Differential expression of TNS1 in the brain metastases of patients with breast cancer.

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Metastasis to the brain is a complication of breast cancer^{1,2} with limited treatment options³. We mined published microarray data^{4,5} to discover genes associated with brain metastases in patients with metastatic breast cancer. We identified significant differential expression of the gene encoding tensin 1, TNS1, in the brain metastases of patients with breast cancer as compared to primary tumors of the breast. TNS1 may be relevant to the underlying biology by which tumor cells of the breast spread to the brain.

Keywords: TNS1, breast cancer, brain metastases, central nervous system metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

One study documented a 34% incidence of metastases to the central nervous system in breast cancer patients treated with trastuzumab⁶. An unbiased and systems-level understanding of the transcriptional makeup of brain metastatic tissues can support identification of therapeutic targets. We performed global transcriptional profiling of brain metastases in patients with brain metastatic breast cancer⁶⁻⁸. We discovered significant differential expression of TNS1 in brain metastases from patients with brain metastatic breast cancer.

Methods

We used datasets GSE125989⁴ and GSE43837⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. 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. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. 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 TNS1 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 sought to discover genes associated with metastasis to the brain in patients with breast cancer by mining published microarray datasets^{4,5}, comparing global gene expression profiles of primary tumors of the breast to brain metastatic tissues from patients with breast cancer.

TNS1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding tensin 1, TNS1, as among the genes whose expression was most quantitatively different in brain metastases when compared to primary tumors of the breast in patients with breast cancer⁴. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, TNS1 ranked 59 out of 22277 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of TNS1 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.16E-04$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of TNS1 in brain metastatic breast cancer. We again found that TNS1 was among the genes whose expression was most quantitatively different when comparing primary tumors of the breast to brain metastases from patients with metastatic breast cancer. When sorting each of the genes expressed in brain metastases based on significance of difference in expression as compared to primary tumors of the breast, TNS1 ranked 369 out of 61359 total transcripts (Table 2), equating to 99.4% differential expression. Differential expression of TNS1 in brain metastases in patients with breast cancer was statistically significant (Table 2; $p=0.00192442$).

TNS1 is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer as compared to primary tumors of the breast.

We obtained exact mRNA expression levels for TNS1 in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in TNS1 gene expression in brain metastatic tissues. TNS1 was expressed at lower levels in brain metastases from patients with breast cancer as compared to primary tumors of the breast (Figure 1). Decreased expression of TNS1 in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1: $p=0.0003$). We calculated a mean fold change of 0.800 ± 0.142 in TNS1 expression in brain metastases when compared to primary tumors of the breast (Table 1).

Thus, through comparative transcriptome analysis of tumor and metastatic tumor tissues of patients with breast cancer using published microarray data^{4,5}, we found that TNS1 was among the genes most differentially expressed in brain metastases, with significantly decreased expression in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

To facilitate discovery of novel therapeutic targets in brain metastatic breast cancer, and to more thoroughly understand the biology of metastasis to the brain in breast cancer, we mined published microarray data^{4,5} to identify genes most differentially expressed in the brain metastases of patients with metastatic breast cancer.

Tensin 1, or tensin, is a cytoskeletal component of focal adhesions and actin-binding protein containing a Src homology 2 (SH2) domain⁷. Tensin can interact with filamentous actin (F-actin), and impair the rate at which F-actin polymerizes in actin filaments⁸ by binding to the barbed-end of the actin filament⁹. Tensin associates with focal adhesions through PTB motif-binding to the tail of integrin $\beta 1$ at NPxY motifs¹⁰. The AMP-activated protein kinase (AMPK) is a negative regulator of tensin expression¹¹. Tensin can co-localize with the p130Cas protein at focal adhesions at the leading-edge of migrating cells¹². Thus, it appears that down-regulation of tensin expression (rather than up-regulation) is important for brain metastatic tissues. This may be to facilitate colonization of the central nervous system by influencing focal adhesion interactions with integrins, or it may be that down-regulation of tensin enables a higher rate of F-actin polymerization during migratory behaviors.

We found here using published microarray data^{4,5} that TNS1 is among the genes whose expression changes most significantly in the brain metastases of patients with metastatic breast cancer and present at significantly lower quantities at the mRNA level in brain metastatic tissue.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
59	221246_x_at	1.16E-04	-4.3470649	1.1642	0.800 ± 0.142	TNS1	tensin 1

Table 1: Differential expression of TNS1 in brain metastases in patients with metastatic breast cancer.

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 TNS1 expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
369	Hs.9973.1.A1_3p_at	0.00192442	-3.305227	-1.35773	TNS1	tensin 1

Table 2: Differential expression of TNS1 in brain metastases in patients with metastatic breast cancer.

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: TNS1 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with brain metastatic breast cancer.

The mRNA expression level of TNS1 in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean TNS1 levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.