Semaphorin 3A is a differentially expressed gene in both lymph node and brain metastases in human 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 for the discovery of genes associated with brain metastasis in humans with metastatic breast cancer. We found that semaphorin 3A, encoded by SEMA3A, was among the genes whose expression was most quantitatively different in the brain metastases of patients with metastatic breast cancer. SEMA3A mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of SEMA3A in primary tumors was significantly correlated with patient distant metastasis-free survival in patients with breast cancer. Modulation of SEMA3A expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, semaphorin 3A, SEMA3A, 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 trial⁶ 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 trastuzumab⁶. 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 differential and decreased expression of the gene encoding semaphorin 3A, SEMA3A, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GS52604⁵ 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 $n=4$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE52604 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer; analysis was performed using platform GPL6480. 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 SEMA3A gene expression was significantly different between primary tumors of the breast and brain metastases in humans with breast cancer using a two-tailed t-test. For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of SEMA3A mRNA expression levels in primary tumors of the breast with distant metastasis-free survival in $n=1803$ breast cancer patients.

Results

We performed global comparative transcriptome analysis of metastatic and primary tumor tissues of patients with metastatic breast, as well as normal tissues of the breast using published microarray data^{4,5} to describe the transcriptional landscape of brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

SEMA3A is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified semaphorin 3A, encoded by SEMA3A, as a differentially expressed gene in the brain metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, SEMA3A ranked 109 out of 17418 total transcripts (Chart 1), equating to 99.4% differential expression. Differential expression of SEMA3A in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.18\text{e-}04$).

Differential gene expression analyses that utilize primary and metastatic tumor tissues to discover genes associated with metastasis can be challenging due to the relatively heterogeneous cellular composition and constitution of the tumor as compared to normal, untransformed (benign) tissues. Thus, to attempt to validate differential transcriptome-wide differential expression of SEMA3A in brain metastases in human metastatic breast cancer, we queried a second microarray dataset⁵, here comparing normal breast tissues rather than primary tumors of the breast to brain metastases. Again, we identified SEMA3A as a differentially expressed gene in the brain metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to normal breast tissues, SEMA3A ranked 13733 out of 41093 total transcripts (Chart 2), equating to 93.1% differential expression. Differential expression of SEMA3A in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=1.96e-02$). Thus, differential expression of SEMA3A, transcriptome-wide, in the brain metastases of women with metastatic breast cancer was conserved across two independent microarray datasets, both when compared to benign and transformed breast tissues. This suggests that SEMA3A differential expression in human brain metastatic breast cancer is a *bona fide* biological process.

SEMA3A is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for SEMA3A, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical significance of change in SEMA3A expression in brain metastatic tissues. We observed significantly decreased expression of SEMA3A in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: SEMA3A was expressed at -1.53 ± 0.80 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -3.40 ± 0.29 AU in brain metastatic tissues (Figure 1). The difference in SEMA3A mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.00062$).

SEMA3A expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in 1803 breast cancer patients in total, to evaluate whether SEMA3A tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of SEMA3A and distant metastasis-free survival (DMFS) in patients with breast cancer, in the lower survival quartile (Figure 2). Patients whose primary tumors expressed low levels of SEMA3A possessed median DMFS of 143.11 months, while patients whose tumors expressed high levels of SEMA3A possessed median DMFS of 92 months. This difference in DMFS based on SEMA3A tumor expression in patients with breast cancer approached statistical significance (Figure 2, Chart 3; logrank p -value 0.055; hazard ratio: 1.25 (0.99-1.58)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified semaphorin 3A, encoded by SEMA3A, as among the genes whose expression was most different, transcriptome-wide, in the brain metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly decreased expression of SEMA3A in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between SEMA3A expression and patient survival outcomes, as distant metastasis-free survival was greater in patients whose primary tumors expressed higher levels of SEMA3A as compared to patients whose primary tumors expressed lower levels of SEMA3A.

Discussion

We provided evidence here that SEMA3A is among the genes whose expression is most different in the brain metastases of patients with metastatic breast cancer, that SEMA3A mRNA is present at significantly decreased quantity in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor SEMA3A expression is correlated with patient survival outcomes in human breast cancer. Semaphorin 3A has been described as a chemorepellent with functions in axonal guidance and inhibition of SEMA3A was shown to enhance regenerative responses in injured spinal cord^{8,9}, suggesting that down-regulation of SEMA3A in brain metastatic breast cancer clones may support migration or survival in the central nervous system by limiting SEMA3A axonal growth inhibition, which further alludes to activation of a neuronal gene expression program in tumor cells of the breast. Evaluation of the effects of ectopic expression of SEMA3A in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of SEMA3A expression may be relevant to the processes by which breast cancer cells exit the breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance, breach the blood-brain barrier and colonize the brain.

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Rank: 109
Probe ID: 16662
p-value: 2.18e-04
t: 4.77
B: 0.810368
Gene: SEMA3A
Gene name: semaphorin 3A

Chart 1: SEMA3A is differentially expressed in brain metastatic breast cancer when comparing brain metastases to primary tumors of the breast.

The rank of global differential expression, the probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Rank: 13733
probe ID: A_23_P317591
p-value: 1.96e-02
t: 2.4173701
B: -3.955278
Gene: SEMA3A
Gene name: semaphorin 3A

Chart 2: SEMA3A is differentially expressed in lymph node metastatic breast cancer when comparing brain metastases to normal breast tissues.

The rank of global differential expression, probe/transcript ID, the *p*-value with respect to differential expression transcriptome-wide, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the gene and gene name are listed in this chart.

Figure 1: SEMA3A is expressed at significantly lower levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of SEMA3A in primary tumors of the breast (left) and in brain metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in SEMA3A expression between primary tumors and brain metastases is $p=0.00062$.

Figure 2: SEMA3A primary tumor expression is correlated with distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival for $n=1803$ total breast cancer patients stratified into two groups, based on low or high expression of SEMA3A in patient primary tumor, in the lower survival quartile. The log rank p -value denoting statistical significance of difference in distant metastasis-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on SEMA3A expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low SEMA3A expression: 143.11 months
High SEMA3A expression: 92 months

Chart 3: Median distant metastasis-free survival is inferior in patients with high primary tumor expression of SEMA3A.

The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of SEMA3A and high primary tumor expression of SEMA3A is listed in this chart, for $n=1803$ breast cancer patients, in the lower survival quartile.