Semaphorin 3D is differentially expressed in the lymph node and 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 growth cone guidance factor semaphorin 3D^{6,7}, encoded by SEMA3D, was among the genes whose expression was most different in the brain and lymph node metastases of patients with metastatic breast cancer as compared to normal breast tissues. SEMA3D mRNA was present at significantly reduced quantities in brain metastatic tissues as compared to normal breast tissues. Down-regulation of the repulsive signals promoted by semaphorin 3D⁸ and interactions with its cognate plexin receptor(s)⁹ may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer. These data combined suggest some level of common origin for metastases that reside in the lymph nodes and colonize the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, lymph node metastasis, SEMA3D, semaphorin 3D, 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 trial 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 primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of semaphorin 3D, encoded by SEMA3D, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE124648⁵ and 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 performed using platform GPL6480. 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 SEMA3D gene expression was significantly between normal breast tissues 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 and lymph node metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

SEMA3D is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Identification of differentially expressed genes across datasets in cancer can be difficult and more challenging than when doing so in benign organ tissue and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked how gene expression was most significantly different between brain metastases from patients with breast cancer and normal breast tissue as opposed to primary tumors of the breast, by comparing global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁴. Using this strategy, we identified the semaphorin 3D, SEMA3D, 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 in expression between brain metastases and normal breast tissues, SEMA3D ranked 62 out of 41093 total transcripts (Table 1), equating to 99.8% differential expression. Differential expression of SEMA3D in brain metastases from

patients with brain metastatic breast cancer in this dataset was statistically significant (Table 1; $p=3.19E-13$).

Next, we queried a second microarray dataset⁵ generated using lymph node metastases and normal breast tissues from patients with breast cancer to determine whether we could validate differential expression of SEMA3D in metastatic tissues of humans with breast cancer, and whether differential expression of SEMA3D 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, SEMA3D ranked 5942 out of 22283 total transcripts (Table 2), equating to 73.3% differential expression. Differential expression of SEMA3D in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Table 2; $p=1.37E-02$). Thus, differential expression of SEMA3D was conserved in the brain and lymph node metastases of patients with metastatic breast cancer in two independent microarray datasets^{4,5}.

SEMA3D is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for SEMA3D, in normal breast tissues and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in SEMA3D expression in brain metastatic tissues. SEMA3D 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 the semaphorin SEMA3D as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer both when compared to the normal breast; SEMA3D expression, as compared to the benign breast, was significantly lower in metastasis to the brain.

Discussion

We provided evidence here that axonal growth cone guidance molecule semaphorin 3D^{6,7}, encoded by SEMA3D, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer. While one study has described significantly correlation of increased SEMA3E expression in primary tumors of the breast with distant metastasis¹⁰, we found that SEMA3D is expressed at significantly lower levels in brain metastases as compared normal breast tissues. Our study utilized blind, comparative transcriptome analysis to discover genes associated with central nervous system metastasis in humans with breast cancer, and we validated our findings across two independently generated microarray datasets. Evaluation of the effects of ectopic expression of SEMA3D in mouse models of metastatic breast cancer on metastasis to the central nervous system are merited. Differential expression and down-regulation of semaphorin 3D in metastasis to the lymph nodes and to the brain suggests some level of common origin for metastases that reside in the lymph nodes, successfully evade immune clearance, and subsequently colonize the brain.

References

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Rank	ID	p-value	t	B	Gene	Gene name
62	A_32_P29118	3.19E-13	-10.0060835	19.936475	SEMA3D	semaphorin 3D

Table 1: SEMA3D is differentially expressed in brain metastases in patients with 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.

Rank	ID	p-value	t	B	Gene	Gene name
5942	215324_at	1.37E-02	2.54744	-3.87345	SEMA3D	semaphorin 3D

Table 2: SEMA3D is differentially expressed in lymph node metastases of patients with metastatic 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: SEMA3D is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to normal breast tissues.

The mRNA expression level of SEMA3D in normal breast tissues (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean SEMA3D levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal tissues of the breast, a p -value, listed above.