Differential expression of the G0/G1 switch gene 2, G0S2, in the brain metastases of patients with breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

In patients with breast cancer, brain metastasis provides limited treatment options¹⁻³. To discover genes associated with brain metastases in patients with metastatic breast cancer, we mined published microarray datasets, comparing global transcription in brain metastases and primary tumors of the breast^{4,5}. Using this methodology, we identified significant differential expression of the G0/G1 switch gene 2, encoded by G0S2, in the brain metastases of patients with breast cancer as compared to primary tumors of the breast. The G0S2 gene product could be of relevance to any one of the numerous processes by which tumor cells in breast cancer metastasize, including exit of the breast, entry into the periphery, breach of the blood brain barrier or colonization of and survival in the brain.

Keywords: G0S2, breast cancer, brain metastases, central nervous system metastases, systems biology of breast cancer, targeted therapeutics in breast cancer.

Trastuzumab-treated breast cancer patients possessed a 34% incidence of central nervous system metastases according to one study⁶. An unbiased understanding of the fundamental transcriptional makeup of brain metastases in patients with breast cancer, as well as the identification of novel therapeutic targets, can be supported by identification of genes associated with brain metastases. We compared brain metastatic tissues to primary tumors of the breast to perform global comparative transcriptional profiling in patients with brain metastatic breast cancer using published microarray datasets⁶⁻⁸. We discovered significant differential expression of the G0/G1 switch gene 2 (G0S2) in the brain metastases of patients with metastatic breast cancer.

Methods

We used datasets GSE43837⁴ and GSE26338⁵ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. 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. 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 G0S2 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

To discover genes associated with brain metastasis in patients with breast cancer, we mined 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.

G0S2 is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the gene encoding growth-arrest specific 1, G0S2, 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, G0S2 ranked 210 out of 61359 total transcripts (Table 1). Differential expression of G0S2 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=0.00099516$).

We queried a second microarray dataset⁵ to attempt to validate transcriptome-wide differential expression of G0S2 in brain metastatic breast cancer. We again found that G0S2 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, G0S2 ranked 234 out of 22575 total transcripts (Table 1). Differential expression of G0S2 in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=8.29E-04$).

G0S2 is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

We obtained exact mRNA expression levels for G0S2, both from primary tumors of the breast and from brain metastatic tissues to evaluate magnitude and direction of change in G0S2 gene expression in brain metastases. In both datasets queried, G0S2 expression was lower in brain metastases than in primary tumors of the breast (Figure 1 and Figure 2). Decreased expression of G0S2 in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1: $p=0.0226$ and Figure 2: $p=0.0327$). We calculated a mean fold change of 0.152 ± 0.261 in G0S2 expression in brain metastases when compared to primary tumors of the breast (Table 1).

Thus, by mining published microarray data to compare global gene expression in primary tumors of the breast and in brain metastases^{4,5}, we found that the G0/G1 switch gene 2, G0S2, was among the genes most differentially expressed in brain metastases; G0S2 was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast.

Discussion

To support identification of novel therapeutic targets in brain metastatic breast cancer, and to describe the transcriptional biology of brain metastasis in an unbiased fashion, we mined published microarray data^{4,5} to discover genes most differentially expressed in the brain metastases of patients with metastatic breast cancer.

G0S2 can interact with the anti-apoptotic protein Bcl-2 and antagonize its control of cell death⁷. In murine embryonic fibroblasts, G0S2 can inhibit transformation induced by oncogenes such as HRas⁸. G0S2 can interact with the adipose triglyceride lipase to exert influence over hydrolysis of adipocyte triacylglycerol. On the other hand, G0S2 can also support the synthesis rather than hydrolysis of triacylglycerol in the liver, utilizing lysophosphatidic acid and acyl-coenzyme A for synthesis of phosphatidic acid¹⁰. Interestingly, G0S2-deficient mice are reported as normal other than what is described as a “possible” lactation defect¹¹. G0S2-null mice manifest increased serum glycerol and decreased body weight, are less active at night and display enhanced cold tolerance¹¹. In human cancer, a subtype of adrenocortical carcinoma is characterized by CpG island hypermethylation, with shorter disease-free and overall survival¹².

We report here that G0S2 is among the genes most differentially expressed in brain metastatic breast cancer in humans, with mRNA for G0S2 present at significantly decreased levels in brain metastases as compared to primary tumors of the breast. G0S2 may be of relevance to pathways underlying metastasis to the brain in human breast cancer.

References

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Rank	ID	p-value	t	B	FC	Gene	Gene name
210	Hs. 95910.0.S1_3p_a_at	0.00099516	-3.5341834	-0.83204	0.152 ± 0.261	G0S2	G0/G1 switch 2

Table 1: Differential expression of G0S2 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 G0S2 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
234	820	8.29E-04	-4.18	-0.40139	G0S2	G0/G1switch 2

Table 2: Differential expression of G0S2 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: G0S2 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 G0S2 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 G0S2 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.

Figure 2: G0S2 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 G0S2 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 G0S2 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.