymph node metastasis. Comparison of global gene expression profiles of 10 normal breast tissues to 44 lymph node metastases⁵ revealed that FIGF was among the genes whose expression was most significantly different transcriptome-wide between the breast and lymph node metastases in patients with metastatic breast cancer (Table 2).

Next, we queried a second microarray dataset generated using lymph node metastases and normal breast tissues from patients with breast cancer to determine whether differential expression of VEGF-D was specific to brain metastases or could be observed in metastases to other sites in patients with metastatic breast cancer. When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues in patients with breast cancer, VEGF-D ranked 283 out of 22283 total transcripts (Table 2), equating to 98.7% differential expression. Differential expression of VEGF-D in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Table 2; $p=8.95E-11$).

VEGF-D is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for VEGF-D, in normal breast tissues and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in VEGF-D expression in brain metastatic tissues. VEGF-D was expressed at lower levels in the brain metastases of patients with breast cancer as compared to normal breast tissues, and this difference was statistically significant (Figure 1; $p<0.0001$).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified VEGF-D as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to the normal breast; VEGF-D was expressed at significantly lower levels in brain metastases as compared to benign breast tissue.

Discussion

We provided evidence here that the vascular endothelial growth factor D, encoded by VEGF-D, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. While expression of VEGF-D has been reported to support tumor metastasis through the lymphatic system^{6,7} we found that VEGF-D is expressed at significantly lower levels in brain metastases as compared to the untransformed breast. Independent evaluation of the effects of ectopic expression of VEGF-D in mouse models of metastatic breast cancer on metastasis to the central nervous system are 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. 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.
5. Sinn, B.V., Fu, C., Lau, R., Litton, J., Tsai, T.H., Murthy, R., Tam, A., Andreopoulou, E., Gong, Y., Murthy, R. and Gould, R., 2019. SET ER/PR: a robust 18-gene predictor for sensitivity to endocrine therapy for metastatic breast cancer. *NPJ breast cancer*, 5(1), pp.1-8.
6. Yamada, Y., Nezu, J.I., Shimane, M. and Hirata, Y., 1997. Molecular cloning of a novel vascular endothelial growth factor, VEGF-D. *Genomics*, 42(3), pp.483-488.
7. Debinski, W., Slagle-Webb, B., Achen, M.G., Stacker, S.A., Tulchinsky, E., Gillespie, G.Y. and Gibo, D.M., 2001. VEGF-D is an X-linked/AP-1 regulated putative onco-angiogen in human glioblastoma multiforme. *Molecular Medicine*, 7(9), pp.598-608.

Rank	ID	p-value	t	B	Gene	Gene name
7	A_23_P45185	3.26E-23	-18.5185799	41.665154	FIGF	c-fos induced growth factor

Table 1: FIGF is differentially expressed in brain metastases in 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, fold change (FC) of FIGF 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
283	206742_at	8.95E-11	7.977436	14.331101	PIR-FIGF///FIGF	PIR-FIGF readthrough///c-fos induced growth factor

Table 2: A FIGF / PIR-FIGF read-through transcript is differentially expressed in lymph node 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, gene and gene name are listed in this chart.

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

The mRNA expression level of FIGF 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 FIGF 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.