Fli1 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 Fli1 as a transcriptional feature of brain metastasis in patients with breast cancer. Fli1 may be of relevance to any one of numerous processes involved in metastasis of breast cancers to the brain, including the generation of tumor clones with sufficient oncogenic potential to colonize the brain.

Keywords: breast cancer, brain metastases, central nervous system metastases, Fli1, 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 *Fli1* 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 *Fli1* 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}.

***Fli1* is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.**

We identified the gene encoding, *Fli1*, 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, *Fli1* ranked 60 out of 22277 total transcripts (Table 1). Differential expression of *Fli1* in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=1.17\text{E-}04$).

We queried a second microarray dataset⁶ to attempt to validate transcriptome-wide differential expression of *Fli1* in brain metastatic breast cancer. We again found that *Fli1* 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, *Fli1* ranked 2928 out of 22575 total transcripts (Table 1). Differential expression of *Fli1* in brain metastases in patients with breast cancer was statistically significant (Table 1; $p=4.04\text{E-}02$).

Fli1 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 Fli1 in primary tumors of the breast and in brain metastases to evaluate magnitude and direction of change in Fli1 gene expression in brain metastatic tissues. Fli1 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 Fli1 in brain metastases as compared to primary tumors of the breast was statistically significant (Figure 1; $p=0.0005$). We calculated a mean fold change of 0.745 ± 0.188 in Fli1 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 Fli1 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.

Discussion

The Fli1 locus was identified as a proviral integration site of the Friend leukemia virus⁷, a pathogen with the capacity to induce the formation of erythroleukemias⁸. Fli1 is a transcription factor of the Ets family⁹. A fusion oncogene, EWS-FLI1, is a classic transcriptional characteristic of the childhood solid tumor Ewing's Sarcoma¹⁰. One report has described the existence of a FLI1 exonic circular RNA in breast cancer that could function to support metastasis through the epigenetic molecules TET1 and DNMT1¹¹.

We used published microarray data to perform global differential gene expression analysis of brain metastases from patients with breast cancer and identified Fli1 as a transcriptional feature of brain metastasis in patients with breast cancer. Fli1 may be of relevance to any number of steps required for metastasis to the brain in human breast cancer, from exit of the breast to colonization of the brain.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
60	210786_s_at	1.17E-04	-4.3433813	1.1546	0.745 ± 0.188	FLI1	Fli-1 proto-oncogene, ETS transcription factor

Table 1: Brain metastases from patients with breast cancer differentially express Fli1.

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 Fli1 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
2928	16840	4.04E-02	-2.25	-3.96723	FLI1	Friend leukemia virus integration 1

Table 2: Brain metastases from patients with breast cancer differentially express Fli1.

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: Fli1 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 Fli1 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 Fli1 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.