TTF1 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 transcription termination factor 1, encoded by TTF1, was among the genes whose expression was most quantitatively different in the brain metastases of patients with metastatic breast cancer. TTF1 mRNA was present at decreased quantities in brain metastatic tissues as compared to primary tumors of the breast. Importantly, expression of TTF1 in primary tumors was significantly correlated with patient distant metastasis-free survival in patients with breast cancer. Modulation of TTF1 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain. These data are one piece of evidence suggesting a common ancestor or tumor clone for brain and lymph node metastases that originate from the primary tumor, alluding to patterns in developmental origin and migratory pathways through the lymph node in human brain metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, transcription termination factor 1, TTF1, 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 transcription termination factor 1, TTF1, in brain 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 $n=11$ primary breast tumors and $n=4$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. 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 TTF1 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 TTF1 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.

TTF1 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 transcription termination factor 1, encoded by TTF1, 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, TTF1 ranked 78 out of 17418 total transcripts (Chart 1), equating to 99.6% differential expression. Differential expression of TTF1 in the brain metastases of patients with metastatic breast cancer was statistically significant (Chart 1; $p=1.57\text{e-}04$).

TTF1 is differentially expressed in the lymph node metastases of patients with breast cancer.

To determine whether differential expression of TTF1 in human breast cancer could be validated in a second microarray dataset, and whether this was specific to brain metastases or could be observed in other anatomical locations, we queried a second microarray dataset⁵, here comparing normal breast tissues to lymph node metastases. Again, we identified TTF1 as a differentially expressed gene in the lymph node metastatic tissues of patients with breast cancer (Chart 2). When sorting each of the genes expressed in lymph node metastases based on significance of difference as compared to normal breast tissues, TTF1 ranked 5051 out of 22283 total transcripts (Chart 2), equating to 77.3% differential expression. Differential expression of TTF1 in the lymph node metastases of patients with metastatic breast cancer was statistically significant (Chart 2; $p=6.94e-03$). Thus, differential expression of TTF1, transcriptome-wide, in metastatic tissues of women with metastatic breast cancer was conserved across two independent microarray datasets, in the brain and in the lymph nodes.

TTF1 is expressed at lower levels in the brain metastases of patients with metastatic breast cancer.

We obtained exact mRNA expression levels for TTF1, 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 TTF1 expression in brain metastatic tissues. We observed significantly decreased expression of TTF1 in the brain metastases of patients with breast cancer as compared to primary tumors of the breast: TTF1 was expressed at -0.40 ± 0.33 arbitrary units (AU) in primary tumors of the breast, while it was expressed at -1.35 ± 0.22 AU in brain metastatic tissues (Figure 1). The difference in TTF1 mRNA levels between primary tumors of the breast and brain metastatic tissues was statistically significant (Figure 1; $p=0.000152$).

TTF1 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 TTF1 tumor expression was correlated with survival outcomes in breast cancer. We observed a correlation between primary tumor expression of TTF1 and distant metastasis-free survival (DMFS) in patients with breast cancer (Figure 2). Patients whose primary tumors expressed low levels of TTF1 possessed median DMFS of 133.68 months, while patients whose tumors expressed high levels of TTF1 possessed median DMFS of 77.23 months. This difference in DMFS based on TTF1 tumor expression in patients with breast cancer was statistically significant (Figure 2, Chart 3; logrank p -value 0.014; hazard ratio: 1.27 (1.05-1.55)).

Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified transcription termination factor 1, encoded by TTF1, as among the genes whose expression was most different, transcriptome-wide, in the brain and 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 decreased expression of TTF1 in brain metastases as compared to primary tumors of the breast. Further, we found a correlation between TTF1 expression and patient survival outcomes, as distant metastasis-free survival was greater in patients whose primary tumors expressed lower levels of TTF1 as compared to patients whose primary tumors expressed higher levels of TTF1.

Discussion

We provided evidence here that TTF1 is among the genes whose expression is most different in the brain and lymph node metastases of patients with metastatic breast cancer, that TTF1 mRNA is present at significantly decreased quantity in brain metastatic tissues as compared to primary tumors of the breast, and that primary tumor TTF1 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of TTF1 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. Modulation of TTF1 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.

References

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Rank: 78
Probe ID: 7003
p-value: 1.57e-04
t: 4.93
B: 1.113518
Gene: TTF1
Gene name: transcription termination factor 1

Chart 1: TTF1 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: 5051

probe ID: 204772_s_at

p-value: 6.94e-03

t: 2.804354

B: -3.259776

Gene: TTF1

Gene name: transcription termination factor 1

Chart 2: TTF1 is differentially expressed in the lymph node metastases of patients with breast cancer when comparing lymph node 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: TTF1 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 TTF1 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 TTF1 expression between primary tumors and brain metastases is $p=0.000152$.

Figure 2: Significant correlation between TTF1 primary tumor expression and 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 TTF1 in patient primary tumor. 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 TTF1 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 TTF1 expression: 133.68 months

2 High TTF1 expression: 77.23 months

3
4 **Chart 3: Median distant metastasis-free survival is inferior in patients with high primary tumor expression of TTF1.**

5 The median DMFS (distant metastasis-free survival) of patients with low primary tumor expression of
6 TTF1 and high primary tumor expression of TTF1 is listed in this chart, for $n=1803$ breast cancer patients.