TCEAL2 is a differentially expressed gene in brain metastatic 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 the transcriptional elongation factor A like 2, encoded by TCEAL2, was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. TCEAL2 mRNA was present at increased quantities in brain metastatic tissues as compared to primary tumors of the breast. Expression of TCEAL2 in primary tumors was significantly correlated with patient recurrence-free survival. Modulation of TCEAL2 expression may be relevant to the biology by which tumor cells metastasize from the breast to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, transcriptional elongation factor A like 2, TCEAL2, 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 increased expression of the gene encoding the transcriptional elongation factor A like 2, TCEAL2, in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE10893⁴ and GSE125989⁵ 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 $n=3$ brain metastases from patients with breast cancer; analysis was performed using platform GPL885. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 array technology with $n=16$ primary breast tumors and $n=16$ brain metastases from patients with breast cancer; the samples utilized by investigators for this microarray dataset were paired, matched tumor and metastasis samples; analysis was performed using platform GPL571. 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 TCEAL2 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 tool for correlation of TCEAL2 mRNA expression levels with recurrence-free survival in $n=3951$ 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 brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

TCEAL2 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 the gene

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2 encoding the transcriptional elongation factor A like 2, encoded by TCEAL2, as a differentially
3 expressed gene in the breast metastatic tissues of humans with breast cancer (Table 1). When
4 sorting each of the genes expressed in brain metastases based on significance of difference as
5 compared to primary tumors of the breast in patients with breast cancer, TCEAL2 ranked 6 out of
6 17418 total transcripts (Chart 1), equating to 99.97% differential expression. Differential
7 expression of TCEAL2 in the brain metastases of patients with metastatic breast cancer was
8 statistically significant (Chart 1; $p=1.53e-06$).

9 We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide
10 differential expression of TCEAL2 in human brain metastatic breast cancer. Again, we identified
11 TCEAL2 as a differentially expressed gene in the brain metastatic tissues of patients with breast
12 cancer (Chart 2). When sorting each of the genes expressed in brain metastases based on
13 significance of difference as compared to primary tumors of the breast in patients with breast
14 cancer, in this dataset, TCEAL2 ranked 985 out of 22277 total transcripts (Chart 2), equating to
15 95.6% differential expression. Differential expression of TCEAL2 in the brain metastases of
16 patients with metastatic breast cancer was statistically significant (Chart 2; $p=2.23e-02$). Thus,
17 differential expression of TCEAL2, transcriptome-wide, in the brain metastases of women with
18 metastatic breast cancer was conserved across two independent microarray datasets.

19 **TCEAL2 expression is significantly correlated with survival outcomes in human breast** 20 **cancer.**

21 We performed Kaplan-Meier survival analysis⁷ to evaluate correlation in 3951 breast
22 cancer patients in total, to evaluate whether TCEAL2 tumor expression was correlated with
23 survival outcomes in breast cancer. We observed a statistically significant correlation between
24 primary tumor expression of TCEAL2 and recurrence-free survival (RFS) (Figure 2). Patients
25 whose primary tumors expressed low levels of TCEAL2 possessed median RFS of 42 months,
26 while patients whose tumors expressed high levels of TCEAL2 possessed median RFS of 60
27 months. This difference in RFS based on TCEAL2 tumor expression in breast cancer was
28 statistically significant (Figure 2, Chart 3; logrank p -value: 0.00011; hazard ratio: 0.81
(0.72-0.9)).

29 **TCEAL2 is expressed at higher levels in the brain metastases of patients with metastatic** 30 **breast cancer.**

31 We obtained exact mRNA expression levels for TCEAL2, in primary tumors of the breast
32 and in brain metastasis of patients with brain metastatic breast cancer to determine direction and
33 statistical significance of change in TCEAL2 expression in brain metastatic tissues. TCEAL2 was
34 expressed at higher levels in the brain metastases of patients with breast cancer as compared to
35 primary tumors of the breast, and this difference was statistically significant (Figure 1;
36 $p<0.00001$).

37 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we
38 identified the transcriptional elongation factor A like 2, TCEAL2, as among the genes whose
39 expression was most different, transcriptome-wide, in the brain metastases of patients with breast
40 cancer when compared to primary tumors of the breast; we observed significantly increased

expression of TCEAL2 in brain metastases as compared to primary tumors of the breast. Further, we found a significant correlation between TCEAL2 expression and patient survival outcomes, as recurrence-free survival was significantly higher in patients whose primary tumors expressed higher levels of TCEAL2 as compared to patients whose primary tumors expressed lower levels of TCEAL2.

Discussion

We provided evidence here that the transcriptional elongation factor A like 2, encoded by TCEAL2, is among the genes whose expression is most different in the brain metastases of patients with brain metastatic breast cancer, and that primary tumor TCEAL2 expression is significantly correlated with patient survival outcomes in human breast cancer. Evaluation of the effects of genetic depletion of TCEAL2 in mouse models of metastatic breast cancer on metastasis to the central nervous system is merited. The phenotypic consequence of modulating TCEAL2 on nascent transcription in circulating tumor cells may be relevant as a target of study.

References

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Rank: 6
Probe ID: 10250
p-value: 1.53e-06
t: -7.44
B: 5.249604
Gene: TCEAL2
Gene name: transcription elongation factor A (SII)-like 2

Chart 1: TCEAL2 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: 985
Probe ID: 211276_at
p-value: 2.23e-02
t: -2.3842197
B: -3.4571
Gene: TCEAL2
Gene name: transcription elongation factor A like 2

Chart 2: TCEAL2 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 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: TCEAL2 is expressed at significantly higher levels in the brain metastases of patients with metastatic breast cancer when compared to primary tumors of the breast.

The mRNA expression level of TCEAL2 in primary tumors of the breast (left) and in brain metastases from women diagnosed with breast cancer (right) is graphically depicted; the result of a statistical test evaluating significance of difference in TCEAL2 expression between primary tumors and brain metastases is $p < 0.00001$.

Figure 2: Significant correlation between TCEAL2 primary tumor expression and recurrence-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of recurrence-free survival for n=3951 total patients stratified into two groups, based on low or high expression of TCEAL2 in patient primary tumors. The log rank *p*-value denoting statistical significance of difference in recurrence-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 TCEAL2 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 TCEAL2 expression: 42 months

2 High TCEAL2 expression: 60 months

3
4 **Chart 3: Median recurrence-free survival is inferior in patients with low primary tumor expression of TCEAL2.**

5 The median RFS (recurrence-free survival) of patients with low primary tumor expression of
6 TCEAL2 and high primary tumor expression of TCEAL2 is listed in this chart.