TSHZ2 is differentially expressed in the brain metastases of patients with metastatic 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 to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that the teashirt zinc finger homeobox 2, encoded by TSHZ2 was among the genes whose expression was most different in the brain metastatic tumor tissues of patients with metastatic breast cancer. We observed significant down-regulation of TSHZ2 in metastasis to the brain when compared to primary tumors of the breast or to normal breast tissues. Down-regulation of TSHZ2 may be important for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, TSHZ2, teashirt zinc finger homeobox 2, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis 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 metastatic breast cancer^{4,5}. We discovered transcriptome-wide differential expression of TSHZ2 in the brain metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE52604⁴ and GSE125989⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. 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 TSHZ2 gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

Results

We performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain 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.

TSHZ2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the teashirt zinc finger homeobox 2, TSHZ2, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to the primary tumors of patients with brain metastatic breast cancer, TSHZ2 ranked 43 out of 22277 total transcripts (Table 1), equating to 99.8% differential expression. Differential expression of TSHZ2 in the brain metastases of patients with metastatic breast cancer was statistically significant (Table 1; $p=2.19E-14$).

We asked whether we could validate differential expression of TSHZ2 in brain metastatic breast cancer in a separate microarray dataset. Comparison of global gene expression profiles of 16 primary tumors of the breast to 16 brain metastases revealed that TSHZ2 was again among the genes whose expression was most significantly different transcriptome-wide in metastases of the brain when compared to tissues of the breast - in this case, in the primary tumors of patients with metastatic breast cancer as opposed to normal breast tissues (Table 2).

1 When sorting each of the genes expressed in brain metastases based on significance of difference
2 in expression as compared to primary tumors of the breast, TSHZ2 ranked 1102 out of 22277 total
3 transcripts (Table 2), equating to 95.1% differential expression. Differential expression of TSHZ2 in the
4 brain metastases of patients with metastatic breast cancer was statistically significant (Table 2;
5 $p=2.73E-02$).

6 **TSHZ2 is expressed at significantly lower levels in the brain metastases of patients with brain**
7 **metastatic breast cancer.**

8 We obtained exact mRNA expression levels for TSHZ2, in primary tumors of the breast and in
9 brain metastasis of patients with brain metastatic breast cancer to determine direction and statistical
10 significance of change in TSHZ2 expression in brain metastatic tissues relative to the untransformed
11 breast. TSHZ2 mRNA was present at lower levels in the brain metastases of patients with breast cancer
12 as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1;
13 $p<0.0001$).

14 Thus, by mining published microarray data^{4,5} in an unbiased and systematic fashion, we identified
15 TSHZ2 as a differentially expressed gene in the brain metastases of patients with metastatic breast cancer
16 when compared to tissues of the breast, transformed and benign. TSHZ2 was expressed at significantly
17 lower levels in brain metastases as compared to primary tumors of the breast.

18 **Discussion**

19 We provided evidence here that the teashirt zinc finger homeobox 2 encoded by TSHZ2 is among
20 the genes whose expression is most different in the brain metastases of patients with metastatic breast
21 cancer. TSHZ2 may be of relevance to any number of steps required for metastasis to the brain, and
22 down-regulation of TSHZ2 in metastatic tissues of the brain suggests that reduction of TSHZ2 expression
23 at some point during progression of primary tumor to brain metastases - including exit from the breast,
24 entry into the vasculature or lymphatics, or breach of the blood-brain barrier - may be important for
25 colonization of the brain with primary tumor-derived clones.
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Rank	ID	p-value	t	B	Gene	Gene name
43	A_32_P221096	2.19E-14	-10.8503911	22.524484	TSHZ2	teashirt zinc finger homeobox 2

Table 1: TSHZ2 is differentially expressed in brain metastases in metastatic breast cancer when compared to normal breast tissues.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change (FC) of TSHZ2 expression between the brain metastases of patients with breast cancer and normal breast tissues, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1102	220213_at	2.73E-02	-2.3054627	-3.5772	TSHZ2	teashirt zinc finger homeobox 2

Table 2: TSHZ2 is differentially expressed in brain metastases in metastatic breast cancer when compared to primary tumors of the breast.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: TSHZ2 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of TSHZ2 in normal breast tissue (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean TSHZ2 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and normal (benign) tissues of the breast, a *p*-value, listed above.