TIGD4 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 tigger transposable element derived 4, TIGD4, 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. Analysis of a separate microarray dataset revealed that TIGD4 was also differentially expressed in brain metastatic tissues⁵. TIGD4 mRNA was present at increased quantities in lymph node metastases as compared to primary tumors of the breast. Importantly, expression of TIGD4 in primary tumors of the breast was correlated with patient distant metastasis-free survival, in lymph node positive patients but not in lymph node negative patients. Modulation of TIGD4 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, metastatic breast cancer, tigger transposable element derived 4, TIGD4, 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 as compared to primary tumors of the breast^{4,5}. We discovered transcriptome-wide differential expression of the gene encoding tigger transposable element derived 4, TIGD4, in lymph node metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE100534⁵ 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. GSE100534 was generated using Affymetrix Human Gene 1.0 ST Array with $n=16$ primary breast tumors and $n=3$ brain metastases from patients metastatic breast cancer; analysis was performed using platform GPL6244. 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 TIGD4 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 TIGD4 mRNA expression levels with distant metastasis-free survival in $n=261$ lymph node positive breast cancer patients and $n=240$ lymph node negative 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.

TIGD4 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 tigger transposable element derived 4, encoded by TIGD4, 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, TIGD4 ranked 205 out of 18034 total transcripts (Chart 1), equating to 98.9% differential expression. Differential expression of TIGD4 in the lymph

node metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=2.47\text{e-}03$).

To attempt to validate transcriptome-wide differential expression of TIGD4 in metastatic tissues of patients with metastatic breast cancer, we queried a second microarray dataset⁵, here comparing primary tumors from patients with breast cancer to brain metastases. Here, we identified TIGD4 as a differentially expressed gene in human brain metastatic 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, TIGD4 ranked 4859 out of 41093 total transcripts (Chart 2), equating to 88.2% differential expression. Differential expression of TIGD4 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=1.52\text{e-}02$). Thus, differential expression of TIGD4, transcriptome-wide, in the metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets. This suggests that TIGD4 differential expression in human metastatic breast cancer is a *bona fide* biological process.

TIGD4 is expressed at higher levels in the lymph node metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for TIGD4, 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 TIGD4 expression in lymph node metastatic tissues. We observed significantly increased expression of TIGD4 in the lymph node metastases of patients with breast cancer as compared to primary tumors of the breast: TIGD4 was expressed at 0.04 ± 0.36 arbitrary units (AU) in primary tumors of the breast, while it was expressed at 0.54 ± 0.74 AU in lymph node metastatic tissues (Figure 1). The difference in TIGD4 mRNA levels between primary tumors of the breast and lymph node metastases was statistically significant (Figure 1; $p=0.002459$).

TIGD4 expression is correlated with survival outcomes in human breast cancer.

We performed Kaplan-Meier survival analysis⁷ in $n=261$ lymph node positive breast cancer patients and $n=240$ lymph node negative breast cancer patients, to evaluate whether TIGD4 tumor expression was correlated with survival outcomes in breast cancer, and whether lymph node status of the patient influenced this correlation. We observed a correlation between primary tumor expression of TIGD4 and distant metastasis-free survival (DMFS) in lymph node positive breast cancer (Figure 2). Lymph node positive patients whose primary tumors expressed low levels of TIGD4 possessed median DMFS of 31 months, while lymph node positive patients whose tumors expressed high levels of TIGD4 possessed median DMFS of 60 months. This difference in DMFS based on TIGD4 tumor expression in lymph node positive patients with breast cancer trended towards statistical significance (Figure 2, Chart 3; logrank p -value: 0.097; hazard ratio: 0.67 (0.42-1.08)). TIGD4 primary tumor expression was not correlated with distant metastasis-free survival in lymph node negative patients (Figure 2, Chart 3; logrank p -value: 0.38; hazard ratio: 0.73 (0.36-1.47)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified TIGD4 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 TIGD4 in lymph node metastases as compared to primary tumors of the breast. TIGD4 was also differentially expressed in brain metastatic tissues. Further, we found a correlation between TIGD4 expression and patient survival outcomes, as TIGD4 primary tumor expression was correlated with distant metastasis-free survival in lymph node positive patients but not in lymph node negative patients.

Discussion

We provided evidence here that the tigger transposable element derived 4, TIGD4, 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 TIGD4 is also differentially expressed in brain metastatic tissues in human breast cancer, that TIGD4 mRNA is present at significantly increased quantities in lymph node metastatic tissues as compared to primary tumors of the breast, and that primary tumor TIGD4 expression is correlated with patient survival outcomes in human breast cancer, in lymph node positive patients but not in lymph node negative patients. Evaluation of the effects of genetic depletion of TIGD4 in mouse models of metastatic breast cancer on metastasis to the lymph nodes and to the central nervous system is merited. Modulation of TIGD4 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: 205
Probe ID: 15226
p-value: 2.47e-03
t: -3.127471
B: -1.5729
Gene: TIGD4
Gene name: tigger transposable element derived 4

Chart 1: TIGD4 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: 4859
Probe ID: 8103235
p-value: 1.52e-02
t: 2.653427
B: -3.392218
Gene: TIGD4
Gene name: tigger transposable element derived 4

Chart 2: TIGD4 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.

Figure 1: TIGD4 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 TIGD4 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 TIGD4 expression between primary tumors of the breast and lymph node metastases, a p -value, is $p=0.002459$.

Figure 2: TIGD4 primary tumor expression is correlated with distant metastasis-free survival in lymph node positive breast cancer patients but not in lymph node negative patients.

Depicted in these Kaplan-Meier plots is the probability of distant metastasis-free survival in patients with breast cancer based on low or high expression of TIGD4 in patient primary tumors. 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 TIGD4 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. Probability of distant metastasis-free survival in $n=261$ lymph node positive patients is shown on the **left** and probability of distant metastasis-free survival in $n=240$ lymph node negative patients is shown on the **right**.

Lymph node positive patients

Low TIGD4 expression: 31 months

High TIGD4 expression: 60 months

Chart 3: Median distant metastasis-free survival is inferior in lymph node positive breast cancer patients with low primary tumor expression of TIGD4.

The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of TIGD4 and high primary tumor expression of TIGD4 is listed in this chart for $n=261$ lymph node positive breast cancer patients.