The death receptor Fas (CD95) is differentially expressed in brain metastases in breast cancer.

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Breast cancer patients with metastasis to the brain are faced with limited treatment options¹. To facilitate enhanced understanding of the transcriptional makeup of brain metastasis in human breast cancer and to support discovery of novel therapeutic targets, we mined published microarray datasets^{2,3} to identify genes whose expression was most markedly different when comparing brain metastases to primary tumors from patients with breast cancer. We identified the Fas receptor as among the genes whose expression was most significantly different in metastases to the brain as compared to primary tumors of the breast. Decreased expression of Fas in brain metastases in patients with breast cancer may be important for any number of steps involved in metastasis, including colonization of the brain.

Keywords: breast cancer, metastasis, brain metastasis, central nervous system metastasis, systems biology of breast cancer.

Brain metastasis is a clinical problem in patients with breast cancer. One study described existence of central nervous system metastasis in 309 out of 1044 patients, with 193 metastases of these patients possessing metastasis specifically to the brain⁴. Overall median survival was reported as 7.4 months in breast cancer patients with central nervous system metastases⁵. Treatment options for patients with central nervous system metastases include whole-brain radiotherapy⁶, surgery⁷ and stereotactic surgery⁸, and treatment options for patients with metastatic breast cancer in general include capecitabine⁹, lapatinib and capecitabine⁹, neratinib and capecitabine¹⁰, trastuzumab¹⁰, pertuzumab¹⁰, and trastuzumab emtansine¹¹. To identify understand the molecular nature of brain metastasis in patients with breast cancer, and to support identification of potential therapeutic targets, we performed differential gene expression profiling using published microarray datasets^{2,3}. We identified the death receptor Fas as among the genes whose expression was most significantly different transcriptome-wide when comparing brain metastases to primary tumor tissues from patients with breast cancer. Fas receptor may be important to the biology of metastasis to the brain in patients with breast cancer whose primary tumors spreads to the central nervous system.

Methods

We used datasets GSE43837² and GSE26338³ for this global differential gene expression analysis 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 Fas 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

Fas is differentially expressed in brain metastases in human breast cancer.

Comparison of global gene expression profiles of 19 primary tumors of the breast to 19 brain metastases² revealed that Fas was among the genes whose expression was most significantly different transcriptome-wide between primary tumors in patients with breast cancer and brain metastases (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and primary breast tumors, Fas ranked 66 out of 61359 total transcripts (Table 1). Differential expression of Fas was statistically significant (Table 1; $p=0.00025439$).

Comparison of global gene expression profiles of 11 primary tumors of the breast to 3 brain metastases³ revealed that Fas was among the genes whose expression was most significantly different transcriptome-wide between primary tumors in patients with breast cancer and brain metastases (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and primary breast tumors, Fas ranked 145 out of 22575 total transcripts (Table 2). Differential expression of Fas was statistically significant in this dataset as well (Table 2; $p=3.88E-04$).

Fas is expressed at significantly lower levels in brain metastases in human breast cancer.

We obtained exact mRNA expression levels for Fas in brain metastases and primary breast tumors to evaluate magnitude and direction of change in Fas expression in metastasis to the brain in patients with breast cancer. Fas expression was lower in metastases to the brain in both datasets analyzed when compared to primary tumors of the breast (Figure 1 and Figure 2). In each case, decreased expression of Fas in brain metastases was statistically significant (Figure 1: $p=0.0028$ and Figure 2: $p=0.0001$). In one dataset, we calculated a fold change in Fas expression of 0.358 ± 0.502 in brain metastases when compared to primary tumors of the breast.

Thus, we identified Fas as among the genes whose expression was most significantly different when comparing global gene expression between brain metastases and primary tumors of the breast in patients with breast cancer; Fas expression was significantly lower in brain metastases as compared to primary tumors of the breast.

Discussion

We undertook a systems-level, unbiased approach to describing the most significant transcriptional features of brain metastases in patients with breast cancer using published microarray datasets^{2,3}, both to further describe the molecular characteristics of brain metastases¹³ and to support identification of novel therapeutic targets in an area of unmet clinical need. We identified the Fas receptor as among the genes whose expression was most significantly different in brain metastases of humans with breast cancer as compared to primary tumors of the breast. Fas was expressed at significantly lower levels at the messenger RNA level in brain metastases as compared to primary tumors of the breast.

Fas is a member of the tumor necrosis factor receptor superfamily, and is able to initiate and transduce signals resulting in cell death through recruitment of the Fas-associated death domain protein FADD and caspase-8. It will be of interest to assess brain metastatic tissues from patients with breast cancer to evaluate caspase-8 expression and activity, and to investigate the possibility that colonization and or survival in the brain requires greater suppression of death signals than in the breast.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
66	g4507582_3p_a_at	0.00025439	-3.9891109	0.25879	0.358 ± 0.502	FAS	Fas cell surface death receptor

Table 1: Fas (CD95) is differentially expressed in the brain metastases of patients with breast cancer.

Rank of differential expression relative to all transcripts measured by microarray, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression when comparing brain metastases to primary tumors of the breast, the fold change of Fas expression in brain metastases when compared to primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
145	13150	3.88E-04	-4.57	0.30426	FAS	Fas (TNF receptor superfamily, member 6)

Table 2: Fas (CD95) is differentially expressed in the brain metastases of patients with breast cancer.

Rank of differential expression relative to all transcripts measured by microarray, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression when comparing brain metastases to primary tumors of the breast, gene and gene name are listed in this chart.

Figure 1: Fas is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

Fas mRNA expression levels in primary tumors of the breast (left) and in metastases to the brain (right) are depicted here with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in Fas mRNA expression between primary tumors of the breast and brain metastases, a *p*-value, listed above.

Figure 2: Fas is expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast in patients with breast cancer.

Fas mRNA expression levels in primary tumors of the breast (left) and in metastases to the brain (right) are depicted here with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in Fas mRNA expression between primary tumors of the breast and brain metastases, a *p*-value, listed above.