TNFRSF9 is differentially expressed in the lymph nodes of patients with metastatic 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 TNF receptor superfamily member 9, TNFRSF9, 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. TNFRSF9 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of TNFRSF9 in primary tumors of the breast was correlated with patient overall survival, in lymph node negative patients but not in lymph node positive patients. Modulation of TNFRSF9 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, TNF receptor superfamily member 9, TNFRSF9, 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. The lymph nodes 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 TNF receptor superfamily member 9, TNFRSF9, 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 TNFRSF9 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 TNFRSF9 mRNA expression levels with overall survival in $n=452$ patients with lymph node positive breast cancer patients and for $n=726$ breast cancer patients with lymph node negative breast cancer.

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.

TNFRSF9 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 TNF receptor superfamily member 9, encoded by TNFRSF9, 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, TNFRSF9 ranked 111 out of 18034 total transcripts (Chart 1), equating to 99.4% differential expression. Differential expression of TNFRSF9 in the lymph

node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=8.72e-04$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of TNFRSF9 in the lymph node metastases of patients with metastatic breast cancer. We again observed TNFRSF9 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, TNFRSF9 ranked 3054 out of 22283 total transcripts (Chart 2), equating to 86.3% differential expression. Differential expression of TNFRSF9 in the lymph node metastases of patients with metastatic breast cancer approached the level of statistical significance (Chart 2; $p=6.55e-04$).

TNFRSF9 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for TNFRSF9, 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 TNFRSF9 expression in lymph node metastatic tissues. We observed significantly increased expression of TNFRSF9 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: TNFRSF9 was expressed at 0.47 ± 0.39 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 1.05 ± 0.70 AU in lymph node metastatic tissues (Figure 1). The difference in TNFRSF9 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.000887$).

TNFRSF9 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=452$ lymph node positive breast cancer patients and $n=726$ lymph node negative breast cancer patients, to evaluate whether TNFRSF9 tumor expression was correlated with survival outcomes in breast cancer and whether lymph node status influenced correlation of TNFRSF9 tumor expression with survival outcomes in human breast cancer. We observed a correlation between primary tumor expression of TNFRSF9 and overall survival (OS) in lymph node negative breast cancer (Figure 2). Lymph node negative patients whose primary tumors expressed low levels of TNFRSF9 possessed median OS of 143 months, while lymph node negative patients whose tumors expressed high levels of TNFRSF9 possessed median OS of 197.62 months. This difference in OS based on TNFRSF9 tumor expression in lymph node positive patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value: 0.039 (hazard ratio: 0.7 (0.49-0.98))). TNFRSF9 primary tumor expression was not significantly correlated with overall survival in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.68; hazard ratio: 1.07 (0.77-1.49)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified TNFRSF9 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; we observed significantly increased expression of TNFRSF9 in lymph node metastases as compared to primary tumors of the breast. Further, we found a correlation between TNFRSF9 expression and patient overall survival, in lymph node negative patients but not in lymph node positive patients.

Discussion

We provided evidence here that TNF receptor superfamily member 9, encoded by TNFRSF9, 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, that TNFRSF9 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor TNFRSF9 expression is correlated with patient survival outcomes in human breast cancer, in lymph node negative patients but not in lymph node positive patients. Evaluation of the effects of genetic depletion of TNFRSF9 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of TNFRSF9 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: 111
Probe ID: 16755
p-value: 8.72e-04
t: -3.4606058
B: -0.6955
Gene: TNFRSF9
Gene name: TNF receptor superfamily member 9

Chart 1: TNFRSF9 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: 3054
Probe ID: 207536_s_at
p-value: 6.55e-04
t: -3.611788
B: -1.062549
Gene: TNFRSF9
Gene name: TNF receptor superfamily member 9

Chart 2: TNFRSF9 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: TNFRSF9 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 TNFRSF9 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 TNFRSF9 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.000887$.

Figure 2: TNFRSF9 primary tumor expression is correlated with overall survival in lymph node negative patients but not in lymph node positive patients.

Depicted in these Kaplan-Meier plots is the probability of overall survival in $n=452$ lymph node positive patients (**left**) and $n=726$ lymph node negative patients (**right**), based on low or high expression of TNFRSF9 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 TNFRSF9 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.

Lymph node negative patients

Low TNFRSF9 expression: 143 months

High TNFRSF9 expression: 197.62 months

Chart 3: Median overall survival is inferior in patients with low primary tumor expression of TNFRSF9.

The median OS (overall survival) of patients with low primary tumor expression of TNFRSF9 and high primary tumor expression of TNFRSF9 is listed in this chart, for $n=452$ patients with lymph node positive breast cancer.