Sulfatase 1 is differentially expressed in brain metastatic breast cancer.

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Brain metastases affect up to 34% of breast cancer patients treated with trastuzumab¹. Limited treatment options are available for clinical control of brain metastatic breast cancer²⁻⁴. We mined published microarray data^{5,6} to identify genes associated with metastasis to the brain in human breast cancer. This unbiased, global gene expression analysis identified differential expression of sulfatase 1, encoded by SULF1, as a transcriptional feature of brain metastasis in patients with breast cancer. We recently identified heparan sulfate proteoglycan 2, HSPG2, as among the genes most differentially expressed when comparing brain metastases to primary tumors of the breast⁷. Thus, a heparan sulfate proteoglycan and an enzyme that catalyzes sulfation of heparan sulfate proteoglycans⁸ are both among the genes whose expression differs most significantly transcriptome-wide between primary tumors of the breast and brain metastases in patients with metastatic breast cancer.

Keywords: breast cancer, brain metastases, central nervous system metastases, SULF1, systems biology of breast cancer, targeted therapeutics in breast cancer.

Brain metastases are a clinical problem for patients with breast cancer²⁻⁴. One study reported a central nervous system metastatic frequency of 34% for patients with breast cancer who had been treated with trastuzumab¹. Unbiased approaches to describe the transcriptional landscape of brain metastases in patients with breast cancer are required. We mined published microarray data^{5,6} to perform blind, global comparative transcriptome analysis of primary tumors and brain metastases in patients with human breast cancer. We identified the sulfatase 1 as among the genes whose expression was most significantly different in the brain metastases of patients with breast cancer as compared to primary tumors of the breast.

Methods

We used datasets GSE125989⁵ and GSE26338⁶ 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. GSE26338 was generated using Human 1A Microarray G4110A technology with $n=11$ primary breast tumors and $n=3$ brain metastases from patients with breast cancer, analysis performed using platform GPL885. 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 SULF1 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 harnessed the power of blind, systems-level comparative transcriptome analysis to discover genes associated with metastasis to the brain in human breast cancer using published microarray data^{5,6}.

SULF1 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding sulfatase 1, SULF1, 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 cancer⁵. 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, SULF1 ranked 30 out of 22277 total transcripts (Table 1), equating to 99.9% differential expression. Differential expression of SULF1 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.59E-05$).

We queried a second microarray dataset⁶ to attempt to validate transcriptome-wide differential expression of SULF1 in brain metastatic breast cancer. We again found that SULF1 was among the genes whose expression was most significantly 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, SULF1 ranked 2769 out of 22575 total transcripts (Table 1), equating to 87.7% differential expression.

Differential expression of SULF1 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=3.7E-02$).

SULF1 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 SULF1 in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in SULF1 gene expression in brain metastatic tissues. SULF1 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 SULF1 in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of $.80 \pm 0.13$ in SULF1 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^{5,6}, we found here that SULF1 was among the genes whose expression was most significantly different when comparing brain metastases to primary tumors of the breast in patients with breast cancer, and that SULF1 was expressed at significantly lower levels in brain metastatic tissues as compared to primary tumors of the breast.

Discussion

We provided evidence here that the sulfatase 1 was among the genes whose expression was most significantly different in metastases to the brain as compared to primary tumors of the breast; we observed significantly decreased expression of SULF1 in brain metastatic tissues. Thus, an enzyme that regulates the sulfation level of heparan sulfate proteoglycans⁸, whose expression results in apoptosis of cancer cells *in vitro*⁷, and whose presence is critical for oligodendrocyte fate choice from ventral neural progenitors⁹, is differentially expressed and its levels are significantly decreased in brain metastases of patients with metastatic breast cancer. We recently described transcriptome-wide differential expression and down-regulation of a heparin sulfate proteoglycan, perlecan, also known as HSPG2, in the brain metastases of patients with metastatic breast cancer⁷. Together the data allude to importance of down-regulation of cellular behaviors associated with sulfated heparan sulfate proteoglycans in metastasis to the brain in humans with breast cancer.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
30	212354_at	1.59E-05	-5.0127009	2.9124	.80 ± 0.13	SULF1	sulfatase 1

Table 1: Brain metastases from patients with metastatic breast cancer differentially express SULF1 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, fold change (FC) of SULF1 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
2769	13806	3.7E-02	-2.29	-3.8887	SULF1	sulfatase 1

Table 2: Brain metastases from patients with metastatic breast cancer differentially express SULF1 when compared to normal breast tissue.

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: SULF1 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

The mRNA expression level of the sulfatase 1 (SULF1) 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 SULF1 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.