Differential expression of VEGF-A in the brain and lymph node metastases of patients with breast cancer.

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Metastasis to the brain is a complication of breast cancer^{1,2} with limited treatment options³. We mined published microarray data^{4,5} to discover genes associated with brain metastases in patients with metastatic breast cancer. We identified significant differential expression of the gene encoding the vascular endothelial growth factor A, VEGFA, in both the brain metastases and lymph node metastases of patients with breast cancer as compared to primary tumors of the breast. VEGF-A, a molecule described to possess the ability to disrupt the blood-brain barrier⁶, may be relevant to the underlying biology by which tumor cells of the breast spread to the lymph nodes and to the brain.

Keywords: VEGFA, breast cancer, metastasis, lymph node metastasis, brain metastases, central nervous system metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study documented a 34% incidence of metastases to the central nervous system in breast cancer patients treated with trastuzumab⁶. An unbiased and systems-level understanding of the transcriptional makeup of brain metastatic tissues can support identification of therapeutic targets. We performed global transcriptional profiling of brain metastases in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential expression of the eukaryotic translation initiation factor VEGFA in brain metastases from patients with brain 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 $n=256$ primary breast tumors from patients with breast cancer and $n=67$ 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 VEGFA 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 sought to discover genes associated with metastasis to the brain in patients with breast cancer by mining published microarray datasets^{4,5}, comparing global gene expression profiles of primary tumors of the breast to brain metastatic tissues from patients with breast cancer.

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

We identified the gene encoding the vascular endothelial growth factor A, VEGFA, as among the genes whose expression was most quantitatively different in brain metastases when compared to primary tumors of the breast in patients with breast cancer⁴. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, VEGFA ranked 337 out of 22277 total transcripts (Table 1), equating to 98.5% differential expression. Differential expression of VEGFA in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=4.24E-03$).

VEGFA is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of VEGFA in metastatic breast cancer. We again found that VEGFA was among the genes whose expression was most significantly different in metastatic breast cancer, here when comparing primary tumors of the breast to lymph node metastases from patients with metastatic breast cancer. When

1 sorting each of the genes expressed in lymph node metastases based on significance of difference in
2 expression as compared to primary tumors of the breast, VEGFA ranked 7929 out of 22283 total
3 transcripts (Table 2), equating to 64.4% differential expression. Differential expression of VEGFA in
4 lymph node metastases in patients with breast cancer approached the level of statistical significance
(Table 2; $p=3.4E-04$).

5 **VEGFA is expressed at significantly higher levels in the brain metastases of patients with brain
6 metastatic breast cancer as compared to primary tumors of the breast.**

7 We obtained exact mRNA expression levels for VEGFA in primary tumors of the breast and in
8 brain metastases to evaluate magnitude and direction of change in VEGFA gene expression in brain
9 metastatic tissues. VEGFA was expressed at higher levels in brain metastases from patients with breast
10 cancer as compared to primary tumors of the breast (Figure 1). Increased expression of VEGFA in brain
11 metastases as compared to primary tumors of the breast was statistically significant (Figure 1: $p=0.0014$).
12 We calculated a mean fold change of 1.13 ± 0.05 in VEGFA expression in brain metastases when
13 compared to primary tumors of the breast (Table 1).

14 Thus, through comparative transcriptome analysis of tumor and metastatic tumor tissues of
15 patients with breast cancer using published microarray data^{4,5}, we found that VEGFA was among the
16 genes most differentially expressed in metastatic breast cancer, with significantly increased expression in
17 brain metastatic tissues and in lymph node metastases as compared to primary tumors of the breast.

18 **Discussion**

19 To facilitate discovery of novel therapeutic targets in brain metastatic breast cancer, and to more
20 thoroughly understand the biology of metastasis to the brain in breast cancer, we mined published
21 microarray data^{4,5} to identify genes most differentially expressed in the brain and lymph node metastases
22 of patients with metastatic breast cancer, discovering the vascular endothelial growth factor A (VEGF-A)
23 as among the genes whose expression changes most significantly.

24 The vascular endothelial growth factor A, as its name implies, functions in the creation of
25 endothelium of the blood vasculature, or blood vessels⁷. Importantly, VEGF-A produced by astrocytes
26 supports breakdown of the blood-brain barrier (BBB), as inactivation of astrocytic VEGF-A reduces
27 breakdown of the BBB^{7,8}. In hepatocellular carcinomas associated with the hepatitis C virus, researches
28 have identified copy number gains in the VEGF-A gene on 6p21 in a subset of tumors⁹. VEGF-A can
bind both vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2¹⁰. VEGF-A can
stimulate both lymphangiogenesis and hemangiogenesis¹⁰, consistent with our finding that VEGFA was
among the genes most differentially expressed in the lymph node metastases of patients with metastatic
breast cancer.

We found here using published microarray data^{4,5} that VEGF-A was among the genes whose
expression changed most significantly in the brain metastases of patients with metastatic breast cancer
and in lymph node metastases, and present at significantly higher quantities at the mRNA level in brain
metastatic tissue as compared to primary tumors of the breast. VEGF-A can disrupt the blood-brain
barrier and stimulate lymphangiogenesis - together with our findings here, this indicates VEGF-A may
likely be important for metastasis to the brain, perhaps through the lymph nodes.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
337	210512_s_at	4.24E-03	3.0618071	-1.9953	1.13 ± 0.05	VEGFA	vascular endothelial growth factor A

Table 1: Differential expression of VEGFA in brain metastases in patients with 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 VEGFA 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
7929	211527_x_at	3.4E-04	-3.621189	-1.10387	VEGFA	vascular endothelial growth factor A

Table 2: Differential expression of VEGFA in lymph node metastases in patients with 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: VEGFA is expressed at significantly higher levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of VEGFA 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 VEGFA 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.