The sphingosine-1-phosphate receptor 4, S1PR4, is differentially expressed in the lymph node metastases of patients with breast cancer.

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Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. Between the breast and the brain reside the secondary lymphoid organ, the lymph nodes. We mined published microarray data^{4,5} to compare primary and metastatic tumor transcriptomes for the discovery of genes associated with metastasis to the lymph nodes in humans with metastatic breast cancer. We found that the sphingosine-1-phosphate receptor 4, S1PR4, was among the genes whose expression was most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. S1PR4 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of S1PR4 in primary tumors of the breast was significantly correlated with patient overall survival. Modulation of S1PR4 expression may be relevant to the biology by which tumor cells metastasize from the breast to the lymph nodes and the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, lymph node metastasis, sphingosine-1-phosphate receptor 4, S1PR4, 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 and lymph node metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. Lymph nodes, a secondary lymphoid organ, reside between the breast and the brain. We performed a global comparative analysis of lymph node metastatic tumor tissues in patients with metastatic breast cancer, compared to primary tumors and normal breast tissues^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding the sphingosine-1-phosphate receptor 4, S1PR4, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE124648⁵ 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 $n=71$ primary breast tumors and $n=7$ lymph node metastases from patients with breast cancer; analysis was performed using platform GPL887. GSE124648 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; analysis was performed using platform GPL96. 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 S1PR4 gene expression was significantly different between primary tumors of the breast and lymph node 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 S1PR4 mRNA expression levels with overall survival in $n=1402$ breast cancer patients.

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 metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

S1PR4 is differentially expressed in the lymph node metastases of patients with metastatic breast cancer.

Through blind, systems-level analysis of published microarray data⁴, we identified the sphingosine-1-phosphate receptor 4, encoded by S1PR4, as a differentially expressed gene in the lymph node metastatic tissues of humans with breast cancer (Table 1). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to primary tumors of the breast in patients with breast cancer, S1PR4 ranked 131 out of 18034 total transcripts (Chart 1), equating to 99.3% differential expression. Differential expression of S1PR4 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.28e-03$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of S1PR4 in the lymph node metastases of patients with metastatic breast cancer. We again observed S1PR4 to be among the genes expressed most quantitatively differently in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, S1PR4 ranked 2256 out of 22283 total transcripts (Chart 2), equating to 89.9% differential expression. Differential expression of S1PR4 in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=1.21\text{e-}04$).

S1PR4 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for S1PR4, in primary tumors of the breast and in lymph node metastases of patients with metastatic breast cancer to determine direction and statistical significance of change in S1PR4 expression in lymph node metastatic tissues. We observed significantly increased expression of S1PR4 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: S1PR4 was expressed at 1.22 ± 0.87 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 2.44 ± 1.47 AU in lymph node metastatic tissues (Figure 1). The difference in S1PR4 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.001518$).

S1PR4 expression is significantly correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=1402$ breast cancer patients in total, to evaluate whether S1PR4 tumor expression was correlated with survival outcomes in breast cancer. We observed a statistically significant correlation between primary tumor expression of S1PR4 and overall survival (OS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of S1PR4 possessed median OS of 78 months, while patients whose tumors expressed high levels of S1PR4 possessed median OS of 118.82 months. This difference in OS based on S1PR4 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.0035; hazard ratio: 0.73 (0.59-0.9)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified S1PR4 as among the genes whose expression was most different, transcriptome-wide, in the lymph node metastases of patients with breast cancer, when compared to primary tumors of the breast and to normal breast tissues, respectively; we observed significantly increased expression of S1PR4 in lymph node metastases as compared to primary tumors of the breast. Further, we found a significant correlation between S1PR4 expression and patient survival outcomes, as overall survival was significantly greater in patients whose primary tumors expressed higher levels of S1PR4 as compared to patients whose primary tumors expressed lower levels of S1PR4.

Discussion

We provided evidence here that the sphingosine-1-phosphate receptor 4, S1PR4, is among the genes whose expression is most different in the lymph node metastases of patients with metastatic breast cancer as compared to primary tumors of the breast and to normal breast tissues, that S1PR4 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor S1PR4 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of S1PR4 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited.

1 Modulation of S1PR4 expression may be relevant to the processes by which breast cancer cells exit the
2 breast, enter the vasculature and/or lymphatics, reside in the lymph nodes, evade immune clearance,
3 breach the blood-brain barrier and colonize the brain.
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Rank: 131
Probe ID: 11884
p-value: 1.28e-03
t: -3.3413563
B: -1.0171
Gene: S1PR4
Gene name: sphingosine-1-phosphate receptor 4

Chart 1: S1PR4 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node 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: 2256
Probe ID: 206437_at
p-value: 1.21e-04
t: -4.136739
B: 0.548029
Gene: S1PR4
Gene name: sphingosine-1-phosphate receptor 4

Chart 2: S1PR4 is differentially expressed in lymph node metastatic breast cancer when comparing lymph node metastases to normal breast tissues.

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.

Figure 1: S1PR4 is expressed at significantly higher levels in the lymph node metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of S1PR4 in primary tumors of the breast (left) and in lymph node metastases of women with metastatic breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in S1PR4 expression between primary tumors of the breast and lymph node metastases, a *p*-value, is $p=0.001518$.

Figure 2: Significant correlation between S1PR4 primary tumor expression and overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1402$ total patients stratified into two groups, based on low or high expression of S1PR4 in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall 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 S1PR4 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.

1 Low S1PR4 expression: 78 months

2 High S1PR4 expression: 118.82 months

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4 **Chart 3: Median overall survival is inferior in patients with low primary tumor expression of S1PR4.**

5 The median OS (overall survival) of patients with low primary tumor expression of S1PR4 and high
6 primary tumor expression of S1PR4 is listed in this chart, for $n=1402$ breast cancer patients.